FINE MAPPING OF SHOOT FLY RESISTANCE AND STAY-GREEN QTLS ON SORGHUM CHROMOSOME SBI-10

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DECLARATION

I hereby declare that the research work presented in this thesis entitled"**Fine mapping of shoot fly resistance and stay-green QTLs on sorghum chromosome SBI-10**", has been carried out by me at the Department of Genetics, Osmania University, Hyderabad under supervision of Proff. P B Kavi Kishor and Dr. C.T. Hash at ICRISAT, Patancheru, Andhra Pradesh, India. This work is original and no part of the thesis has been submitted earlier for the award of any degree or diploma of any university.

Date:

Place: Hyderabad **(K. N. S. USHA KIRANMAYEE)**

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OUT LINE OF WORK

ABBREVATIONS

˚C	:	degree Celsius
µl	:	microliter
AFLP	:	Amplified Fragment Length Polymorphism
bp	:	base pair
cM	:	centiMorgan
CTAB	:	Cetyl Trimethyl Ammonium Bromide
CIM	:	Composite Interval Mapping
DAF	:	Days after 50% flowering
DA	:	Days after anthesis
DNA	:	Deoxyribonucleic acid
DArT	:	Diversity Array Technology
EST	:	Expressed Sequence Tag
FM	:	Fine Mapping
F_1	:	First filial generation
F_4	:	Fourth Filial generation
GAPIT	:	Genome Association and Prediction integrated Tool
GWAS	:	Genome Wide Association Mapping
GS	:	Genomic Selection
GBS	:	Genotyping by sequencing
Gls	:	Glossiness
GDW	:	Grain Dry Weight
GNpP	:	Grain number per Panicle
GNP	:	Grain Number per Plot
gms	:	Grams
HPR	:	Host Plant Resistance
HGM	:	Hundred Grain Mass
ICRISAT	:	International Crops Research Institute for the Semi-Arid Tropics
IM	:	Interval mapping
ISEP	:	International Sorghum EST Primer
JGI	:	Joint Genome Institute
JM	:	JoinMap
kb	:	kilo bases
LSP	:	Leaf Sheath Pigmentation
LD	:	Linkage Disequilibrium
LG	:	Linkage Group
LOD	:	Logarithm of odds (base10)
MTA	:	Marker Trait Association
MAB	:	Marker-Assisted Breeing
MAS	:	Marker-Assisted Selection
mM	:	milliMolar
Mb	:	Million bases
MAGIC	:	Multi advanced
ML	:	Maximum likelihood
NAM	:	Nested Association mapping
NGS	:	Next Generation Sequencing
PnDW	:	Panicle Dry Weight

PHI	:	Panicle Harvest Index
%GL	:	Percentage Green Leafarea
%SFDH	:	Percentage Shoot Fly Dead Heart
PlHt	:	Plant Height
PCR	:	Polymerase Chain Reaction
PCoA	:	Principal co-ordinate analysis
QTL	:	Quantitative Trait Loci
RAPD	:	Random Amplified Polymorphic DNA
RIL	:	Recombinant Inbred Line
RFLP	:	Restricted Fragment Length Polymorphism
F_2	:	Seconf Filial generation
SV	:	Seedling vigour
SFR	:	Shoot Fly Resistance
SSR	:	Simple sequence Polymorphism
SNP	:	Single-Nucleotide Polymorphism
SNPeff	:	SNP Effect
SBI-10	:	Sorghum Bicolor chromosome-10
STG	:	Stay-green
F_3	:	Third filial generation
TASSEL	:	Trait Analysis by aSSociation, Evolution and Linkage
TDL	:	Trichome Density Lower
TDU	:	Trichome Density Upper

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INTRODUCTION

1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a cultivated tropical crop plant belonging to the family Poaceae, tribe Adropoganeae and genus *Sorghum*. Sorghum is largely a self-pollinated diploid crop (2n=2x = 20) with fully sequenced genome length of ~735Mb. It is the fifth most important cereal crop globally (Dicko *et al.*, 2006), and chemical/biofuels feed-stocks across a range of environments and production systems. Its remarkable ability to produce grains under adverse conditions, which are characteristic of arid and semi-arid regions, in particular with much less water and at high temperatures than most other grain crops, makes sorghum an important 'fail-safe' source of food, feed, fiber, and fuel in the global agro-ecosystem.Sorghum grain is the second most important feed-stock for bio-ethanol production in USA, after maize grain. Sorghum and maize are closely related and diverged from the common ancestor ~12 million years ago (MYA). Genome sequences for both species have recently been published (Paterson *et al.*, 2009; Schnable *et al.*, 2009).

1.1.Distribution of sorghum

As a model for tropical grasses, sorghum is a logical complement to rice (*Oryza sativa*). Sorghum has similarities with maize and sugar cane which may support that they are derived from a similar ancestor (Paterson *et al.*, 2008). USA, India, México, Nigeria, Sudan and Ethiopia are the major producers of sorghum. Other sorghum producing countries include Australia, Brazil, Argentina, China, Burkina Faso, Mali, Egypt, Niger, Tanzania, Chad and Cameroon. Grain is mostly used as food (55%), in the form of breads and porridges in Asia and Africa, and as feed (33%) in America. Its stover is an increasingly important source of dry season fodder for livestock, especially in Asia (http://www.icrisat.org/crop-sorghum.htm). Total sorghum production from all sorghum producing countries was 57 million tonnes in 2012. The world average annual yield for sorghum was 1.08 tonnes per hectare in the year 2012. FAO reported the United States of America as the top sorghum producer with a harvest of 1.22 million tonnes

followed by India, Nigeria, Mexico and Sudan (FAOSTAT, 2012). In India, with its large population and fragile balance in the production and demand equation for food grains, sorghum plays a crucial role in national food security. Attempts to increase the production of sorghum with the introduction of new high-yielding varieties and hybrids since 1966, was largely unsuccessful because of the susceptibility of the improved cultivars to various biotic and abiotic stresses (Young and Teetes, 1977; Ajayi, 1987; Sharma *et al.*, 1993; Prem Kishore, 2001).

1.2.Adoptability to climate change

Sorghum is called "the camel of crops". It has earned this name because of its ability to grow in arid soils and withstand prolonged droughts. Sorghum is highly efficient in using of water, carbon dioxide, nutrients and solar light (Kundiyana, 1996). This crop is considered one of the most drought resistant, making it one of the most successful in semi-desert regions from Africa and Asia (Serna-Saldívar, 2010). This resistance is due mainly to its photosynthetic C_4 metabolism that allows sorghum to accumulate CO_2 during the night, to lower the photorespiration rate in the presence of light, to reduce the loss of water across the stomata and the waste of carbon (Keeley and Rundel, 2003). The leaves of sorghum and maize are similar but in the case of sorghum they are covered by a waxy coat that protects the plant from prolonged droughts. The sorghum grain is grouped in panicles and the plant height ranges from 120 to 400 cm depending on the type of cultivar and growing conditions. An advantage of sorghum compared to maize is that it has a comparatively lower seed requirement because only 10 to 15 kg/ha are used compared with 40 kg/ha required by other cereals (Kundiyana, 1996). In some regions it is possible to produce multiple crops per year, either from seed (replanting) or from ratoon (Saballos, 2008; Turhollow *et al.,* 2010). The crop plays a major role in the food security of millions of people in marginal agricultural areas. It occupies 25% or more of arable land in Mauritania, Gambia, Mali, Burkina Faso, Ghana, Niger, Somalia and Yemen, and globally it is the fifth largest cereal crop after wheat, rice, maize, and barley.

1.3.Drought stress and stay-green phenotypes

Abiotic stresses are the most harmful constraints concerning the growth and productivity of crops worldwide. After soil nutrient deficiency, drought stress is the most important abiotic constraint for sorghum production globally (Haussman *et al.,* 2002b). Sorghum is well adapted to semi-arid environments and regarded as model crop for studying drought stress tolerance among grass species. So, breeders mostly have been focusing on improving drought stress tolerant varieties of sorghum (Kassahun *et al*., 2010). Drought stress during and after flowering typically causes premature leaf senescence which in turn lead to stalk lodging, stalk rot disease, reduced grain filling, and significant grain and stover yield losses. Drought has been explained in many ways depending on the timing and intensity of water stress relative to reproductive stage of the crop. Drought can occur in any stage of crop due to, environment factors, management factors, genetic factors (Jordan *et al.,* 2012). If plants withstand drought spell occurring at post-flowering and grain filling stages, it is defined as terminal drought tolerance. Plants exhibit drought tolerance, drought resistance and also drought avoidance (Yue, 2006). If plant characters are best associated with post-flowering drought tolerance, then it may be due to delayed leaf senescence or non-senescence or "stay-green" trait (Borrell *et al.*, 2001; Jordan *et al.,* 2003, 2012; Harris *et al.*, 2007; Kassahun *et al.,* 2010; Borrell *et al.,* 2014a, Rama Reddy *et al.,* 2014). The "stay-green" trait is the ability to maintain functional green leaf area (GL) to improve quality of residues (Van Oosterom *et al.,* 1996), support the continuation of carbon fixation and supply of starch (McBee *et al.,* 1983), prevent premature death and lodging (Rosenow and Clark, 1981), sustain grain-filling under water stress (Rosenow *et al.,* 1983; Rajcan and Tollenaar, 1999a,b), and improve grain yield under stress (Borrell *et al*., 1996). Stay-green of sorghum is of three types. Type A stay-green phenotypes have a delayed onset and a normal rate of senescence following its onset. Type B stay-green phenotypes initiate leaf senescence normally but the rate of senescence is comparatively slower. Type C stay-green phenotypes retain chlorophyll despite the normal onset and progression through senescence. Many crop plants like rice, wheat, maize, barley cotton,

tobacco have been reported till date with stay-green character (Thomas and Howarth, 2000). Mechanism of regulation and expression of stay-green phenotype is still incomplete. Rosenow (1983) observed positive impact on crop performance of plants having delayed leaf senescence under water stress during grain filling. Presence of stay-green phenotype is a result of balance between nitrogen (N) demand by grain and nitrogen captured by vegetative parts of plant like increasing the supply of water by modified root architecture which increases water extraction from soil or reducing water demand by reducing transpiration loss. Nitrogen remobilization from leaves maintain longer photosynthetic activity and supply adequate carbohydrates to developing grains (Borell *et al.,* 1999, 2000a, 2001). Many explanations have been given for delayed leaf senescence which may be due to photosynthetic activity regulated by stomata during carbon fixation. As sorghum is a C_4 plant, phosphoenolpyruvate (PEP) carboxylase is used along with RuBisCO enzyme for carbon fixation. Where nitrogen (N) supply to plant is mechanized by RuBisCO, carbon nitrogen ratios and ABA levels are the most likey to affect senescence. Increased production of cytokinins may also lead to delayed leaf senescence (Gan and Amasino, 1995).

1.4. Marker assisted selection for stay-green

Research advances and studies on stay-green trait helped breeders in developing drought resistant cultivars. Stay-green trait has been manipulated by using marker assisted backcross. Transferring of target alleles from stay-green to senescent cultivars were majorly focused on sorghum breeding activities. Recent advent of molecular markers (RFLP, AFLP, SSR, SNP, DArT, CAPS)and marker-assisted selection and identifying quantitative trait loci (QTL) of related traits laid the steps for transfer of targeted QTLs from donor parent to the recurrent parent. Wild type plants expressing stay-green trait, will have linkage drag and QTL interactions. Presence of epistatic interactions among stay-green and between stay-green loci and genes in other regions of sorghum genome lead to linkage drag. Near isogenic lines (NILs) can be used to get rid of complex genetic interactions and phenotypes associated with it (Harris *et al.,* 2007). Many stay-

green drought resistant sources are available at ICRISAT, Patancheru, Hyderabad, India, in the gene bank of the institute and large scale MABC programmes were initiated for terminal drought tolerance in sorghum. Overall six sources such as E36-1, B35, QL41, SC56, SC283 and SDS1948-3 have so far identified QTLs of stay-green trait. Donors with elite susceptible cultivars are being used in ICRISAT breeding programmes. Haussmann *et al.,* (2002b) reported stay-green QTLs in sorghum. Kassahun and colleagues reported stay-green QTLs from the donor B35 (Kassahun *et al.,* 2010). Major problem for utilizing E36-1 as a source for breeding was availability of less number of polymorphic markers, as E36-1 belongs to the same set of zera-zera landrace which contributes mostly for the development of agronomically elite caudatum derivatives across sorghum breeding programmes. Further, major stay-green QTL mapping to SBI-10 has very large confidence interval and is linked with unfavourable alleles at neighbouring shoot fly resistant component QTLs associated with seedling leaf glossiness score and seedling leaf blade trichome density (Vadez *et al.,* 2013). Grand Parents involved in RSG04008-6 x J2614-11 cross are BTx623 which belong to kafir race, IS18551, R16 belong to race Durra, E36-1 belongs to Guinea-Caudatum hybrid race of sorghum bicolor. With advances in sorghum genomics, increase in availability of various marker systems, simple sequence repeats (SSR) markers (Ramu *et al.,* 2010), DArT markers (Mace and Jordon, 2010), and wide range of single nucleotide polymorphisms (SNPs) by genotyping-by-sequencing (GBS) have been developed by next generation sequencing utilizing Illumina platform (Elshire *et al.,* 2011, Nelson *et al.,* 2011).

1.5.Biotic stresses and shoot fly resistance

Major biotic constraints of sorghum yield and production are insect pests and diseases. More than 150 species of insect pests damage sorghum, of which sorghum shoot fly *Antherigonia soccata* (Rondani), is the major insect pest in Africa, Asia and Mediterrian Europe (Sharma, 1993). During early stages of crop growth and adaptation, shoot fly causes “dead heart” symptoms in plants. It mostly attacks tropical grass species like wheat, barley and sorghum. Shoot fly

lays white, elongated, cigar-shaped eggs singly on abaxial (lower) surface of the leaves parallel to mid-rib around 7-28 days after seedling emergence. The eggs hatch in 1-2 days of incubation and larvae enter the seedling's whorl of the central leaf, where it cuts the growing point, and feeds on the decaying leaf tissue, resulting in a typical wilting and drying of the central whorl leaf, a condition called 'dead heart' (Pont, 1972). As a result of dead heart formation, the young seedlings may be killed outright or they may produce axial tillers, which are rarely productive. The axial tillers serve as a mechanism of recovery resistance if they remain undamaged, but if shoot fly infestation continues, the seedling may die or present a rosette appearance and fail to produce any grain (Dhillon *et al.,* 2005). The pest is especially serious in late-sown crops, but sometimes appears with early sowing also, when the preceding dry season is interrupted by frequent showers of rain (Nimbalkar and Bapat, 1987). The levels of infestation may go up to 90–100% under delayed sowing (Hiremath and Renukarya, 1966, Satish *et al.,* 2009). Host plant resistance is one of the cheapest and sustainable methods for managing the insect pests and diseases. Improvement in stress resistance will increase ecological fitness, reduces pesticide use, and facilitates creation of a sustainable production system with increased efficiency, profitability and enhances grain quality traits. An integrated synergistic system involving plant breeding and genomics research using advanced molecular tools could increase the efficiency and precision of crop improvement. Earlier studies on the genetics of shoot fly resistance suggested that the component traits of resistance are complex and quantitatively inherited (Goud *et al.,* 1983; Hallali *et al.,* 1983; Agrawal and Abraham 1985), with predominantly additive gene effects (Nimbalkar and Bapat, 1992). Shoot fly resistance in sorghum was classified into three components, viz., non-preference for oviposition, antibiosis and tolerance (Soto, 1974). Under field conditions, resistance to shoot fly is primarily due to non-preference for oviposition (also called antixenosis, observed as reduction in the number of eggs laid on the seedling) (Jotwani *et al.,* 1971). Many other important component traits (Sukhani, 1987) such as leaf glossiness, leaf trichomes, seedling vigor, epicuticular wax (Nwanze *et al.,* 1992) and

biochemical factors (Singh *et al.,* 2004) are also associated with shoot fly resistance in sorghum which are quantitative in nature.

The severity of shoot fly infestation can be reduced by good management practices, of which the use of resistant cultivars is the most effective, economical and an eco-friendly approach to control the pest. Although many notable successes have been achieved through conventional breeding in the improvement of plant resistance to insects, the breeding process is often slow and laborious, and sufficient levels of resistance have not been achieved due to the quantitative nature of resistance (Tao *et al.,* 2003). However, concerted efforts toward breeding for shoot fly resistance have resulted in some progress, and a number of genotypes with resistance to shoot fly have been identified (Singh and Rana, 1996; Kumar *et al.,* 2000; Sharma *et al.,* 2003). Unfortunately, all high-yielding sorghum cultivars presently under cultivation in India are highly susceptible to shoot fly, prompting the national program to fix a threshold level of resistance before any cultivar can be officially released for cultivation.

1.5.1. Shoot fly resistance and QTLs

Agronomic practices (timely sowing), natural and synthetic insecticides, natural enemies and host plant resistance (HPR), are all components of integrated pest management practices used to minimize sorghum losses due to shoot fly infestation (Kumar *et al.,* 2008); but HPR and timely sowing remains the most preferred as they are cost-effective, eco-friendly and easily adapted by farmers. Host plant resistance to shoot fly is mediated by a number of morphological, biochemical and genetic factors. Of the many important morphological components of sorghum HPR identified, seedling leaf blade glossiness (Maiti *et al.,* 1984), seedling leaf blade trichome density (Maiti and Bidinger, 1979), seedling vigor, and leaf sheath pigmentation are all positively associated with Shoot Fly Resistance (SFR) (Tarumoto, 2005). Further, these SFR component traits have been mapped and the putative QTLs identified for individual traits and subsequently validated by marker-assisted backcross (MABC)-introgression into

genetic backgrounds highly susceptible to shoot fly. Using the cross BTx623 × IS18551, Sajjanar (2002) and Folkertsma *et al.* (2003) mapped shoot fly resistance (SFR) QTLs on SBI-01, SBI-05, SBI-07, and SBI-10. Similarly, using cross 296B × IS18551, Satish *et al.* (2009, 2012) mapped SFR QTLs on SBI-01, SBI-03, SBI-04, SBI-05, SBI-06, SBI-09, SBI-07, and SBI-10. Aruna *et al.* (2011) also mapped SFR QTLs on SBI-01, SBI-02, SBI-03, SBI-04, SBI-06, SBI-07, SBI-09, and SBI-10 using shoot fly resistance source IS2122. In a reciprocal cross IS18551 × 296B, Apotikar *et al.* (2011) found SFR QTLs on SBI-01 and SBI-03. Five putative QTLs for SFR component traits from IS18551 were then validated by MABC-introgression into the genetic backgrounds of elite shoot fly-susceptible hybrid seed parent maintainer lines 296B and BTx623 (Jyothi, 2010). Molecular marker assisted breeding programmes at ICRISAT, Patancheru, involved in transferring shoot fly resistance QTLs from donor parent IS18551 to different elite sorghum backgrounds of which 296B and IS18551 were the shoot fly susceptible and recurrent parents (Jyothi, 2010). Out of these, a near isogenic line (NIL) J2614-11 (parent 2) is having QTLs for shoot fly resistance (leaf blade glossiness and leaf blade trichome density) on sorghum chromosome SBI-10. Whereas, Kassahun (2006) stated that marker assisted breeding programme has transferred stay-green QTLs from E36-1 donor parent into R16 post-rainy *rabi* sorghum variety. Introgression line with stay-green QTL present on LG-G (SBI-10) was named as RSG04008-6, and used as a recurrent parent (parent 1).

1.6.QTL/gene pyramiding for resistance

Gene pyramiding is a breeding strategy that serves to combine favourable alleles at multiple genetic loci into a single plant genotype. This process of stacking of genes/QTL into a single elite cultivar background can now be efficiently performed by marker-assisted selection (MAS), using backcrossing or pedigree approaches. This approach expedites the varietal development process by providing the opportunity to select for all desirable genes/QTLs simultaneously, as well as eliminating the time-consuming process of inoculation for different races or isolates at different time intervals (Kole, 2006). Pyramiding of multiple

genes or common major QTLs for biotic, abiotic stresses are important approaches for genetical improvement of any genotype. Fully sequenced genome of sorghum (Paterson *et al.,* 2009), location of major QTLs for insect pests such as shoot fly (Satish *et al.,* 2009; Aruna *et al.,* 2011; Satish *et al.,* 2012), green bug (Agrama *et al.,* 2002; Katsar *et al.,* 2002; Nagaraj *et al.,* 2005; Wu and Huang 2008) head bug (Deu *et al.,* 2005) and midge (Tao *et al.,* 2003) which infest at different stages of plant development from seedling to panicle maturity, have been identified previously. Similarly, major stay-green QTLs were mapped (Haussmann *et al.,* 2002b) and introgression line developed (Mahalakshmi and Bidinger, 2002). Stay-green QTLs on sorghum chromosome SBI-10 are overlapping with the shoot fly resistance QTLs. So, this is the place of interest for fine mapping. Further, fine mapping of the identified QTLs into small intervals (Paterson *et al.,* 1990) provides meaning for QTL identification with increased marker density QTL region. Fine mapping studies reveals the smallest region contributing for the phenotypic traits and lays the basis for gene identification. Fine mapping can be achieved by large scale population with more markers showing more recombination events. In early generation populations like F_2, F_3, huge recombination events are available but, heterozygosity, segregation distortion, dominance and epistasis need to be overcome to fine map the interested regions. A genome-wide association study (GWAS) is a further advanced method to understand the marker trait associations based on linkage disequilibrium and can identify the SNP associated with the candidate genes (Visscher*et al.,* 2012).

Candidate genes underlying the target QTLs like seedling leaf blade glossiness and trichome density have been reported by Satish *et al.,* (2009, 2012) and Aruna *et al.,* (2011), but newer version of annotation of sorghum (2.1V) and present studies on trichome density and glossiness in different crops have more interesting results to support the identified QTLs. Identification of genes, pathways and mechanism involved in sorghum seedling leaf blade glossy and trichome density have not yet been clearly studied nor the candidate genes cloned

in sorghum. Majority of the studies were carried out in model crop plants like Arabidopsis and maize. Wax deficient mutant loci in *Zea mays* (maize), *Brassica napus* and sorghum are defined as '*glossy'* loci whereas in *Arabidopsis thaliana* and *Hordeum vulgare* (barley) they were named as '*ceriferum*' (cer) mutant loci (Kunst and Samuels, 2003). In Arabidopsis, many studies were reported as shine (*shn*) mutants which were isolated, characterized and found that the *shn* gene encodes for APETALA2 (AP2)/ethylene response element binding protein (EREBP) transcriptional factors that act in up- and down-regulation of lipid biosynthesis (Aharoni *et al.,* 2004). More than 30 '*glossy*' loci have been identified and a few were cloned (*gl1, gl2, gl3, gl4, gl8, gl13* and *gl15*) in maize (Li L *et al.,* 2013) and their functional role in glossiness has been reported. Similarly, for trichome density, many studies reported that WRKY, MYB transcription factors play important roles (Eulgem *et al.,* 2000; Johnson *et al.,* 2002; Ishida *et al.,* 2007; Liang G *et al.,* 2014).

Finding out candidate genes responsible for the major QTLs can be located with the help of high-throughput genotyping assays like single nucleotide polymorphism (SNP) that helps to map and study candidate gene associations with a biotic and abiotic stress related traits. Fine characterization or fine mapping approach for candidate genes associated with target traits can be evaluated with the help of historical recombination events occurring in QTLs and QTL flanking regions during transfer of desired traits to recurrent parent background. This will improve efficiency of introgression and its components by reducing the number of breeding cycles but look huge into genotyping recombinant (F_2) data sets (Satish *et al.,* 2012). However, Genotyping-By-Sequencing (GBS) approach gave huge marker-density and genome-wide coverage which has been possible with GBS-SNP platform that helps in the identification of SNPs closest to or inside the individual genes or regulatory elements associated with it. GBS data helps in constructing high resolution map of the target region and map based tagging of candidate genes identified (Vadez *et al.,* 2013). It would be desirable to generate recombinants having the favorable alleles at all three loci (for a high level of

glossiness, good green leaf area retention, and high trichome density), since such recombinants could be used as donors of the "cassette" of these three genes in applied marker-assisted breeding programmes targeting the Post-rainy (*rabi*) sorghum production environments of Peninsular India, where both shoot fly resistance and terminal drought tolerance are essential traits of well-adapted sorghum cultivars. In the course of producing such a recombinants from the cross of the BTx623-background, shoot fly resistance QTL introgression line (J2614-11) and the R16-background stay-green QTL introgression line (RSG04008), it should be possible to fine-map (and perhaps identify the underlying genes) for all three of these components of the cassette. Taking the above background into consideration, the following objectives have been framed for the present investigation.

OBJECTIVES

1. To produce $F_{2:3}$ fine-mapping populations by crossing the (J2614-11) BTx623- and (RSG04008-6) R16-background introgression lines, and then selfing themto produce the F_2recombinant population by one further generation of selfing and finally to produce test units for phenotyping.
2. To fine-map the SBI-10 component QTLs for seedling leaf blade glossiness, seedling leaf blade trichome density and for post-flowering green leaf area retention (stay-green) by combining genotyping and phenotyping data sets for selected subset of the fine-mapping populations.
3. To conduct bioinformatics searches for candidate genes underlying the three target QTLs, and use SNP haplotypes within these candidate genes for selected fine-mapping population segregants to assess which candidate genes are best associated with variation in these traits.
4. To recombine selected progenies from the fine-mapping population and self them to generate the desired genotype homozygous lines for favorable alleles at all three target QTLs.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Sorghum

2.1.1. Importance and crop improvement

Sorghum belongs to the Poaceae family (tribe Andropogoneae), which includes rice, maize, barley, oat, rye, millet, and wheat. Despite the separation of sorghum from maize and rice approximately 15-20 and 50 million years ago respectively, significant conservation of gene order exists among the genomes of these plants, which facilitates comparative genome mapping approaches (Bennetzen, 2000). Sorghum has a diverse germplasm, unusual tolerance to hot and dry environments, and a relatively small diploid genome of approximately 760-810 Mb (Arumuganathan and Earle, 1991), well suited for genomic approaches. A high-density integrated genetic and physical map of sorghum has been created using a combination of AFLP technology, six-dimensional bacterial artificial chromosome (BAC) pooling, and BAC DNA fingerprinting (Klein *et al.,* 2000). A dense genetic map of sorghum was obtained by scoring 2454 AFLPs, 203 RFLPs, and 136 SSRs in a recombinant inbred (RI) population, consisting of 137 lines derived from a cross of two *S. bicolor* genotypes BTx623 and IS3620C (Menz *et al.,* 2002). The physical map was generated using three different BAC libraries with BAC contigs anchored every 1.5cM across all ten sorghum chromosomes. The integrated genetic and physical map permits map-based cloning of important genes (Childs *et al.,* 1997; Klein *et al.,* 2005; Mace *et al.,* 2009; Mace and Jordan, 2011). Despite of its growing importance and molecular studies, improvement of sorghum in terms of production has been affected due to insect pest and drought stress. The most destructive insect pest of this crop is shoot fly out of 150 insect pests (Sharma *et al*., 2003) and drought stress causes heavy loss to the crop yield.

The present study was aimed at utilizing recently developed GBS SNPs and SSR molecular markers to map and fine map the genomic regions associated with stay-green and shoot fly resistance component traits for utilization in drought tolerance and shoot fly resistance breeding programs. The literature pertaining with the emphasis on the utility of conventional and molecular approaches for breeding cultivars resistant to drought and shoot fly are reviewed here.

2.1.1.1.Molecular markers and its importance

Molecular markers are necessary for locating the genomic regions of interest based on the marker polymorphism detected due to genomic sequence polymorphism which can be possible with co-dominant markers. Co-dominant markers differentiate homozygotes with a heterozygote which has key role in marker assisted selection as well as in marker assisted breeding. So based on the priority of DNA-based markers they were categorized as dominant molecular markers (RAPD, AFLP and DArT) and co-dominant molecular markers

2.1.1.1.1.1. Dominant markers are able to detect presence or absence of alleles i.e., allele 'A' or allele 'B' types can be identified but not heterozygotes.

2.1.1.1.1.2.Random amplified polymorphic DNA (RAPD)

This method amplifies genomic DNA with single random primer. In sorghum, many studies were reported (Tuinstra *et al.,* 1996; Subudhi and Nyugen 2000; Hausmann *et al.,* 2002a; Agrama *et al.,* 2002) on RAPD marker assisted breeding methods.

2.1.1.1.1.3.Amplified fragment length polymorphic DNA (AFLP)

It is a PCR-based multi-locus finger printing technique without prior sequence information. Many studies were carried out in Sorghum using this technique (Klein *et al.,* 2001; Haussmann *et al.,* 2002a, 2004; Menz *et al.,* 2002; Mace *et al.,* 2008; Ritter *et al.,* 2008; Shegro *et al.,* 2013; Gerrano *et al.,* 2014).

2.1.1.1.1.4.Diversity array technology (DArT)

It is a micro array based technology which detects all types of DNA variation (SNP, methylation, indels). A pool of genomes representing the germplasm of interest was cloned and individual inserts are arrayed on microarray resulting in a "discovery array". Labeled genomic imprints of germplasm of interest were genotyped by hybridizing to the discovery array. As this behaves as a dominant marker, the presence of variable DNA fragments will be detected with array-based technology which only found in few but not all. DArT was extensively used by sorghum molecular breeders (Mace *et al.,* 2008, 2009, 2012; Mace and Jordan 2010, 2011; Sabadin *et al.,* 2012; Bouchet *et al.,* 2012; Phuong *et al.,* 2013; Fiedler *et al.,* 2014) as it is a cost-effective high throughput method and independent of sequence information and electrophoresis.

2.1.1.1.2. Co-dominant molecular markers are capable to differentiate heterozygotes and homozygotes based on polymorphic genotyping data.

2.1.1.1.2.1. Restriction fragment length polymorphism (RFLP)

RFLP detects differences in individuals with the help of fragmentation sizes obtained by a single restriction enzyme activity on a group of individuals. This produces varied length fragments (Beckmann and Soller, 1986) and presence or absence of restriction site leads to fragment length polymorphism.RFLPs are challenging but time-consuming as they are more cost-effective PCR-based markers. In sorghum, RFLP studies (Pereira *et al.,* 1994; Xu *et al.,* 1994; Cui *et al*., 1995; Boivin *et al.,* 1999; Peng *et al.,* 1999; Bhattramakki *et al.,* 2000; Subudhi and Nguyen, 2000; Schloss *et al.,* 2002, Menz *et al.,* 2002) were used to construct linkage maps.

2.1.1.1.2.2.Microsatellite markers/Simple sequence repeat markers (SSR)

Microsatellites are tandem repeats of one to six nucleotide long DNA motifs that occur in all eukaryotic and prokaryotic genomes with frequent variation in a

number of repeat unit loci. SSRs are ubiquitous in nature and are used in high throughput genotyping, linkage map development, diversity analysis, QTL analysis, fine mapping, gene tagging and candidate gene identification. Nucleotide sequences of flanking regions of microsatellites are known, specific primers (20-25 bp) can be designed to amplify the SSRs using PCR. SSRs are cost-effective, PCR based markers and abundant in plant genomic DNA. In sorghum, microsatellite markers were exceptionally used (Brown *et al*., 1996; Taramino *et al.,* 1997; Tao *et al.,* 1998a, 2000, 2003; Kong *et al.,* 2000; Bhattramakki *et al.,* 2000; Schloss *et al*., 2002; Hausmann *et al.,* 2002a, 2004; Menz *et al.,* 2002; Agrama *et al.,* 2002; Bowers *et al.,* 2003; Wu and Huang, 2007; Mace *et al.,* 2008, 2009, 2010, 2011; Srinivas *et al.,* 2008, 2009a, b; Satish *et al.,* 2009, 2012; Billot *et al.,* 2013; Burow *et al.,* 2009; Yonemaru *et al*., 2009; Aruna *et al*., 2011; Ramu *et al.,* 2009, 2010, 2013; Nagaraja reddy *et al.,* 2012, 2013, 2014; Kiranmayee *et al.*,2016).

2.1.1.1.2.3. Single nucleotide polymorphism (SNP): SNPs are most abundant forms of genetic variation and distributed throughout the genome in various plants and animals. SNPs are more common mutations which occur between related genomes which are being used as markers in advanced molecular mapping methodologies (Fiedler *et al.,* 2015). Different technologies have been adopted for scoring SNPs which varies from low throughput to ultra highthroughput methods. Unigenes and EST databases were highly targeted for SNP discovery as they are the genomic regions of interest but, the frequency of SNPs in conserved regions like mRNA is low. Recently Elshire *et al.,* (2011) identified a new approach which digs out a huge number of SNPs using genotyping-by-sequencing (GBS) methods with low cost and more advance genome wide association studies (GWAS) performed. GBS SNPs are highly applicable in genome profiling (Morris *et al*., 2013a, b; Perez *et al.,* 2014; Lasky *et al.,* 2015) and direct re-sequencing utilizes SNPs as genotyping markers to detect the polymorphism using high throughput whole genome re-sequencing (Nelson *et al.,* 2012; Zou *et al.,* 2012; Bekele *et al.,* 2013; Mace *et*

al., 2013b), high density linkage map development (Zou *et al.,* 2012), QTL mapping (Zou *et al*., 2012; Phoung *et al*., 2013), fine mapping (Caniato *et al.*, 2014; Han L *et al*., 2015), diversity analysis and association mapping (Morris *et al*., 2013 a, b; Lasky *et al.,* 2015) in sorghum.

2.1.1.2. Mapping population

Mapping population development is the major step towards linkage mapping, QTL mapping, and fine mapping studies. The population itself decides the results as more diverse parents for traits of interest gives more clear results. Both the parents should be polymorphic for a trait of interest and cannot be too distant which may cause sterility or may lead to high level of segregation distortion during linkage analysis (Semagn *et al.,* 2006). A donor parent could be distant from an elite parent at least in genomic regions carrying for putative QTLs of interest and an elite parent for pedigree or backcross. Elite parent, weak for biotic and abiotic stresses mapped QTLs and high yielding economically important genotypes were selected for crossing in breeding programs in order to develop mapping populations for the traits of interest. Parents were assessed for polymorphism for the traits of interest and then crossed to produce F_1 heterozygous hybrid seeds which may be having high plant vigor. These mapping populations developed based on the intensity of trait study, a number of generations advanced for observing the trait and the time. For mapping population size of the population is also plays major role in the identification of variant genomic regions and their association with markers. Vales *et al.,* 2005 have demonstrated that as the population size increases, the number of QTLs will also increase. Sub-populations of 300, 200, 150, 100, 50 lines from a full population (409) with high recombination were selected and studied to confirm an increase in population resulted in the identification of a minor effect QTLs. All the mapping populations derived confirmed true by F_1 heterozygote's selfing. Lin *et al.,* (2004) stated heritability values also depend on the number of individuals included in the analysis. Different types of mapping populations were used based on the type of study, duration and cost of the experiment.

Commonly used mapping populations are second filial generation (F_2), back cross (BC), double haploids (DHs), recombinant inbred line (RIL) population, Near-isogenic lines (NILs), Nested association mapping population (NAM) and Multi-parent advanced generation intercross population (MAGIC).

2.1.1.2.1. F_2 Population

F_2 populations are derived from F_1 true hybrids by selfing or inter mating among F_1s. All possible types of recombinants are derived from the F_2 population due to meiotic crossovers or recombination or Chiasma occurrence. F_2 population has been extensively used in sorghum for construction of linkage maps and QTL analysis (Paterson *et al*., 1995b; Pereira and lee, 1995; Pereira *et al*., 1995; Due *et al.*, 2005; Cuevas *et al.*, 2014; Guo *et al.*, 2015; Kiranmayee *et al.*, 2016; Pfeiffer and Rooney, 2016). As each individual F_2 has different recombination with other individuals, we cannot replicate nor do we restrict to any experimental design except augmented design with parents as the checks (Kiranmayee *et al.*, 2016). In order to reduce F_2 experimental errors, F_3 phenotyping data means were assigned to F_2 genotype families. This design is called $F_{2:3}$ (F_2 derived F_3) design in plant genetics (Zhang and Xu, 2004; Kao, 2006). F_2 individual's genotyping data was utilized to map the QTL from the F_3 phenotyping data of their corresponding F_2 individuals to increase accuracy.

2.1.1.2.2. Back cross (BC) population

When F_1 hybrids were crossed with any one of the parent and the resulted population is named as backcross population. It is a segregating population derived by backcrossing of F_1 hybrid to one of its parents (recurrent or recipient). If backcross selection is repeated up to six generations, 99% genome of recurrent parent is retrieved. This population is near to F_2 population but, recombination was conserved only in one gamete. This population further advanced to near-isogenic lines (NILs) in sorghum (Harris *et al*., 2007; Kassahun *et al.*, 2010; Jyothi, 2010; Yohannes *et al.*, 2015).

2.1.1.2.3. Near iso-genic lines (NILs)

Repeated backcross of genetically distinct parent lines with extensive genotyping lead to near isogenic lines (NILs) are also a type of immortal populations consisting of introgression lines (ILs). Such populations consist of a single fragment or small number of genomic introgression fragments from a donor parent into a homogenous genetic background. NIL population resolution can be improved by minimizing the introgression size of each NIL and requires large population size for genome wide coverage as NILs have single introgression line which increases the power to detect small effect QTLs (Keurentjes *et al.,* 2007). The backcrossed population up to six generations (BC6) and more derived from the recurrent parent are used by Babu *et al*., (2004), and Semagn *et al*., (2006). Tuinstra *et al.,* (1998) identified post-flowering drought and pre-flowering drought QTLs using NILs. Sorghum and maize NILs were utilized for studying the overall effect of glycine betaine synthesis and its accumulation under conditions of osmotic stress (Peel *et al.,* 2010) and Harris *et al.,* (2007) fine mapped B35 stay-green QTLs using NILs in sorghum. Borrell *et al*., (2014a,b) utilized sorghum stay-green NILs and identified the stay-green role in leaf plant drought tolerance by enhancing the canopy development, leaf anatomy, root growth and water uptake and their individual QTLs role in crop water usage patterns and grain yield under terminal drought.

2.1.1.2.4. Multi-parent advanced generation inter-cross (MAGIC) population

Conventional QTL mapping studies are used for identification of QTLs from bi-parental crosses developed from F_2, BC or RILs. Recently, a multi-parent advanced generation intercross (MAGIC) strategy came into light to dissect multiple alleles and to provide increased recombination and mapping resolution (Cavanagh *et al.,* 2008). The main objective of developing MAGIC populations is to promote intercrossing and shuffling of the genome. The advantages of using multi-parent populations are that: (1) more targeted traits from each of the

parents can be analyzed based on the selection of parents used to make the multi-parent crosses; and (2) increased precision and resolution with which QTLs can be detected due to the increased level of recombination (Cavanagh *et al.,* 2008). Multi-parent populations are now attractive for researchers due to the development of high-throughput SNP genotyping platforms and advances in statistical methods to analyze data from such populations (Bandillo *et al.,* 2013). MAGIC populations have been first studied in Arabidopsis (Cavanagh *et al.,* 2008) and then rice (Bandillo *et al.,* 2013), wheat (Huang *et al.,* 2012; Cavanagh *et al*., 2013; Rebetzke *et al.,* 2014), chickpea (Gaur *et al.,* 2012), maize (Pea *et al.,* 2013), pigeon pea, groundnut and sorghum from ICRISAT.

2.1.1.2.5. Recombinant inbred lines (RILs)

RIL population developed from F_2 individuals from a bi-parental cross is based the genotyping and phenotyping for the trait of interest. It is a fixed population for traits of interest selected for at least six to eight generations. For genetic mapping studies like QTL identification, RIL populations are widely used and the mapping resolution highly depends on population size. Each RIL contains several introgression segments and on average, each genomic region is represented by an equal number of both parental genotypes of a population (Keurentjes *et al.,* 2007). RILs have increased power to detect QTLs when compared to $F_{2:3}$ populations (Moreno Gonzalez, 1993) but less when compared to NILs. In sorghum, many studies based on RIL population have been carried out (Tuinstra *et al.,* 1996, 1997; Tao *et al.,* 1998a, b; Boivin *et al.,* 1999; Peng *et al.,* 1999; Kong *et al.,* 2000; Tao *et al.,* 2000; Xu *et al.,* 2000; Bhattaramakki *et al.,* 2000; Klein *et al.,* 2001a, b; Haussmann *et al.,* 2002b; Agrama *et al*., 2002; Tao *et al.,* 2003; Mace *et al.,* 2008, 2009, 2010, 2011; Srinivasa Reddy *et al*., 2008; Srinivas *et al.,* 2008, 2009a, b; Satish *et al*., 2009, 2012, Aruna *et al.,* 2011; Naga Raja Reddy *et al*., 2013, 2014; Hayes *et al*., 2016; Gelli *et al*., 2016). These studies have identified a huge number of QTLs with the large effect on molecular breeding. Complete homozygous RILs are difficult to obtain and is time consuming and costs more space and money.

2.1.1.3. Selective genotypingand phenotyping

Selective genotyping is the term used when the term linkage between marker loci and QTL affecting some particular trait is carried out by genotyping only individuals from high and low phenotypic tails of entire experimental population (Darvasi and Soller, 1992). Large population sizes are advantageous for linkage mapping, QTL mapping, fine mapping and association studies. Selective genotyping and phenotyping approaches could be effective strategies for cost reduction associated with conducting QTL analysis in large population experiments and is superior to random sampling (Vales *et al.*, 2005). Selective recombinant genotyping can be utilized for high-resolution QTL mapping (Ronin *et al.*, 2003). Selective genotyping is also known as distribution extreme analysis or trait-based marker analysis (Collard, 2005). Xu and Hu (2009) demonstrated a combined approach for QTL mapping and segregation distorted loci resulted from selective genotyping. Recently, in watermelon for identification of Fusarium wilt, QTL mapping studies associated with GBS utilized selective genotyping approach was adapted (Lambel *et al.*, 2014).

2.2. Linkage maps and high density linkage maps

Genetic mapping involved ascertainment of phenotype in a genetically segregating population followed by association analysis between phenotype and genotype at marker loci spanning entire genome (Jin *et al.*, 2004). Mapping is a process of identifying genomic variant regions with the particular phenotype. Molecular data of crop genome is usually presented in the framework of genetic linkage maps useful to tag or locate genes of interest and facilitate marker assisted selection (MAS) and map- based cloning. Linkage mapping is keeping the markers in order, indicating the relative distance between them and assigning them to their linkage groups based on recombination values from all their pairwise combinations between marker loci in the segregating families like F_2 population, BC population, DH or RIL population of the experimental cross.

Markers or genomic tags or loci are assigned to linkage groups using different computer programs like MAPMAKER/EXP (Lander *et al.,* 1987), JoinMap (Van Oojen and Voorrips, 2001), ICIM mapping (Li H *et al*., 2007, THREAD Mapper Studio (Cheema et al., 2010), MAP DISTO (Lorieux, 2012) and computational programming developments including R/qtl package (Broman*et al*., 2003) were used to develop linkage and QTL maps. Many methods and software's have been developed to map the trait of interest. Inorder to calculate the important genomic regions, the order of the markers need to be determined in the derived population. Recent studies and NGS technologies have increased the marker data points in many crop plants. Re-sequencing technologies like GBS SNPs and RAD sequencing technologies have increased the marker density and ultra high density maps were generated in sorghum along with SSR and DArT markers. Recent advances in sorghum genomics and the availability of sorghum genome sequence (Paterson *et al.,* 2009) and the high throughput tools for whole genome sequencing at reduced prices year after year for data point generation for single nucleotide polymorphisms (SNPs), using Skim sequencing technologies like genotyping by sequencing (GBS) (Elshire *et al.,* 2011; Morris *et al.,* 2013a, b; Lasky *et al.,* 2015) have laid the path for genomic selection. Such technologies have increased the selection gain and improved the effectiveness of molecular breeding. In sorghum, various molecular markers (RFLP, RAPD, AFLP, SSR, DArT and SNPs) have been utilized for constructing linkage maps (Taramino *et al.,* 1997; Tao *et al.,* 1998, 2000, 2003; Kong *et al.,* 2000; Bhattramakki *et al.,* 2000; Hausmann *et al.,* 2002a, 2004; Menz *et al.,* 2002; Agrama *et al.,* 2002; Bowers *et al.,*2003; Wu and Huang 2007; Mace *et al.,* 2008, 2009, 2010, 2011; Srinivas *et al*., 2008, 2009a, b; Satish *et al.,* 2009, 2012; Billot *et al.,* 2013; Burrow *et al.,* 2009; Aruna *et al*., 2011; Ramu *et al.,* 2009, 2010; Perez *et al.,* 2014; Nelson *et al.,* 2012; Zou *et al.,* 2012; Bekele *et al.,* 2013; Nagaraja reddy *et al.,* 2012, 2013, 2014; Kiranmayee *et al.,* 2016; Gelli *et al*., 2016). The summary of different linkage maps studied are represented in Table 1.

Table 1 Review of literature for linkage mapping and QTL mapping in sorghum

S No	Reference	Mapping population Type	Mapping population Size	Parental lines	Molecular marker type (SSR/SNP)	LG (chromosome)	QTLs mapped	PURPOSE-comparitive mapping/LG/QTL map/Fine map/diversity
1	Lin *et al.*, 1995	F_2	370	S. bicolor / S. propinquum	RFLP	SBI-01,02,03,06, 07,09	9	plant height and maturity
2	Paterson *et al.*, 1995a	F_3	3488	S. bicolor / S. propinquum	RFLP	SBI-01,02,03,08, 10	5	kernal weight
3	Paterson *et al.*, 1995b	F_2		S. bicolor / S. propinquum	RFLP	SBI-01,02,04,06, 07,08,09,10	22	stem morphology
4	Pereira and Lee1995	F_2	152	CK60/PI229828	RFLP	SBI-06,07,09,10	4	Plant height
5	Pereira *et al.*, 1995	F_2	152	CK60/PI229828	RFLP	SBI-01,02,03,06, 07,08,09,10	25	Plant height,panicle architecture,Seed weight
6	Tuinstra *et al.*, 1997	RI F_{5-7}(HIF)	98	Tx7078/B35	RAPD, RFLP	SBI-01	1	seed weight
7	Rami *et al.*, 1998	RI F_{5-7}		IS2807/379,IS2807/249	RFLP	SBI-01,02,03,04, 06,07,10	53	Days to 50% flowering,plant height,grain yeild trits
8	Tao *et al.*, 1998b	RILs	160	QL39/QL41	RFLP,SSR	SBI-01, 02,03,08	4	Rust resistance
9	Crasta *et al.*, 1999	RI F_{6-7}	96	B35/Tx430	RFLP	SBI-01, 03,05,09, 10	7	DAF and stay-green
10	Peng *et al.*, 1999	RILs	137	BTx623/IS3620C	RFLP			High density map
11	Bhattramakki *et al.*, 2000	RILs	137	BTx623/IS3620C	SSR,RFLP			High density map
12	Subudhi and Nyugen 2000	RILs	98	B35/Tx7000	RFLP,SSR,RAPD	SBI-02, 03,05,07	4	Stay-green
13	Tao *et al.*, 2000	RILs	160	QL39/QL41	SSR,RFLP	SBI-01, 02,03,09,10	5	Stay-green

Table 1 (Contd...)

S No	Reference	Mapping population Type	Mapping population Size	Parental lines	Molecular marker type (SSR/SNP)	LG (chromosome)	QTLs mapped	PURPOSE-comparitive mapping/LG/QTL map/Fine map/diversity
14	Xu *et al.*, 2000	RILs F_7	98	B35/Tx7000	RFLP	SBI-02, 03,05	7	Stay-green
15	Chantereau *et al.*, 2001	RILs F_7	85	IS2807/IS7680	RFLP	SBI-01, 02,10	10	Maturity
16	Hart *et al.*, 2001	RI F_{6-8}	136	BTx623/IS3620C	SSR,RFLP	SBI-01, 02,03,04, 05,06,07,08, 09,10	29	leaf,stem,grain maturity,panicle QTL map
18	Klein *et al.*, 2001	F_5	125	RTx430/Sureno	SSR,AFLP	SBI-01, 02,04,06,07, 09,10	14	Grain mould,plant height,Zonate leaf spot, Bacterial leaf spot, resistance to anthracnose
19	Agrama *et al.*, 2002	RILs	93	GBIK/Redlan	SSR,RAPD	SBI-02, 04,07,09,10	5	Green bug resistance QTL mapping
20	Hausmann *et al.*, 2002b	$F_{3:5}$	225/226	N13/E36-,IS9830/E36-1	SSR,AFLP, RFLP,RAPD	SBI-01, 02,03,04,07, 08,10	36	Stay-green QTL mapping
21	Katsar *et al.*, 2002			BTx623/S. propinquum, RTx430/PI550607		SBI-01, 04,05,06,07, 09,10	6	Green bug resistance (Biotypes I & K)
22	Menz *et al.*, 2002	RILs	137	BTx623/IS3620C	SSR,AFLP,RFLP			High density map
23	Tao *et al.*, 2003	RILs	120	B890562/ICSV745	SSR,RFLP	SBI-03,07,09	3	Midge resistance
24	Hausmann *et al.*, 2004	$F_{3:5}$	225/226	N13/E36-,IS9830/E36-1	SSR,AFLP,RFLP,RAPD	SBI-01,02,03,04, 05,06,07,08, 09,10	29	striga resistance
25	Deu *et al.*,2005	F_2	217	Malisor 84-7/S34	RFLP	SBI-01,02,03,04, 08,09	9	Head bug resistance

Table 1 (Contd...)

S No	Reference	Mapping population Type	Mapping population Size	Parental lines	Molecular marker type (SSR/SNP)	LG (chromosome)	QTLs mapped	PURPOSE-comparitive mapping/LG/QTL map/Fine map/diversity
26	Brown *et al.*, 2006	RILs	119	BTx623/IS3620C	SSR,AFLP,,RFLP	SBI-01,03,04,06,07,08,09	19	Agronomic traits QTL mapping
27	Feltus *et al.*, 2006	RILs/F_2	137/370	BTx623/IS3620C	SSR,RFLP	SBI-01,02,03,04,05,06,07,08,09,10	76	Leaf,stem,grain maturity,panicle QTL map
28	Wu *et al.*,2007	$F_{2:3}$	277 families	Westland/PI550610	SSR	SBI-09	1	Green bug resistance
29	Knoll *et al.*, 2008	RILs	146	Shan Qui Red/SRN39	SSR,RAPD, RFLP	SBI-01,02,03,04,07	10	cold tolerance
30	Mace *et al.*, 2008	F_5 RILs	92	R931945-2-2/IS8525	DArT,SSR,AFLP	SBI-01,02,03,04,05,06,07,08,09,10		High density map
31	Murray *et al.*, 2008	RILs	176	BTx623/Rio		SBI-01,02,03,04,05,06,07,08,10	34	Brix,sugr related traits, stem morphology,grain composition,leaf,tillering, 1000kernal weight
32	Parh *et al.*, 2008	RILs	146	IS8525/R931945-2-2	SSR,AFLP,DArT	SBI-01,02,04,06,07,08,09,10	17	Ergot resistance
33	Ritter *et al.*, 2008	F_6 RILs	184	R9188/R9403463-2-1	AFLP,SSR	SBI-01,02,03,04,05,06,07,08,10	29	Brix,sugr related traits, stem morphology,grain yeild,days to flowering
34	Srinivas *et al.*, 2008	F_7 RILs	168	296B/IS18551	EST-SSRs			High density map
35	Srinivasa reddy *et al.*, 2008	F_9 RILs	93	IS22380/E36-1	SSR,RAPD	SBI-01,02,04,06	9	charcoal rot resistnce
36	Wu & Huang, 2008	$F_{2:3}$	277	Westland/PI55061	SSR	SBI-	4	Green bug resistance

Table 1 (Contd...)

S No	Reference	Mapping population Type	Mapping population Size	Parental lines	Molecular marker type (SSR/SNP)	LG (chromosome)	QTLs mapped	PURPOSE-comparitive mapping/LG/QTL map/Fine map/diversity
			families	0		01,03,09		
37	Burow *et al.*, 2009	F_2/F_3	100/200	KFS2021/BTx623	SSR	SBI-10	1	Bloom cuticle (BLMC)
38	Mace *et al.*, 2009			SSM249/SARIASO 10	DArT,SSR,AFLP	SBI-01,02,03,04,05,06,07,08,09,10		High density consensus map
39	Perumal *et al.*, 2009	$F_{2:3}$	71 families	SC748-5/BTx623	AFLP	SBI-05	1	Anthracnose resistance
40	Satish *et al.*, 2009	F_7 RILs	168	296B/IS18551	SSR	SBI-01,03,04,05,06,07,09,10	29	Shoot fly resistance
41	Srinivas *et al.*, 2009a	F_7 RILs	168	296B/IS18551	EST-SSRs			High density map
42	Srinivas *et al.*, 2009b	F_7 RILs	168	296B/IS18551	Genomic and genic SSRs	SBI-01,02,03,04,05,06,07,08,09	47	Stay-green, plant height,maturity,plant height, panicle architecture
43	Winn *et al.*, 2009	F_4 RILS	277	P850029/Sureno	SSR	SBI-01	2	Protein digestibility
44	Mohan *et al.*, 2010	F_7 RILs	168	296B/IS18551	SSR	SBI-06	3	Target leaf spot resistance,Zonate leaf spot resistance, Drechstera leaf blight resistance
45	Shiringani *et al.*, 2010	RILs	188	M71/SS79	EST-SSRs,AFLP	SBI-01,02,03,04,05,06,07,08,09,10	86	Brix,sugar related traits,Plant height,maturity,Grain and panicle yeild
46	Aruna *et al.*, 2011	RILs	210	27B/ IS2122	Genomic and genic SSR	SBI-01,02,03,04,06,07,09,10		Shoot fly resistance
47	Nagaraja reddy *et al.*, 2012	RILs (F_9)	245	M35-1/B35	Genic SSRs			High density map

Table 1 (Contd...)

S No	Reference	Mapping population Type	Mapping population Size	Parental lines	Molecular marker type (SSR/SNP)	LG (chromosome)	QTLs mapped	PURPOSE-comparitive mapping/LG/QTL map/Fine map/diversity
48	Nelson *et al.,* 2011	diverse accessions	8	BTx623,BTx430; P898012; Segaolane; SC35; SC265; PI653737,12- 26 (Sorghum)	RAD sequencing			diversity and SNP identification
49	Sabadin *et al.,* 2012	RILs	90	BR007/SC283	DArT,RFLP,SSR and STS	SBI-02,03,04,06, 08,10	17	Stay-green, flowering time nd plant height
50	Satish *et al.,* 2012	F_7 RILs	168	296B/IS18551	SSR	SBI-01, 03, 04, 05, 06, 07, 09, 10	49	Shoot fly resistance
51	Bekele *et al.,* 2013	diversity	564		Re-sequencing			Diversity and SNP identification
52	Fakruddin *et al.,* 2013	RILs	184	E36-1/SPV570	SSR,SNPs		28	Root traits and yeild traits
53	Kong *et al.,* 2013	F_5	161	S. bicolor / S. propinquum	SSR	SBI-04, 08, 09	3	Plant archtecture, growth, reproduction
54	Morris *et al.,* 2013a	diversity	142/336		GBS SNP			Sorghum flavonoid pigmentation GWAS
55	Nagaraja reddy *et al.,* 2013	RILs (F_9)	245	M35-1/B35	Genomic and genic SSRs	SBI-01, 02, 03, 04, 05, 06, 07, 08, 09, 10	91	Agronomic traits and yeild related traits
56	Alam *et al.,* .2014	RILs	214	R931945-2-2/*S.verticilliflorum*		SBI-01,02,03,04, 05,06,07,08, 09,10	61	Tillering
57	Caniato *et al.,* 2014	diversity	254		Sequencing			Diversity and SNP identification
58	Naga raja reddy *et al.,* 2014	RILs (F_9)	245	M35-1/B35	Genic SSRs	SBI-01, 02, 03, 04, 05,	61	Stay-green and grain yeild

Table 1 (Contd...)

S No	Reference	Mapping population Type	Mapping population Size	Parental lines	Molecular marker type (SSR/SNP)	LG (chromosome)	QTLs mapped	PURPOSE-comparitive mapping/LG/QTL map/Fine map/diversity
						06, 07, 09, 10		
59	Guo *et al.*, 2015	F_2		B140/CK60B,MS 138B/B140	SSR	SBI-04, 07	6	Seed dormancy QTL mapping and expression analysis
60	Kong *et al.*, 2015	F_5	161	S. bicolor / S. propinquum	SSR	SBI-01, 03, 07, 08, 09	7	Number of Rhizomes and vegetative branching
61	Lasky *et al.*, 2015	diversity	1943		GBS SNP			diversity and SNP identification
62	Gelli *et al.*, 2016	RILs	131	CK60/China17	GBS SNP		38	Agronomic traits

2.2.1. QTL mapping and fine mapping

A QTL is a region of interest of any genome that is responsible for variation in the quantitative trait of interest. Identification of QTL is a complicated process due to QTL interactions or epistasis because of many additional sources of variation (Doerge, 2002). QTL analysis is based on the association between phenotype and genotype of the markers. For initial QTL mapping, we need population segregation for the trait of interest and markers for identification of the trait. Traditional and simple statistical approach is to assess the differences in the phenotypic means for single -marker genotypic classes (Doerge, 2002). QTL mapping provides a means to dissect complex phenotypic characters into their component traits (QTLs) and allow the identification of molecular markers linked to desirable QTLs, so that these can be directly used in marker-assisted selection. Identified QTLs need to be tested for the significant level based on the logarithm of odds (LOD) values. High LOD values signified the existence of the QTL with more confidence level and minimum LOD will be 2. The probability of occurrence of a QTL is achieved by using permutation testing. Phenotypes are shuffled among the individuals and genome scans are performed on thousands of such data sets (Georges, 2007). The percentage of phenotypic variance obtained for the identified QTL makes the level of importance of a QTL; the more the phenotypic variance, the more the probability of the presence of genes related to the trait of interest.

2.2.1.2. Interval mapping

Interval mapping methods are widely used for mapping of QTLs in segregating generations derived from crosses between inbred lines. Interval mapping (Lander and Botstein, 1989) uses an estimated genetic map as framework for identification of QTLs. Interval mapping searches through the ordered genetic markers in a systematic, linear fashion, testing the likelihood of occurrence of QTL. As the likelihood is a mixture of normal distribution, it may fail standard statistical distributions and it is difficult to declare a QTL with confidence

(Deorge, 2002). The efficiency of detecting and the accuracy of mapping multiple QTLs by using genetic markers are much increased by employing multiple QTL models instead of single QTL models used in interval mapping (Jansen, 1993). QTL interval mapping can be calculated using maximum likelihood method or regression approach. Incase of maximum likelihood method, the goodness of fit can be tested using the method of maximum likelihood (ML). The flanking markers will have four genotypes (AABB, AAbb, aaBB, aabb), each having a mixture of QTL genotypes. LOD threshold values are determined separately for each experiment and it ranges from 2-4 (Churchill and Doerge, 1994; Doerge and Churchill, 1996). Incase of QTL interval mapping, using regression approach, linear regression can be used for interval mapping. Regression method is based on the estimation of the proportion of variance (AABB-0.99, AAbb-0.75, aaBB-0.25, aabb-0.01) explained by QTL. The regression method is faster in computation, especially when the number of QTLs considered in the model is large. Knowledge of the factors affecting the differences between regression and ML interval mapping can help in developing an efficient strategy, using both methods in QTL mapping (Kao, 2000). Maximum likelihood approach is more preferred than regression method.

2.2.1.3. Composite interval mapping

Composite interval mapping (CIM) (Jansen and Stam, 1994; Jansen 1994; Zeng, 1994) and multiple QTL mapping (MQM) (Jansen, 1993) have similar results by reducing the number of similar models. Both the methods extend the idea of interval mapping to include additional markers as cofactors-outside a defined window of analysis for the purpose of removing the variation associated with other QTLs in the genomes. CIM combines the maximum likelihood approach with multiple regressions, using marker co-factors to reduce the bias in estimates of QTL map positions and to increase the power to detect QTL by decreasing within marker class phenotypic variation (Mackay, 2001). The background genetic variation in a population can be controlled in this analysis combining interval mapping with multiple regression approaches and it is more

advantageous than previous methods. Linkage map also referred to as Quantitative trait mapping (Guo *et al.*,2010). Different softwares are available for QTL mapping PlabQTL version 1.2 (Utz and Melchinger, 1996), QTL cartographer V2.5 (Wang *et al.*, 2010), Inclusive Composite Interval Mapping 1.2V (ICIM) (Li H *et al*., 2007), Map QTL and R/qtl (Broman*et al*., 2003).

2.2.2. QTL mapping in sorghum

QTL mapping is an important approach that received increased attention in plant breeding for identifying polygenic traits which are important agronomically. QTL studies in sorghum identified many genomic regions associated with agronomically important traits like flowering time (Brown *et al.*,2006; Thurber *et al.*,2013; Higgins *et al.*,2014), plant height (Brown *et al.*,2008; Srinivas *et al.*,2009b; Thurber *et al.*,2013; Higgins *et al.*,2014), grain yield (Shehzad *etal*., 2014; Zhang *et al.*,2015), drought tolerance (Pre-flowering, Post-flowering drought tolerance) (Tuinstra *et al.*, 1996, 1997; Crasta *et al*., 1999; Subudhi *et al.*, 2000; Tao *et al.*, 2000; Kebede *et al.*, 2001; Haussmann *et al.*,2002b; Harris *et al.*,2007; Kassahun *et al.*, 2010; Naga raja reddy *et al.*,2014), heat tolerance (Johnson *et al.*,2014), cold tolerance (Knoll *et al*., 2008); ergot resistance (Parh *et al.*,2008) shoot-fly resistance (Jyothi, 2010, Deshpande, 2005, Satish *et al.*,2009, 2012; Aruna *et al.*,2011; Kiranmayee *et al.*,2016), stem borer resistance (Vinayan, 2010), midge resistance (Tao *et al.*,2003), rust resistance (Tao *et al*., 1998b),*Striga* resistance (Haussmann *et al.*,2004; Yohannes *et al.*, 2014), bloom cuticle (BLMC) (Burow *et al*., 2009), brassinosteroids QTLs (Perez *et al.*,2014), nodal root angle QTL (Mace *et al.*, 2012; Singh *et al.*,2012), aluminum tolerance (Magalhaes *et al.*,2007; Caniato *et al*., 2007, 2011, 2014) , male sterility (Klein *et al*., 2005; Jordan *et al.*,2010) and seed dormancy QTL (Guo *et al.*, 2015). Many traits were well studied in sorghum and their quantitative loci were identified and summarized in Table 1.

2.2.2.1.Fine mapping in sorghum

In sorghum, fine mapping studies are advancing recently due to increased availability of NGS technologies and available sorghum genome sequencing data. Most important and well-studied traits are agronomic traits like flowering time which has been studied and fine mapped using NAM population (Mace *et al.*, 2013a), plant height DW1 region confined to GA2 oxidase on chromosome 9 (Higgins *et al.*,2014). Another QTL for plant height (qHT7.1) was identified near genomic region harboring the known auxin transporter DW3 gene (Li *et al*., 2015). Altsb fine genetic mapping reveals *SbMATE*, aluminum activated citrate transporter at Altsb locus on chromosome 3 and is responsible for the aluminium tolerance (Caniato *et al*. 2011, 2014). Grain weight QTL fine mapped in sorghum delimited qGW1 region to 101kb on chromosome 1 short arm and has 13 putative genes (Han L *et al.*,2015), stem water controlling locus qSW6 was fine mapped using QTL analysis and bulk sergeant analysis and deep sequencing technologies which reduced 339 kb on chromosome 6 (Han Y *et al*., 2015).

2.2.2.2.Physical map and bin mapping in sorghum

Availability of sorghum genome sequence (Peterson *et al.*,2009) has led to the construction of physical sorghum maps based on sequencing data and re-sequencing data. Based on sequencing data available in public databases of sorghum like Phytozyme, Gramene, Pfam, Uniprot, new primers were blasted against the sorghum genome database for the location of physical positions/Insilco mapping (Li *et al.*, 2009; Ramu *et al.*, 2010). Mace and Jordan, (2010, 2011) have also utilized the sorghum genome database for locating the marker physical positions. GBS SNP physical positions were also determined by the availability of reference genome data. Whereas re-sequencing, RAD sequencing, and Array technology completely depend on physical positions and genome sequences (Mace *et al.*,2013b).

Over the past three decades, QTL mapping studies have become major tools for plant breeders identifying genomic regions controlling traits of economic interest

– largely because the molecular marker revolution rapidly reduced costs per marker data point dramatically improving both genome coverage and marker density that can be achieved with a given operational budget. Initially, single markers, and then intervals between adjacent markers were used to identify QTLs, but theory and recent studies demonstrate increased marker density and increased population size further improve QTL mapping resolution. Many QTL mapping approaches e.g. composite interval mapping (CIM) and multiple interval mapping (MIM) exhibit defects when used with high marker densities. In case of GBS-SNPs, huge (and highly incomplete) marker data sets are generated. They need considerable curation before their effective use for mapping is possible for example, one always needs to remove duplicate and ambiguous markers and to identify recombinant regions. Bin mapping strategies have spread widely across plant and animal genetics communities as they facilitate clear recombination break-point identification for genetic mapping and genomic selection. Genetic mapping using binned data points is a new genetic analysis method that can assist in fine-mapping QTLs. In order to overcome huge SNP data errors, a method which is recently emerging is the highly computational approach known as "bin mapping" (Huang *et al.*,2009). The bin-mapping approach has been widely used in recent studies on maize, rice, sorghum, barley, wheat and chickpea to improve their genetic maps. Refined linkage maps with recombinant bins address many queries and have advantages over traditional linkage maps for QTL mapping.

2.2.2.3.NAM (Nested association mapping)

An approach that combines the strength of linkage mapping and association mapping referred as Nested association mapping (NAM) has been proposed to identify functional markers (Guo *et al*., 2010). NAM was first demonstrated by Yu *et al.*, (2008) and maize flowering QTL was dissected using NAM population (Buckler *et al*., 2009) and maize leaf architecture by Tian *et al.*,(2011). In sorghum, very few studies have been reported on NAM population. Initially,Jordan *et al.*,(2011) developed NAM population and Mace *et al*.,

(2013a) developed back cross NAM population for dissecting flowering time in sorghum.

2.3.NGS technologies

Molecular marker technology in recent generations has led to the development of high-throughput technologies which can produce huge data points with low cost. Illumina Miseq and Hiseq 2500 (Bentley *et al.*,2008), Ion torrent PGM (Rothberg *et al*., 2011), Roche 454 FLX Titanium (Thudi *et al.*,2012) are few sequencing platforms which were used recently in many sequencing programs (Laidlaw *et al.*,2010).

2.3.2. Genotyping-by-sequencing (GBS)

Low cost and multiplexed genotyping by GBS is beneficial to breed superior cultivars in many crop species (Kim *et al.*,2016). Lasky *et al.,* (2015) has proved that GBS can be even a very high efficient approach which can be applied to any species with local adaptation. GBS is the new methodology developed with the advancement of next generation DNA sequencing technology development leads to genome-wide SNPs detection and application in various plants (Deschamps *et al*., 2012) in recent years by Elshire *et al.*,2011. GBS technology involves the digestion of genomic DNA with restriction enzymes followed by ligation of barcode adaptors, PCR amplification and sequencing of the PCR amplified product. GBS data are interpreted and analyzed with the help of bioinformatics pipelines (He *et al.*,2014). Reduced cost of GBS was helpful in skim-sequencing of not only parents but also their progeny/RILs. GBS was also used in implementing GWAS, genomic diversity study, genetic linkage analysis, new SNP discovery, and genomic selection during massive plant breeding programs. One advantage of GBS is, knowledge og genome sequence is not necessary, but SNPs are identified at the same time. Plants like maize, wheat, barley, rice, potato, and cassava have also been optimized by the GBS (Poland and Rife, 2012; Narum *et al.*,2013; He *et al.*,2014). Initially, GBS was developed for high-resolution association studies in maize but the low cost and the powerful NGS

approach on discovering SNPs in plants and their population made it a robust technology available for the researchers. Nearly, 5000 RILs were subjected to re-sequencing using a restriction endonuclease-based approach and Illumina technology, which resulted in 1.4 million SNPs and 200,000 indels in maize (Gore *et al.*,2009). A total of 2815 maize inbreed accessions were genotyped and 681,257 SNPs were detected across the whole genome. Few detected SNPs were found associated with candidate genes for kernel color, sweetness and flowering time also (Romay *et al.,* 2013). A major QTL for *Fusariumoxysporium* resistance was identified with the help of GBS technology in water melons (Lambel *et al.*,2014). Brix, sucrose, glucose, fructose are agronomically important traits for water melon which were discovered with the help of GBS technology (Ren *et al.*,2014). Nearly, 2,65,000 SNPs were identified in 971 worldwide accessions in agro-climatic diversity studies which lead to the identification of candidate genes for plant height and inflorescence in sorghum (Morris *et al.*,2013a) and for genome environment interactions of 1943 diversity accessions (Lasky *et al.*,2015). Kim *et al.,* 2016 has stated that GBS typically shows good results when it is applied to an inbred diploid species with well-established reference genome like sorghum. GBS provides a rapid and robust tool for genotyping largely-homozygous sorghum populations.

2.3.3. Genome wide association studies (GWAS)

The development of next generation sequencing technologies has enabled genome wide association studies (GWAS) of many complex traits in plants. The main research objective of genetics and genomics include identification of the causal relationship between genetic polymorphism within a species and the phenotypic differences observed between individuals. Any phenotypic differences identified are connected back to the underlying causative loci using various mapping approaches including QTL mapping. In this aspect, GWAS is considered as a powerful tool for connecting the genotype-phenotype associations (Korte and Farlow, 2013). GWAS was first reported in Arabidopsis for flowering time and pathogen resistance (Aranzana *et al.,* 2005) and then for

wheat, barley, rice, maize and few other crops (Soto-Cerda and Clutier, 2012). GWAS is based on linkage disequilibrium (LD) and is also named as association mapping (Guo *et al.*,2010). Association mapping methods were performed on genome-wide scale in plants (George and Cavanagh, 2015). LD is the non-random association between alleles at different loci which may be due to genetic drift, natural selection or some evolutionarily forced mutations which lead to recombination breaks. Larger the population size, weaker the LD for a given genetic distance at which LD decay determines the number of markers needed to tag a haplotype (Visscher *et al.*,2012). GWAS is complementary to QTL mapping but when they both performed together, they may reduce each other's limitations (Zhao *et al.*, 2007, Brachi *et al.*, 2010). GWAS require large number of markers for locating marker-trait associations. Many types of softwares like Gappit and Tassel are widely used for GWAS studies. In sorghum, using GWAS plant height components and inflorescence architecture were analyzed in diversity landraces (Morris *et al.*, 2013a; Thurber *et al.*,2013; Higgins *et al.*,2014), and flavonoid pigmentation traits and seed tannins were interlinked and dissected with high resolution by GBS SNP (Morris *et al.*,2013b). Aluminum tolerance in sorghum was determined using GBS SNP and GWAS methodology (Caniato *et al.*,2014). Rhodes *et al.*,(2014) have utilized GWAS for improving sorghum crop nutritional values by identifying proanthocyanidins and 3-deoxyanthocyanidins, polyphenols with antioxidants for crop biofortification. In sorghum, using GWAS, Perez *et al.*, (2014) have shown that brassinosteroid candidate genes have more impact on plant architecture. Genomic signatures of environment adaptation may be useful for crop improvement, enhancing germplasm identification and marker assisted selection. Genome-environment associations and phenotypic analyses may reveal environmental adaptation (Lasky *et al.*,2015). During the next few years, GWAS and next generation based genotyping technologies will be used for generating SNP data especially in unsequenced genomes. GWAS in plants will be challenging to analyze, and the results that will be of great use (George and Cavanagh, 2015).

2.4.Stay-green phenotype an integrated drought adaptation trait

Drought is a serious agronomic problem and the single greatest factor contributing to crop yield loss in the world today. Early senescent genotypes (less chlorophyll and low photosynthetic activity) have fewer yields when compared to lines with delayed senescence. Stay-green/delay in senescence is one of the drought tolerance mechanisms adapted by plants. Stay-green phenotype is due to loss of functional chlorophyll and photosynthetic activity exhibited by plants like sorghum, maize, wheat, rice, barley etc. Stay-green is the delay in senescence and this phenotype has the ability to sustain drought conditions and prevent lodging and charcoal rot in order to maintain normal grain filling (Rosenow and Clark, 1981; Rosenow *et al.,* 1983; Sanchez,*et al.*,2002; Borrell *et al.*,2014a, b). Stay-green is the best characterized component for drought tolerance (Borrell *et al.,* 2001; Jordan *et al.,* 2003, 2012; Harris *et al.,* 2007; Kassahun *et al.,* 2010; Borrell *et al.,* 2014a, Naga raja reddy *et al.,*2014) and retention of green leaf area (stay-green) positively correlates with higher grain yield and gains more importance and need to study more extensively. Under terminal drought conditions with limited water levels, grain yield has increased stay-green phenotypes when compared to senescent and intermediately senescent lines (Borrell *et al.*,1999, 2000b, 2014a, 2014b,Harris *et al.,* 2007, Haussmann *et al.,* 2002b; Jordon *et al.,* 2003, 2012). Many morphological (Sabadin *et al.*,2012), pre-flowering (Tuinstra *et al.*,1996, Phuong *et al.,* 2013), phenological factors, environmental factors (Vadez*et al.,* 2011, 2013; Kholova *et al.,* 2013; Borrell *etal* 2014a, 2014b) also influence stay green trait. Tuinstra *et al.*,(1997) reported stay-green QTLs under drought stress conditions in sorghum. They also reported yield related QTLs under fully irrigated conditions which are associated with stay-green QTLs indicating the pleiotropic nature of the detected stay-green QTLs. Several yield-related traits associated with stay-green were studied and mapped in sorghum (Crasta *et al.,* 1999; Kebede *et al.,* 2001; Rami *et al.,* 1998; Klein *et al.,* 2001; Feltus *et al.,* 2006; Srinivas *et al.,* 2009b). Many numbers of epistatic interactions were observed between stay-green loci and genes (Subudhi *et al.*,2000; Harris *et*

al.,2007). Inheritance of stay-green is complex as it is influenced by environment and shows both dominant and recessive pattern of inheritance. Onset of senescence was additive and rate of delay in senescence is dominant (Van Oosterom *et al.*, 1996, Tuinstra *et al.*,1997, Harris *et al*., 2007, Kasahun *et al.*,2010). Drought stress may be alleviated by developing crops that are well adapted to dry-land environments with marker assisted breeding programs. In order to be successful in marker assisted breeding, increasing marker density and identifying QTLs are necessary which would narrow down the QTLs to smaller regions.QTL mapping studies for stay-green trait has been identified long ago. Genomic regions responsible for stay-green trait were detected with the help of molecular markers and the phenotypic data of the stay-green lines locate the variations in the genomic regions which are important for breeding programs aimed at developing drought tolerance. QTLs for stay-green have much importance in improving the productivity under drought stress conditions (Borrell *et al.,* 2000a). Several QTL mapping studies contributing to stay-green expression under drought stress conditions have been evaluated and studied in mapping populations (Tuinstra *et al.,* 1996, 1997, 1998; Crasta *et al.*,1999; Subudhi *et al.,* 2000; Tao *et al.,* 2000; Xu *et al.,* 2000; Kebede *et al.,* 2001; Sanchez *et al.,* 2002; Haussmann *et al.,* 2002b; Hash *et al.,* 2003; Harris *et al.,* 2007),introgressed lines (Blummel *et al*., 2015; Kiranmayee *et al*., 2016) and near-isogenic lines (Tuinstra *et al.,* 1998, Subudhi *et al.,* 2000; Harris *et al.,* 2007). Several stay-green sources have been field evaluated that were developed through crosses (Mahalakshmi and Bidinger, 2002; Reddy *et al.,* 2007). Reduced canopy development, crop water usage, alterations in leaf anatomy, and grain filling have been observed under terminal drought stress conditions (Vadez *et al.*,2011; Borell *et al.,* 2014a; 2014b). Stay-green QTLs have also been reported to be co-localized with nodal root angle (Mace *et al.*,2012) and also affect the spread of lateral roots after maturation under drought stress (Singh *et al.*,2012). In addition to sorghum, stay-green has been extensively studied in maize (Wang A*et al.,* 2012), wheat (Chen *et al.,* 2010), barley (Gous *et al.,* 2013), rice (Huang *et al.,* 2015), Arabidopsis (Sakuraba *et al.,* 2014) etc.

The ex-ante economic impact of developing and disseminating a drought tolerant sorghum cultivar in target countries of Africa and Asia has been ascertained (Nedumaran *et al.*, 2014).

2.5.Shoot fly resistance

Shoot fly,*Atherigona soccata* (Rondani) is one of the major insect pests of sorghum grown in Africa, Asia, and Mediterranean Europe. In peninsular India, sorghum is cultivated during rainy and post-rainy seasons where shoot fly attacks the crop and damages early stages of crop growth, adversely affecting establishment and productivity (Sharma *et al.*, 2003). Shoot fly infests sorghum seedlings from 7 days after emergence (DAE) to 30 DAE. The female shoot fly has just 30-days' life span and lays white, elongated cigar-shaped eggs singly on the abaxial (lower) surface of leaf blades parallel to the midrib (Dhillon *et al*., 2006). Eggs hatch into maggots following 1-2 days of incubation, and each larva/maggot enters the central leaf whorl of the seedling on which it hatched. The larva reaches and cuts the seedling growing point, and feeds on the decaying tissue, resulting in drying of the central whorl causing a typical 'dead heart' symptom. Among several components of integrated pest management practices used to minimize losses due to shoot fly infestation of sorghum, host plant resistance (HPR) and timely sowing remain the most preferred options as they are cost-effective, eco-friendly and easily adopted by farmers (Kumar *et al.*, 2008). HPR to shoot fly is mediated by a number of morphological, biochemical and genetic factors. Shoot fly morphological component traits include seedling leaf blade glossiness (Maiti *et al.*, 1984), seedling leaf blade trichome density (Maiti and Bidinger, 1979), seedling vigor and leaf sheath pigmentation which are positively associated with shoot fly resistance (SFR) (Tarumoto, 2005). Further, these SFR component traits have been mapped, putative QTLs identified for individual traits, and subsequently validated by marker-assisted backcrossing (MABC)-based introgression into genetic backgrounds highly susceptible to shoot fly (Kiranmayee *et al*., 2015). Using a sorghum recombinant inbred line (RIL) population derived from the cross

(BTx623 × IS18551), Sajjanar (2002) and Folkertsma *et al.,* (2003) mapped SFR QTLs on SBI-01, SBI-03, SBI-05, SBI-07, SBI-09 and SBI-10. Similarly, using (296B × IS18551)-based RIL population, Deshpande(2005), Mehtre (2006) and Satish *et al.,* (2009, 2012) mapped SFR QTLs on SBI-01, SBI-03, SBI-04, SBI-05, SBI-06, SBI-09, SBI-07, and SBI-10. Aruna *et al.,* (2011) mapped SFR QTLs on SBI-01, SBI-02, SBI-03, SBI-04, SBI-06, SBI-07, SBI-09, and SBI-10 using shoot fly resistance source IS2122. In a RIL population based on a reciprocal cross IS18551 × 296B, Apotikar *et al.,* (2011) found SFR QTLs on SBI-01 and SBI-03. Similarly, five putative QTLs for SFR component traits from IS18551 were validated by MABC into the genetic backgrounds of elite shoot fly-susceptible hybrid seed parent maintainer lines 296B and BTx623 (Jyothi, 2010). Probable candidate genes underlying the target QTLs for seedling leaf blade glossiness and trichome density have been reported by Satish *et al.,* (2009, 2012) and Aruna *et al.,* (2011). In the present study we attempted to refine QTL intervals for trichome density and glossiness on SBI-10 by comparing whole sorghum genome sequence (Paterson *et al.,* 2009) annotation and a sequence-based physical map integrated with sorghum linkage maps (Ramu *et al.,* 2010), with genetic and physical maps available from different QTL mapping studies and whole genome sequence information (Mace and Jordan, 2011). We also tried to compare earlier shoot fly resistance QTL mapping studies on sorghum chromosome SBI-10 with the present study based on genetic and physical maps. Identification of genes, pathways, and mechanisms involved in sorghum phenotypes for seedling leaf blade glossiness and trichome density have not yet been completed in sorghum. Many such studies have been carried out in model plants like *Oryza sativa* (rice), Arabidopsis and *Zea mays* (maize) but not in sorghum. Wax deficient mutant loci in maize,*Brassica napus* and sorghum are defined as '*glossy'* loci, whereas in *Arabidopsis thalina* and *Hordeum vulgare* (barley), they were named as *ceriferum* (cer) mutant loci (Kunst and Samuels, 2003). In Arabidopsis, many studies have reported shine (*shn*) mutants, which were isolated and characterized, determining that the *shn* gene encodes AP2/EREBP (ethylene

responsive element binding protein) transcriptional factors that act in up- and down-regulation of lipid biosynthesis (Aharoni *et al.,* 2004). More than 30 '*glossy*' loci have been identified and a few were cloned (*gl1, gl2,gl3,gl4,gl8,gl13* and *gl15*) in maize (Li *et al.,* 2013) and their functional roles in glossiness have been reported. Similarly, for trichome density, many studies have reported that WRKY and MYB transcription factors play important roles (Eulgem *et al.,* 2000; Johnson *et al.,* 2002; Ishida *et al.,* 2007; Liang *et al.,* 2014).

2.6.Advantages of MAS

Marker assisted selection (MAS) refers to the use of molecular markers to assist phenotypic selection in crop improvement. Various types of molecular markers have major roles in plant breeding (He *et al.,* 2014). Molecular genetic maps are the basics for marker assisted plant breeding and crop improvement. In most sorghum breeding programs, the implementation of MAS is limited (Hash *et al.,* 2003). Sorghum breeders are aiming to increase the crop productivity under biotic and abiotic stress conditions by understanding the genetic and molecular basis with the help of both conventional trait-based approaches, a newly developed molecular marker approach. Inorder to achieve success in detecting QTLs, a population is needed which is segregating for the desired traits and the accurate phenotyping with efficient DNA markers distributed uniformly in the sorghum genome.

2.6.1. Gene pyramiding

Gene pyramiding is a breeding strategy that serves to combine favorable alleles at multiple genetic loci into a single plant genotype. This process of stacking of genes/QTL into a single elite cultivar background can know be efficiently performed by marker-assisted selection (MAS), using backcrossing or pedigree approaches. It expedites the varietal development process by providing the opportunity to select for all desirable genes/QTLs simultaneously as well as eliminating the time-consuming process of inoculation for different races or

isolates at different time intervals (Kole, 2006). If we know the location of series of genes of interest then gene pyramiding method will assist in many ways (Servin *et al*., 2004). Recent advances in genomics are helpful in marker assisted selection to produce NILs and for gene pyramiding in several cereals including sorghum (Witcombe and Hash, 2000). A number of reports have demonstrated successful pyramiding of blast or bacterial leaf blight resistance genes in rice (Huang *et al.,* 1997; Hittalmani *et al.,* 2000; Singh *et al.,* 2001). Huang *et al.,* (1997) pyramided four bacterial blight resistance genes,*Xa4*,*xa*5,*xa13* and *Xa2*1, in different combinations. Breeding lines with two, three, and four resistance genes were developed, and these pyramid lines showed a wider spectrum and a higher level of resistance than lines with only single genes. Another marker-aided pyramiding experiment involving the above three BB genes into PR106, a widely grown cultivar in India, was conducted by Singh *et al.,* (2001). Hittalmani *et al.,* (2000) pyramided three major genes,*Pi1*,*Piz5* and *Pita,* for blast resistance located on chromosomes 11, 6 and 12, respectively, using DNA markers. For *Piz5,* the PCR-based sequence amplified polymorphic (SAP) marker was used, whereas flanking markers were used for the other two. Field testing of the pyramided lines in the Philippines and India showed enhanced resistance against leaf blast in comparison with the lines with a single gene. In pearl millet, Hash *et al.,* (2006) demonstrated pyramiding of favorable alleles at two major QTLs for host plant resistance to pearl millet downy mildew, from donor parent ICMP 451-P6 in the genetic background of elite pollinator H 77/833-2 (Breese *et al.,* 2002). This has substantially improved resistance reactions across 9 diverse pathogen isolates in the product lines (ICMR 01004 and ICMR 01007) compared to their donor and recurrent parents. In maize, very recent reports have shown that they have pyramided eight QTLs/genes for four grain quality traits and three for rust resistance traits, which need to be further evaluated in multi-year/multi-location trials for commercial cultivation (Tyagi *et al*., 2014). Thus, the knowledge and genetic markers developed herein provide tools to initiate the pyramiding of multiple biotic and abiotic resistance loci through marker-assisted selection (Kiranmayee *et al*., 2015).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Development of fine mapping population

3.1.1. Plant materials

BTx623 is an elite, high yielding, homozygous advanced breeding line released by Texas A&M University, USA. It belongs to the Kafir race of *Sorghum bicolor*, and is susceptible to senescence and shoot fly. Plants are short/dwarf at maturity. Leaves of seedlings are dark (non-tan), dull, broad, drooping without trichomes. Panicle is having semi-loose stiff branches. Grains are white (thick white mesocarp and reddish-purple spotted white pericarp) and glumes are reddish brown in colour. Its genome has been sequenced (Paterson *et al.,* 2009) and is used as the reference genome in the present study. IS18551 is a shoot fly resistant (SFR) line belonging to race Durra. Leaves of seedlings are light green, narrow, and erect, with high trichome density on abaxial (lower) and adaxial (upper) surfaces, tall at maturity. It originates from Ethiopia, and has straw-colored grains with large black glumes. Panicle has semi-compact branches. E36-1, a stay-green (stg) source, belongs to the Guinea-Caudatum hybrid race, and originated from Ethiopia. It is well adapted to tropics and also resistant to charcoal rot and lodging (Haussmann *et al*., 2004). Grains are pinkish white, glumes are brown (straw) in colour. Panicle shape is semi-compact, elliptical. R16 is highly senescent and belongs to race Durra. It is an agronomically elite, post-rainy seasonj (*rabi)*-adapted sorghum released restorer line. Grains are creamy/lustrous, bold in nature with thin mesocarp. Panicle shape is semi-compact and elliptical.

3.1.2. Parents

RSG04008-6 (non-glossy, less trichomed) is a single-plant selection from a high yielding, drought tolerant but shoot fly susceptible introgression line (IL) with E36-1 alleles for stay-green-associated drought tolerance in highly senescent R16 background (R16 x E36-1) (C.T. Hash and colleagues) (Kassahun, 2006). Parent J2614-11(glossy, highly trichomed) is a single plant selection from a shoot fly resistant introgression line derived from IS18551 alleles introduced by MABC into BTx623 background (BTx623 × IS18551) having validated donor alleles for seedling leaf blade glossiness and trichome density on SBI-10 (Jyothi, 2010).

3.1.2.1 Confirmation of parental intogression lines for their donor alleles

As both of the parents derived from different MABC projects, there is a need to confirm the seed material before crossing. SSR markers present in the long-arm of sorghum chromosome-10 (SBI-10L) were selected and screened on parents (RSG04008-6, J2614-11) and grandparents (R16, E36-1, IS18551, and BTx623) to confirm parents with alleles of donor grandparents selected for crossing. Parents and markers were chosen carefully. Markers must be polymorphic between parents but not for grandparents. Grandparent's donor allele should be similar with parental alleles which indicate presence of donor allele in introgressed lines. Polymorphism between parents is necessary. Selected parents are crossed to generate F_1 heterozygous hybrid seed, that is then advanced by selfing to generate the segregating mapping population.

3.1.3. Development of F_2population, recombinant $F_{2:3}$ and $F_{2:4}$ progenies

At ICRISAT,Patancheru, a manually emasculated and pollinated plant × plant cross was made between RSG04008-6 and J2614-11 (U1000019) during *rabi* 2010 to produce F_1 seeds. The F_1 seeds were harvested from female parent RSG04008-6 and then sown for generation advance. Morphologically and genotypically confirmed F_1 plants were self-pollinated using selfing bags to produce F_2 seed lots. A moderately-large, high-resolution mapping population of

1,894 F_2 individuals, derived from a single selfed F_1 plant (U110055), was grown in three batches in plastic pots during *rabi* 2011-2012 (Feb - Jun 2012) with triply-repeated parents for each F_2 sowing thinned to 3 plants per plot per sowing. Plants were labelled individually (with plant number starting from U120001 to U121941) for F_2 progenies, while parents were tagged with their names. In total, DNA samples of 1,894 F_2 plants, and parents (9 repeats in total for each parent), were isolated and genotyped initially with 5 simple sequence repeat (SSR) markers namely *Xgap001,Xnhsbm1044,Xiabt340,Xisep630* and *Xtxp141* distributed across the 37cM (4Mbp) SBI-10 interval where QTLs for seedling leaf blade glossiness and trichome density from donor parent IS18551 had previously been mapped, and then introgressed into BTx623 background to produce parent J2614-11. Some 369 individual homozygous and nearly homozygous recombinant F_2 plants were identified. All of these 369 selected recombinant F_2 individuals were advanced by selfing to the F_3 generation (Fig .1). Based on F_2genotyping data of 7 co-dominant SSR markers and $F_{2:3}$ phenotyping data, we have selected a further reduced subset of 152 most informative recombinants and selfed their corresponding F_3 progenies to produce F_4 seed which were sent to field trials in three replications for fine mapping.

3.1.3.1. Phenotyping in F_2 and $F_{2:3}$

At ICRISAT, Patancheru, Hyderabad, F_2 plants were tagged and scored individually for the traits, whereas in $F_{2:3}$ generation, the plants were segregating for the traits within the family; so maximum group of plants with similar phenotype were scored for each genotype. Seedling leaf blade glossiness was scored visually at 12-15 days after emergence (DAE) as described in Sharma *et al.,* (1992) where 1 = shiny, pale green, pointed, narrow and erect leaves (glossy) and 5 = dull, dark green, broad and droopy leaves (non-glossy). Leaf blade trichome density was scored by visual appearance of trichomes on leaves as described in Bourland *et al.,* (2003), but based on the trait variation, in the present population, scores were defined as follows. As trichomes are hairy leaf structures, leaf surface roughness indicated degree of trichome density and

smooth leaf surfaces indicated the absence of trichomes. Scores were given as 0 = absent, 1= very low density, 2 = low density, 3 = medium density, 4 = high density, 5 = very high density. All observations were recorded only by first author.

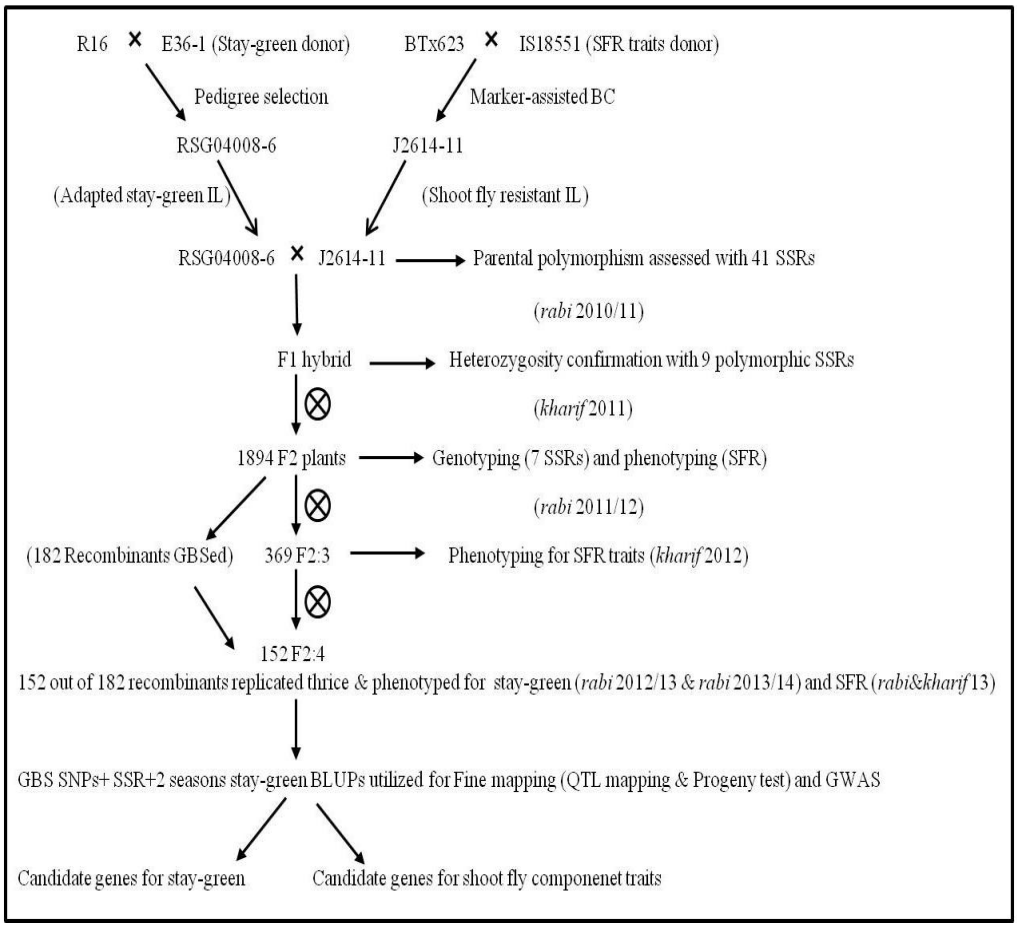

Fig .1 Schematic of parental introgression line (IL) development and derivation of population for fine mapping of shoot fly resistance and stay-green traits on SBI-10L

3.2. Evaluation of $F_{2:4}$ stay-green progenies in the field

3.2.1. Location- and season-wise-experiment andfield experimental design

Field experiments were conducted during post-rainy season (*rabi*) of 2012-2013 and 2013-2014 at ICRISAT, Patancheru (17° 30' N, 78° 16' E, altitude 545 m). The post-rainy season is ideal for terminal drought stress tolerance assessment as the plants utilize stored soil moisture for maturation and grain filling under progressively severe drought stress and moderate evaporative conditions. Onset of stress was manipulated by controlling supply of water before flowering. One irrigation was given to the experimental field after sowing in order to facilitate uniform seed germination. The experiments reported here were sown on a shallow (40 to 60 cm) vertic inceptisol (very fine montmorillontic isohyperthermic) overlying a loose, decomposing granite-base material that is permeable to roots but contains limited plant-available water (Haussmann *etal*., 2002b). During the 2012-2013 and 2013-2014 experiments, 152 genotypes + RSG04008-6-6 (twice) + J2614-11-11 (twice), a total of 156 entries were arranged in an alpha lattice design with 39 blocks per replication × 12 entries per block (Patterson and Williams, 1976) with three replications. The experimental units were 2-row plots (length 2 m) with 45 cmand 15 cminter- and intra-row spacing, respectively. A basal application of 20 kg ha^{-1} N and 20 kg ha^{-1} P_2O_5 as di-ammonium phosphate was given before sowing. In the 2012-2013 experiment, sowing was carried on 31st December 2012 and in 2013-2014 experiment, the day of sowing was done on 20th November 2013. The seeds were machine sown and the field was irrigated with overhead sprinklers to ensure germination. The crop was thinned 10 days after emergence to about 60,000 plants ha^{-1}. The temperature regime was not very similar in the two seasons due to dates of sowing and exposure of stress variations. Climatic difference between the 2 test years was that the 2012-2013 season received 57 mm rain in April and the crop was harvested during June 2013. The season 2013-2014 did not receive any rains and the crop was harvested during May 2014.Traits assessed in the two trials included the number of sorghum plants, the time from emergence to 50%

flowering (DF), plant height (PlHt), plant count/plot (PtNP),panicle count/plot (PnNP), productive tillers/plant (ETNP), panicle dry weight in grams/plot (PnDW/plot), grain dry weight grams/plot (GDW/plot), panicle harvest index (PHI), mean 100-grain mass (g) (HGM), grain number per plot (GNP/plot), grain number per panicle (GNPP) and for senescence, weekly percent of senescence was observed on plot basis after days to 50% flowering.

3.2.2. Percent green leaf area 7, 14, 21, 28, 35, 42, 49 days after flowering (% GL 7 DAF, % GL 14 DAF, % GL 21 DAF, % GL 28 DAF, % GL 35 DAF, % GL 42 and % GL 49 DAF) observations

Visual score rating was given plot wise, 7 days after 50% flowering and the values were recorded as % GL 7 DAF. Visual score ratings were recorded 14 DAF by comparing the plots and score was given as % GL 14 DAF. Visual score ratings were recorded 21 days after 50% flowering and recorded as % GL 21 DAF. Weekly visual score ratings were given 28 DAF and observations were recorded as % GL 28 DAF. Visual score ratings were recorded as 35 DAF and recorded as % GL 35 DAF. Visual score ratings were recorded after 42 DAF recorded as % GL 42 DAF. Visual score ratings recorded after 49 DAF recorded as% GL 49 DAF.

3.2.2.1. Estimation of senescence

Leaf senescence pattern was assessed plot-wise under partially-irrigated conditions. The percent green leaf area (% GL) of each leaf of the tagged plants was estimated visually on a weekly basis from anthesis to harvest. The visual senescence readings were deducted from 100 to get the percent green leaf area (% GL). For example, leaf 1 % GL = 100-leaf senescence and % GL was converted to GL as: leaf 1 GL = Leaf 1 % GL/100 × Leaf 1 area. Weekly weighted (by leaf size) average GL per plant was calculated and averaged first on a plot and then on a genotype basis. Logistic curves were fit to the genotype × date means using the logistic curve fitting routine of GENSTAT, and the fitted curves were used to compare genotype senescence patterns and to predict % GL at key times during the grain-filling period (Mahalakshmi and Bidinger, 2002).

3.3. Evaluation of Agronomic traits in the feild

3.3.1. Time to 50% flowering (days): This is calculated based on duration from the date of sowing to anthesis of 50% plants in a single plot.

3.3.2. Plant height (cm) [PlHt]: Plant height was measured from two weeks after flowering and measured from ground to the panicle top (PlHt, cm) with the help of a large scale indicated in centimeters by selecting three different plants from the 2m × 2 row plot of three different heights. Mean values were calculated for each plot and used.

3.3.3. Plant count/number (PtNP): Total plants in each 2m × 2row plot were counted and measured as PtNP.

3.3.4. Panicle count/plot (**PnNP)**: This is a post-harvest observation. Total number of panicles per plot after harvesting were counted and measured as PnNP.

3.3.5. Productive/Effective tillers/plot (**ETNP):** This is a derived trait. Total number of panicles per plot out of the number of plants per plot was counted. (PnNP/PtNP = ETNP).

3.3.6. Panicle dry weight in grams (PnDW): Total weight of mature panicles per plot (2m × 2 row) was measured in grams with the help of balance.

3.3.7. Panicle dry weight in grams/plot (**PnDW/plot):** It is a derived trait. In 2m × 2 row ideal plant count per plot (26) was divided by total panicle count multiplied by panicle dry weight in grams. Total weight of mature panicles per plot (2m × 2 row) is measured in grams with the help of balance.

3.3.8. Grain weight in grams/plot (GDW/plot): It is a derived trait. It is calculated as ratio of GDW with PnDw multiplied by PnDw/total plot. Total thrashed grain per plot is weighed using balance and measured as GDW.

3.3.9. Panicle harvest index (PHI): This is a derived trait. It is calculated as ratio of grain weight (g) (GDW) to panicle weight in grams (PnDW) multiplied with hundred.

3.3.10. Mean 100-grain mass (g)(HGM): Hundred seeds were counted per sample in three replicates and weighed on balance, all the three readings were noted and mean of three was calculated.

3.3.11. Grain number per plot (GNP/plot): This is calculated as grain weight grams/plot (GDW/plot) divided by mean 100-grain mass (g)(HGM), multiplied with hundred.

3.3.12. Grain number per panicle (GNPP): This is calculated as grain number/plot (GNP/plot) divided by panicle count number per plot (PnNP). Pre-harvest and post-harvest observations data were collected and arranged in an Excel spreadsheet in GenStat 14th edition for analysis.

3.4. Field experimental designs for shoot fly screening

Field experiments were conducted at ICRISAT-Patancheru, research fields during *kharif* (rainy) 2013 and *rabi*(post-rainy) 2013/14 seasons. On 8th July 2013, plant material was sown for shoot fly screening in an alpha lattice design with three replications, 32 blocks, and 5 entries per block for a total of 160 entries per replication. Entries included 152 F_4 individuals, along with their parents (RSG04008-6 and J2614-11), a susceptible control (Swarna), and resistant control (IS18551), that were repeated twice (total 160 entries), and were replicated thrice. They were arranged in 2m × 2row plots. The rows were 60cm apart and plants were spaced 15 cm apart within each row. All standard cultural

practices were followed to raise a successful crop, except no insecticide was applied. The *rabi* trial was sown on 29th of October 2013, under conditions otherwise similar to the *kharif* trial.

3.4.1. Shoot fly resistance screening technique

To attain uniform shoot fly pressure under field conditions, interlard fish meal technique (Nwanze, 1997) was followed for resistance screening (Fig .2). Four rows of susceptible cultivar (Swarna) were sown 20 days before sowing the test material and referred to as interlards. This was carried out to allow multiplication of shoot fly for one generation. Ten days after seedling emergence of test material, polythene bags containing moistened fish meal were kept in the field of test material at uniform intervals covering the entire area to attract the emerging shoot flies from interlard rows. Plant protection measures were avoided until the shoot fly infestation period was complete.

3.4.1.1. Phenotypic observations- leaf blade glossiness

Observations of leaf glossiness score (glossy=1 and non-glossy=5), trichome density on abaxial and adaxial counts (number per microscopic field), seedling vigour (1-5 scale), deadhearts (%) were recorded across two seasons: Rainy (*kharif)* 2013 and Post-rainy (*rabi*) seasons in 2013. Leaf glossiness was visually scored on a scale of 1-5 at 7-12 days after emergence (DAE), where, 1= highly glossy, 2= glossy, 3= moderatly glossy, 4= non-glossy, 5= highly non-glossy. Leaf glossiness was recorded during early morning hours where light reflection is maximal, before sunlight intensity is too high. Leaves are pale green, narrow, shiny, and erect in nature for glossy, and dark green, broad, dull,droopy in nature for non-glossy.

Fig .2 Interlard fish meal technique in field for shoot fly infestation

3.4.1.2. Leaf blade trichome density (numbers/microscopic field)

Trichome density on adaxial leaf surface (upper), trichome density on abaxial leaf surface (lower) was recorded on 14-17 DAE on the central portion of fifth leaf from the base,in three randomly selected plants from the plot of both screening environments. For measuring need visual scoring observations scale from1-5 scale or for microscopic observations need to collect (approximately 2 cm^2) leaf and cleared in Acetic acid:alcohol (2:1) for 12-24 h to clear the chlorophyll of leaves. Then cleared samples were transferred into 90% lactic acid in small vails and stored for later observations. For microscopic observations the leaf samples were mounted on a slide with a drop of water and observed under stereomicroscopeat 10X magnification. Each entry has three leaf samples with both abaxial and adaxial observations (3 leafs x 2 observations=6 total observations) recorded as trichomes per microscopic field for both the *kharif* 2013 and *rabi* 2013 seasons.

3.4.1.3. Seedling vigour

Seedling vigour (a combination of height, leaf growth and robustness) was evaluated for each plot at 9 DAE (seedling vigour I) and 16 DAE(seedling vigour II) on a scale from 1 to 3 where 1 represents plants with highvigour (plants showing maximum height, leaf expansion and robustness), and 3 represents plants with low vigour (plants showing minimum growth, less leaf expansion and poor adaptation). The scores are as follows:

1 = **90-60%** of the maximum seedling growth,

2 = **60-30%** of the maximum seedling growth,

3 = **< 30%** of the maximum seedling growth.

3.4.1.4. Leaf sheath pigmentation

Plumule and leaf sheath purple pigmentation scores of sorghum genotypes at 7 days after seedling emergence are as follows. 1 = plumule or leaf sheath with dark pink/purple pigment, 2 = plumule or leaf sheath with light pink pigment, 3 = plumule or leaf sheath with green color.

3.4.1.5. Shoot fly dead heart percentage (% SFDH)

This is a direct measure of shoot fly infestation. The number of dead hearts per plot was counted and percent shoot fly dead hearts was calculated based on total plant count with the ratio of deadhearts count. 100% SFDH = Highly susceptible to shoot fly,> 80% SFDH = Suceptible to shoot fly,> 60% SFDH = Moderatly susceptible to shoot fly,< 40% SFDH = Resistant to shoot fly,< 20% SFDH = Highly resistant to shoot fly.

3.5. Statistical analysis of F_2 and $F_{2:3}$ seedling leaf blade glossiness and trichome density

In F_2 and F_3 generations, observed phenotyping data were analyzed using SAS software package (SAS Institute, Cary, NYC, USA). Augmented design using PROC–MIXED with entries random was used to estimate covariance parameter estimates and Best Linear Unpredicted means (BLUPS) were derived with

'Z'values calculated. The huge F_2 population sown in 3 blocks, and each block with 600-650 individual F_2 along with 3 times replicated parental checks were included. Block effect was estimated from repeated checks means and removed from the corresponding F_2 population values in each block in order to minimize the rate of error.

3.6. Statistical analysis of F_4 phenotyping data

F_3-derived F_4 progenies were screened for delay in senescence observations, agronomic traits and shoot fly resistance morphological traits.

3.6.1. Analysis of variance (ANOVA)

The analyses of variance for F_4 progeny phenotypic data sets were performed using the residual maximum likelihood algorithm (ReML), which provides best linear unbiased predictions (BLUP) of the performance of the genotypes (Patterson and Thompson, 1971). ReML estimates the components of variance by maximizing the likelihood of all contrasts with zero expectation. For each trait and for each entry, the predicted means were calculated with replication as fixed effects for both individual environments (seasons); and blocks within replications and genotypes (Rep/Block+Geno) as random effects. Alpha-lattice design data were analysed by unbalanced analysis of variance. For a single season data replications + genotypes (Rep+Geno) were used as the treatment structure and for block structure number of blocks within replications (Rep/Block). Data across both the seasons were pooled in single excel file with Env1 and Env2 in the single column (environment) for all the replicated data including block structure for unbalance design Anova. Similarly, BLUPs calculated across season utilized for Genotype × Environment effect estimation. The data were analyzed using the GenStat (14^{th} edition) package.

3.6.2. Heritability

It was estimated in RILs for all resistance components as well as the ratio of total genotypic variance to the phenotypic variance (Falconer, 1989).

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

where, H^2 - % Heritability coefficient,σ_g- Genotypic variance,σ_p- Phenotypic variance. The heritability percentage categorized as low, moderate and high as given by Robinson *et al.*,(1949):

0-30% - low, 30-60% - moderate, 60% and above high.

3.6.3. Standard error (SE)

$$S.E = \frac{\sqrt{(N-1)}}{N} \frac{\sqrt{(\text{Error MS})}}{r}$$

Where,

N = Number of Individuals

Error MS = Error mean sum of square

3.6.4. Coefficient of Variation (CV):

$$CV = \frac{\sqrt{\text{Error MS}}}{GM} \times 100$$

Where,

Error MS = Error mean sum of square

GM = Grand mean.

3.6.5. Phenotypic correlation

Correlation coefficient (r) among the agronomic traits to % green leaf area and shoot fly morphological traits to shoot fly dead heart percentage was estimated by using software Statistica. The observed value of correlation coefficient was compared with the tabulated value for (n-2) degrees of freedom to test for significance.

3.7. High-throughput DNA extraction

The steps involved in the DNA extraction protocol are explained below:

Around 1894 germinated selfed seeds from F_1 and their parents were grown in pots in a green house. Three seeds (F_2) per pot were planted. Staggered sowing was done in three stages for isolating DNA at regular intervals. In first sowing, a total of 465 samples (5*93 +2 parent's bulk; separate pots+1 blank check (plates), second sowing, 465 samples fit in 5 plates with parents in each plate with 1 blank check per plate and in third sowing, a total of 558 (6*96) seeds were sown along with parents. One week gap was maintained between every sowing for ideal DNA extraction without mixing of samples. F_2 plants were tagged with a serial no.U120001 – U121894 (U-Usha, 12-2012, 0001-plant serial number). Totally, 1,894 plant materialswere sown as mentioned in (plate records, serial number and isolated DNA images). Single plant DNA was extracted from each seedling of F_2 and parents and grandparents, using CTAB method (Mace *et al.*,2003) with slight modifications. DNA was further purified by RNase digestion followed by extraction with phenol:chloroform:isoamyl alcohol (25:24:1) and ethanol precipitation as described by Mace *et al.*,(2003). The reagents required for DNA extraction are listed in Appendix and the adopted procedure for 96-well plate mini-DNA extraction is described here.

Steel balls (4 mm in diameter and 2 numbers per extraction tube), pre-chilled at –20°C for about 30 minutes, were added to the 12×8 well strip extraction tubes with strip caps (Marsh Biomarket, USA) that were kept on ice. Before starting

DNA extraction, 3% CTAB buffer was preheated at 65°C in a water bath (Precision Scientific model: shaking water bath 50). Leaf blades of six inches size were collected from 2-3-week-old seedlings. The leaves were cut into small pieces and these pieces (approximately 30 mg) were then transferred to extraction tubes that were fitted in a box. 450 µl of preheated, 3% CTAB buffer was added to each extraction tube containing leaf sample. Grinding was carried out using a Sigma Geno-Grinder (Spex Certiprep, USA) at 500 strokes/minute for 2 minutes. Grinding was repeated until the color of the solution became pale green and leaf strip pieces were sufficiently macerated. After the first round of grinding, the boxes were checked for leakage by taking them out from the Geno-Grinder and were shaken for proper mixing of leaf tissue with buffer. After grinding, the box with the tubes was fixed in a locking device and incubated at 65°C in a water bath for 30 minutes with shaking at every 10 min. 450 µl of chloroform:isoamyl alcohol (24:1) mixture was added to each tube, tubes were inverted twice and the samples were centrifuged at 5500 rpm for 15 min (Sigma Laboratory Centrifuge 4K15C with QIAGEN rotor model NR09100:2×120 g) at 23°C (room temp). After centrifugation, the aqueous layer (approximately 300 µl) was transferred to a fresh tube (Marsh Biomarket). To each tube containing aqueous layer, 0.7 volume (approximately 210 µl) of cold (kept at –20°C) isopropanol was added. The solution was carefully mixed and the tubes were kept at –20°C for 30 min. The samples were centrifuged in a centrifuge (same as earlier) at 5500 rpm for 15 min at 4°C. The supernatant was decanted carefully without dropping the pellet under the fume hood and pellets were kept for drying. In order to remove co-isolated RNA; pellets were dissolved into 200 µl of low salt T1E0.1 buffer and 3 µl of RNase A (10 mg/ml). The solution was incubated at 37°C for 30 min or overnight at room temperature. After incubation, samples were brought to room temperature, then equal volumes of 200 µl of phenol: chloroform: isoamyl alcohol (25:24:1) were added to each tube, mixed and centrifuged (same as earlier) at 5000 rpm for 5 minutes. The aqueous in each tube was transferred to a fresh tube (Marsh Biomarket) and 200 µl of chloroform: isoamyl alcohol (24:1) was added to each

tube, mixed and centrifuged at 5000 rpm for 10 min (same as earlier). The aqueous layer was transferred to fresh tube. 15 µl (approximately 1/10th volume) of 3 M sodium acetate (pH 5.2) and 300 µl (2 volumes) of absolute ethanol (kept at –20°C) were added to each of the tubes and the mixtures were subsequently incubated in a freezer (–20°C) for 20 min. Following the incubation at –20°C, the tubes were centrifuged (same as earlier) at 5500 rpm for 15 min at 4°C. After centrifugation, the supernatant was carefully decanted from each tube in order to ensure that the pellet remains inside the tube. Subsequently, 200 µl of 70% ethanol was added to each of the tubes and this was followed by centrifugation (same as earlier) at 5000 rpm for 5 min. The supernatant was carefully decanted and pellet was allowed to dry in a speed vacuum evaporator (SPD Speed vac, Thermo scientific product, SPD111V). Dried pellets were re-suspended in 100 µl of $T_1E_{0.1}$ buffer and kept overnight at room temperature to dissolve completely. The re-suspended DNA samples were stored at 4°C.

3.8. Quantification and normalization of DNA

The quality of DNA in each sample was checked using 0.8% agarose gels stained with 0.5 µl/10 ml ethidium bromide (10 mg/ml). For checking the quality of the extracted DNA, each well of the agarose gel was loaded with 5 µl of sample (3 µl distilled water + 1 µl orange dye + 1 µl DNA sample) with the standard λ DNA molecular weight markers (2.5 ng/µl, 5 ng/µl, 10 ng/µl) on 0.8% agarose gel and gel was allowed to run at 80 V for 15 min. After completing the electrophoresis, DNA banding patterns on the gel were visualized under UV light using Gel Documentation and the image was saved. A smear of DNA indicated poor quality whereas a clear band indicated good quality DNA. Samples of poor quality DNA were re-extracted. The quantity of DNA in each experimental sample was assessed using a fluorescence spectrophotometer (Spectrafluor plus, Tecan, Switzerland) by staining DNA with PicoGreen™ (1/200 dilution) (Juro, Supply Gmbh, Switzerland). Based on the relative fluorescence unit (RFU) values and using a calibration graph, DNA

concentration of each experimental sample was calculated (DNA concentration = -2.782763 + 0.002019 × RFU). The DNA was normalized to 5 ng/µl concentration with visual comparison by loading DNA samples.The DNA concentration of each experimental sample was then normalized to 2.5 ng/µl to produce working samples for use in PCR reactions.

3.9. Polymerase chain reaction (PCR) amplification

PCR reactions were conducted in 96 and 384-well plates in a GeneAmp PCR system 9700 Perkin Elmer (Applied Biosystem, USA) DNA thermo cycler. For separation of amplicons using capillary electrophoresis m-13 tailed and direct flourophore labeled primers were used. The m-13 (5'CACGACGTTGTAAAACGAC3') tailed forward primer from each primer pair was labeled with different flourophores - 6-FAM™ (Blue), VIC® (Green), PET® (RED) and NED™ (Yellow) (Applied Biosystems) before amplification. The reactions were performed in volumes of 5 µl using three different protocols. A touchdown (61-51) PCR program was used to amplify the DNA fragments. Reaction conditions for the PCR program were as follows: denaturation at 94°C for 15 min, denaturation at 94°C for 15 sec, annealing at 61°C for 20 sec, (temperature reduced by 1°C for each cycle), extension at 72°C for 30 sec, denaturation at 94^0C for 10 times, denaturation at 94°C for 10 sec, annealing at 54°C for 20 sec, extension at 72°C for 30 sec, denaturation at 94^0C for 40 times, extension of 20 min at 72°C, store at 4°C (Smith *et al.*,1995). PCR reaction mixtures used in DNA amplification was as follows.

Component	Working concentration	Final conccentration	Volume
DNA	2.5 ng/µl	2.5 ng	1.0 µl
PRIMER			
M13-label	2.0 pm/µl	0.08 pm/µl	0.4 µl
M13-tailed forward	2.0 pm/µl	0.16 pm/µl	0.2 µl
Reverse primer	2.0 pm/µl	0.16 pm/µl	0.4 µl
MgCl2	25mM	2mM	0.4µl
dNTPs	2 mM	0.12 mM	0.25µl
Buffer	10 X	1 X	0.5 µl
Enzyme(Sib enzyme®)	5 U/µl	0.1 U	0.02 µl
water			1.83 µl
Total			5

3.9.1. Fragment analysis

The amplified PCR products were separated by capillary electrophoresis using ABI prism 3730 automatic DNA sequencer (Applied Biosystems Inc.). The capillary electrophoresis technique has a resolution of less than 2 bp and hence can be used to clearly distinguish polymorphisms of less than 2 bp. Moreover, as this technique is a fluorescence based detection system, it dispenses with the need for radioactive or laborious manual polyacrylamide gel screening techniques. Prior to electrophoresis multiplexing was carried out *i.e.* the amplified products of primers labeled with different dyes or same flourophores-labeled primers with non-overlapping amplicons (in terms of size range) were pooled. Multiplexing of numerous fragments and pool-plexing of numerous samples increased the throughput of this technique. For multiplexing, 1.0 µl of each of the amplified products were pooled and each of the pooled PCR product

were then mixed with 0.25 µl of GeneScan™ -500 LIZ® internal size standard (Applied Biosystems) and 7.0 µl of Hi-Di formamide (Applied Biosystems). The final volume was made up to 10 µl with sterile distilled water. This final product was then denatured for 5 min at 95°C (Perkin Elmer 9700, Applied Biosystems) and cooled immediately on ice for ABI runs.

3.9.2. Fragment size fractionation

The denatured DNA amplicons were separated using capillary electrophoresis with the help of automatic DNA sequencer ABI 3730. In this technique, as the DNA migrates through the detection cell, the capillaries are simultaneously illuminated from both sides of the array by an argon-ion laser. To accomplish this, a beam from a single laser source is split using a series of mirror to form a dual pathway. The fluorescent emissions are then spectrally separated by a spectrograph and focused onto a charged couple device, which are then converted to digital information that is processed by the "collection software". The fluorescent internal size standard in each capillary eliminates variability. The capillary runs on ABI 3730 were performed using "Microsatellite Default" analysis method and "Genemapper-POP7" run module. The fragments were separated on a 36 cm capillary array using POP7 as a separation matrix.

3.9.4. Data processing

For PCR products electrophoresed on ABI 3730 DNA sequencer, the Genemapper® v4.0 software (Applied Biosystems) provides a series of automatic fragment sizing, allele scoring, bin-building and auto panelize algorithms. GeneMapper® combines the precision sizing capabilities of allele calling power and helping in accurate genotyping of the samples. Sizing of the PCR products of 35-500 base pairs was performed using the GeneScan Liz 500 internal lane size standard with fragment sizes of 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490 and 500 base pairs. Data analysis was carried out using GeneMapper® software version 4.0 where the allele(s) of each genotype in the form of peaks were size corrected.

3.9.5. Scoring of amplified products

The amplified PCR products of the SSR markers screened on the RILs were scored as follows: A = Homozygote carrying allele from female parent (RSG04008-6), B = Homozygote carrying allele from male parent (J2614-11), H = Heterozygote carrying alleles from both parents, - = Missing data for individual at a locus. Initially, F_2 mapping was carried out with five SSR markers equally distributed across the chromosome-10, long arm target region. All the five SSR markers were genotyped on 1894 isolated DNA samples with the above-mentioned touchdown PCR protocol. After scoring, the dataset was assembled in Microsoft Excel spreadsheet. Final data set was then arranged on an Excel sheet in a format suitable for linkage mapping.

3.10. Genotyping by sequencing (GBS)

Genomic DNAs were digested individually with ApeKI (recognition site: G|CWCG). The resulting fragments were ligated with sample-specific "barcodes" called "restriction site associated DNA tags" (RAD tags), and restricted, barcoded DNA samples were then multiplexed at 96- or 384-plex. GBS libraries were constructed and subjected to skim sequencing to a depth of 0.1X. The resulting 66-base pair sequence reads were sorted by barcodes. DNA sequencing was performed either on the Illumina Genome Analyzer IIx or HiSeq2000. Sequences were mapped to the BTx623 sorghum reference genome by using BWA (43), and SNPs were called with the TASSEL v4.4.10 GBS pipeline (Glaubitz et al., 2014) (www.maizegenetics.net/tassel/). Sequence tags, 64-bp sequences that included a leading 4-bp C[T/A]GC signature from the cut site, were identified, and tags with at least 10X total coverage were retained (Elshire *et al*., 2011).Functional annotation of SNPs was performed with reference gene feature information using snpEff v3.6 (Cingolani *et al*., 2012)

3.11. Selection of fine-mapping population recombinants by genotyping

The fine-mapping populations (F_4 lines derived from corresponding F_2 individuals) were genotyped (using DNA samples collected from 2000 F_2) with the available genomic (Brown *et al.*, 1996; Bhattramakki *et al.*,2000; Schloss *et al.*,2002) and genic (Srinivas *et al.*, 2008, 2009b; Ramu *et al.*, 2009) SSRs distributed across the long arm of SBI-10, to identify the small fraction (369 F_3 and selected 152 F_4 progenies) homozygous or nearly homozygous for recombination events in the target region between *Xgap001* and *Xtxp141*.

3.12. Linkage map construction

For the selected five markers, genotypic data were generated and used as an input for JoinMap V3.0 (Van Ooijen and Voorips, 2001) for constructing a linkage map using Kosambi function to convert recombination fractions into centiMorgans (cM) (Kosambi, 1943). Marker order was assigned at minimum LOD 3 and segregation distortion and chi square values were calculated using JoinMap V3.0 (Van Ooijen and Voorips, 2001).QTL mapping for F_2 lines was performed with genotyping data and phenotypic mean values data by composite interval mapping (CIM) in QTL cartographer Windows V2.5 (Wang *et al.*,2010). Default window size 10 cM, walking speed 1 cM, control marker 5 and backward regression method was used for this purpose. LOD 2 was used as criteria for QTL analysis and significance of each QTL interval with the threshold level performed at 1000 permutations, was determined, and the significance level was observed. F_3 phenotyping data with F_2 genotyping data were used and QTLs were positioned, and their effects were estimated by composite interval mapping (CIM) (Zeng, 1994; Jansen, 1994) by PLAB QTL version 1.2 (Utz and Melchinger, 1996) on both individual traits.

3.12.1. GBS-SNP integration into the SSR genetic linkage map

Huge GBS dataset with 60% missing data points was obtained from the selected recombinants. Only SNP data from target region was utilized for genetic map construction with GBS-SNP markers. RSG04008-6-6 was coded as ‘A’, J2614-11-11 as ‘B’ and heterozygote was coded as ‘H’. In few genotypes, the parents

and grandparents data that mismatched with the controversial SNPs were also discarded. SNP markers were arranged with the help of their physical map positions and the genotypic data were observed clearly for data duplication. 50% duplicated data were found and such SNPs were discarded. Remaining markers were used for development of linkage map.

3.12.1.1. Distance matrix and principal coordinate analysis (PCA)

Distance matrix between all selected pairs of markers was calculated using THREaD Mapper Studio: a novel, visual web server for the estimation of genetic linkage maps (Cheema *et al.,* 2010). Due to huge data set, only distance matrix was calculated in the THREaD Mapper Studio and the data were utilized for principal coordinate analysis. Principle coordinate analysis of distance matrix data results in a horseshoe arch are also known as 'horseshoe effect' (Podani and Miklos, 2002) and this is the basics of the THREaD Mapper method (Cheema *et al*., 2010). The linear arrangement of markers along the 'horseshoe curve' of the PCA plot corresponds to the marker order and can be used for constructing a dense linkage map. SNPs placed centrally and not along the horseshoe have more missing data points need to be eliminated as they confuse the map order.

3.12.1.2. SSR-SNP linkage map

The markers in linear order of horshoe arch were used for linkage map construction with the help of JoinMap V3.0 (Van Ooijen and Voorips 2001). The Kosambi map function was used to convert recombination fractions into centi-Morgans (cM) (Kosambi, 1943). Marker order was assigned at minimum LOD 3 and segregation distortion and chi-square values were estimated using JoinMap V3.0 (Van Ooijen and Voorips, 2001).

3.13. QTL mapping

Single marker analysis (SMA) and composite interval mapping (CIM) were conducted using QTL Cartographer for Windows v2.5 (Wang *et al.,* 2010) on the observed traits in $F_{2,}$ $F_{2:3,}$ and $F_{2:4}$ populations with, 1894, 369, and 154

entries (genotypes), respectively. Linkage between individual markers and each trait was initially evaluated using SMA, prior to analysis using CIM. Background markers for inclusion in the CIM model were selected by forward stepwise regression for each trait. The five most significant background markers were then used for analysis (default). The 'walking speed' was set at 2 cM and the 'window size' at10 cM for CIM. A conservative permutation threshold at the 0.01 significance threshold was obtained for each trait using 1,000 permutations. 2-LOD and 3-LOD support intervals were determined, as described by Lander and Botstein (1989). The additive effects and percentage of variation explained (R^2) for all significant QTL were determined at their peak LOD values. QTL mapping with SSR markers for mapping QTLs and to fine map the QTL and which SNPs are associated with which QTLs were analyzed. Candidate genes for leaf blade glossiness, leaf blade trichomes, stay green QTLs and agronomic trait QTLs were identified with the help of SNPs flanking to the QTLs. SNPs linked to generic regions and functional role of the genes reveal the target genes using bioinformatics search.

3.13.1. QTL cluster analysis and fine mapping

QTLs sharing common marker interval with common peak position were grouped into QTL clusters. For fine mapping F_2, 152 selected recombinants were aligned with their phenotypic data and sorted with phenotypic values. Both the phenotypic extreme haplotypes were aligned to clearly visualize the recombination break point which helped in clear identification of genomic regions responsible for the stay-green traits by avoiding environment and genetic background effect and other unknown factors. The haplotype of the target region was compared for identification of informative recombinant break points with the help of phenotyping data. Use of phenotypic extremes will always be helpful and avoid the effect of problems associated with environment variation and genetic background effect in early generations (Li *et al.*, 2004).

3.13.2. Fine mapping and genome wide association studies (GWAS)

Skim-sequence GBS SNP genotyping data and phenotyping data sets (BLUPs) for the selected recombinants were analysed to determine the most likely positions for one or more glossy gene(s), trichome gene(s), and the stay-green gene(s) underlying the target QTLs on the long-arm of SBI-10, and identify closely-linked marker loci for each QTL. This information was used to identify desirable individual homozygous lines for favourable alleles at all three target QTLs. QTLs were fine mapped by comparing the phenotypic means of recombinant genotype classes (Paterson *et al*., 1990). Genome wide association mapping can be used to identify the significant association between molecular markers (SSR, SNP) and phenotype trait using R package GAPIT (Lipka *et al*., 2012). The genotyped individuals with GBS data were used for GWAS studies to identify marker trait associations (MTAs) and possible candidate genes for the shoot fly morphological trait and stay green QTLs on sorghum chromosome SBI-10 QTLs identified.

3.13.3. Comparison of QTL mapping with GWAS studiesfor confirmation of fine map region

Selected recombinant SNP data were used for construction of linkage map and for QTL mapping with F_4 two seasons phenotyping data for seedling leaf blade glossiness, trichome density and percent green leaf area for all the weeks. QTL mapping results were compared with GWAS studies and fine mapping data for confirmation and exact locations.

3.14. Candidate gene identification

Information on map positions of GBS-SNP markers most closely flanking each target QTL (assumed to be under the control of a single gene per QTL) can be combined with the annotated aligned sorghum genome sequence to identify candidate genes that might be associated with each of the trait. Candidate genes responsible for stay-green according to earlier studies in other plants like maize, Arabidopsisand rice were selected based on their functional role. GWAS MTAs

associated with candidate genes as well as fine mapped regions having candidate genes were searched (http://phytozome.jgi.doe.gov/pz/portal.html) for their function and reported as probable candidate genes based on their physical and genetic position.

3.14.1. Assessment of candidate genes underlying each target QTL

SNP analysis of the candidate genes in the parental lines and selected recombinants were used to further reduce the number of candidate genes, resulting in identification of a single functional gene controlling observed variation in each of the three target traits: seedling leaf blade glossiness, seedling leaf blade trichome density, and green leaf area retention under conditions of terminal drought stress. Candidate genes responsible for stay-green, glossy and trichome density according to earlier studies in other crops like maize, Arabidopsis, rice were selected based on functional role.

3.15. Recombinants of 3-gene/QTL selection

Selection of plants with desired combinations were identified and selected for further analysis. It would be desirable to generate recombinants having the favorable alleles at all three loci (for a high level of glossiness, good green leaf area retention, and high trichome density), as such recombinants could be used as donors of the "cassette" of these three genes in applied marker-assisted breeding programmes targeting the *rabi* sorghum production environments of peninsular India, where both shoot fly resistance and terminal drought tolerance are essential traits of well-adapted sorghum cultivars. In the course of producing such a recombinant from the cross of the BTx623-background, shoot fly resistance QTL introgression line and the R16-background stay-green QTL introgression line, it should be possible to fine-map (and perhaps identify the underlying genes) for all three of these components of the cassette.

3.15.1.Marker-assisted breeding for pyramiding of shoot fly morphological traits and stay-green traits on SBI-10L

From the results of objective 2, the map order of the three target QTLs was determined, and marker genotypes of the recombinants generated. Fine-mapping population progenies (F_4) with allelic compositions required to generate segregants homozygous for favorable alleles at all three target QTLs (that is, stay-green plants with trichomed glossy seedlings) were identified and advanced (by selfing and/or crossing followed by a further generation of selfing) to produce the required segregating population(s). Individuals of the population(s) were then genotyped to identify the desired triple-homozygotes (pyramided), which were selfed.

4. RESULTS

The present experimental study was carried out with main focus on fine genetic mapping of stay-green and shoot fly resistance QTLs on sorghum chromosome SBI-10 using an introgression line cross of RSG04008-6 (stay-green donor) and J2614-11 (shoot fly resistant donor) parents. F_2 genotyping, F_2, F_3 and F_4 phenotyping evaluations for fine mapping using GBS and GWAS were performed and the results are presented here.

4.1. Confirmation of parents and F_1s

J2614-11 is a donor parent for seedling leaf blade glossiness and trichome density in the cross RSG04008-6 × J2614-11. Parents were clearly differentiated visually as glossy *vs* non-glossy and trichomed *vs* non trichomed. In order to confirm the genetic composition, all the 21 polymorphic markers out of 41 co-dominant SSR markers (Annexure 1) were assessed and compared between genomic regions of parent (RSG04008-6 and J2614-11) and grandparents (R16, E36-1, BTx623 and IS18551) for target 37cM interval (Table 2). Marker alleles for each of parent-grandparent pair of E36-1, RSG04008-6 and J2614-11, IS18551 were monomorphic across the SBI-10 target region but these marker alleles are polymorphic between the two different parents and the two different grandparent's pairs, which confirm that the introgressed parental target regions under study were derived from their respective grandparents. A total of seven plant × plant crosses were executed and seed from a single cross was planted with one seed per hill. All the crosses along with parents (and grandparents) were screened with 9 polymorphic SSRs but one cross RSG04008-6 × J2614-11 (U100019) was successful and confirmed to be a true heterozygous for parental alleles. From the cross RSG04008-6 × J2614-11 twelve F_1 seeds were produced. All the 12 F_1 plants were screened for heterozygosity in the initial interval markers. A total of 9 polymorphic co-dominant SSR markers were screened on all 12 F_1s and 11 F_1s having heterozygous alleles but one plant was homozygous

which was discarded (Table 3). High quality grade 1 marker allele profiles were obtained for all markers (Fig .3).

4.2. Developing F_2s and selection of informative $F_{2:3}$ progenies

The confirmed true F_1s were selfed to produce F_2 seed. Out of selfed eleven F_1s, a single F_1 plant (U110055) with 1958 seeds was selected for advancement during late *rabi* season 2011/12 and used as a high-resolution recombinant mapping population.

A total of 1,894 F_2 individuals (survived after sowing) from the high resolution cross (HRC) along with its parents RSG04008-6 and J2614-11, were genotyped with 5 SSR markers covering the target SFR QTL target region on the long arm of sorghum chromosome SBI-10. The five markers were selected in particular for genotyping the population because the introgressed line parent J2614-11 was bred using *Xgap001* and *Xtxp141* as flanking markers for transferring of a two-component shoot fly resistance QTLs by MABC from donor IS18551 into recurrent parent BTx623 background. Though 9 polymorphic SSR markers detect loci between the two flanking markers *Xgap001* and *Xtxp141*, the markers *Xnhsbm1044* and *Xisep0630* were reported to be associated with the trait trichome density that conferred shoot fly resistance in sorghum population 296B × IS18551. Marker *Xiabt340* is a middle third marker, which is not associated with either of the two shoot fly resistance component target QTLs. We have selected 369 F_2 homozygous and nearly homozygous recombinant F_2 plants based on these 5 SSR markers and genotypes with complete homozygosity for i) RSG04008-6 alleles ii) J2614-11 alleles across this target region, or complete heterozygosity across this region were discarded. The selected F_2 progenies were genotyped with 3 additional markers to increase the flanking regions for safer detection of exact location of the target QTL regions and sub-subset selection for fine mapping (Fig .4). All the 369 selected recombinant F_2 individuals were selfed to produce F_3 seed during late *rabi* season 2011/12. Phenotypic data for the F_3 progenies was collected similarly to F_2 phenotyping data, except that the

F_3 data were collected on a progeny-basis, whereas the F_2 data were collected from individual plants phenotyping.

4.3. F_3derived highly informative F_4 recombinantprogenies

Out of 369 F_3 progenies, 182 highly informative double recombinants for the favourable alleles were selected based on 7 markers genotyping data along with phenotyping data (Fig .4). F_3 self-seed of 182 recombinants were not sufficient. Only 152 recombinants have sufficient seed sets for multiplicated field trials for two seasons, and two traits (stay-green and shoot fly morphological traits) evaluation.

4.4. Trait variation of shoot fly resistance component traits, stay-green and agronomic traits

Parental introgression lines which have different genetic backgrounds and different introgressed segments differ significantly with each other for both glossiness and trichome density (Fig .5). Parent RSG04008-6 is a pedigree-derived introgression line in R16 background with E36-1 stay-green alleles at a QTL on SBI-10 and J2614-11 is a backcross-derived introgression line in BTx623 background with IS18551 shoot fly resistance alleles at QTLs on SBI-10. Trait variation was observed for all the shoot fly resistance component traits, stay-green, and agronomics traits in F_4 progeny for both seasons (*rabi*2013 and 2014).

4.4.1. Mean performances of parents, F_2 and F_4 populations

The mean performances of the parental introgression lines RSG04008-6 and J2614-11 differed phenotypically for all the observed traits. The parental lines differed for shoot fly morphological traits in *kharif* 2013,*rabi*(post-rainy) 2013 and agronomic traits but for stay-green scores, low variation was noticed across two environments *rabi* 2012-2013 and *rabi* 2013-2014. As predicted, the RSG04008-6 has low mean values for all shoot fly resistance component trait values and increased agronomic and stay-green performance across both the

environments indicating it is a shoot fly susceptible stay-green elite cultivar. J2614-11 is a shoot fly resistant male parent with moderately stay-green or less green leaf area mean values when compared to RSG04008-6 parent (Tables 4 and 7,Fig .6).

4.4.1.1. F_2 mean performances

1894 F_2 individual plants were observed for shoot fly morphological traits like seedling leaf blade glossiness and trichome density to confirm the QTL genomic regions segregation in F_2 population.

4.4.1.1.1. Seedling leaf blade glossiness

The trait glossiness was also scored visually and the results were divided into two categories- (i) glossy and (ii) non-glossy. The complex glossiness trait was characterized by narrow, erect, pale, shiny green leaves and 1457 individuals (76.92%) exhibited glossy leaves. A total of 437 F_2 individuals (23.08%) with non-glossy leaves were characterized by broad, dull, droopy leaves. For this trait, the individuals followed the Mendelian genetics and segregated in 3:1 ratio (Fig .6a) having χ^2 = 0.99 not significant, with the glossy phenotype of shoot fly resistant introgression line parent J2614-11 being dominant (Table 4).

4.4.1.1.2. Seedling leaf blade trichome density

In the present study, 1,894 individual F_2 plants were scored for the morphological component traits of shoot fly resistance. It is observed that there are substantial variations in trichome density score in the F_2 population (Fig .6b) and this information was combined with the SSR genotype data to locate the QTLs for the trait. By doing so, we understand that significant variations exist for these traits. Very low trichome density scores ranging from 0.0 to 1.0 were observed in 285 F_2 individuals (13.72%). Likewise, for 124 individuals (6.54%) medium trichome density score, for 863 individuals (45.56%) high trichome density score and for 362 individuals (19.11%) very high trichome density score were noted, of which the highest number of individuals (863) showed high trichome density scores (Fig .6b). The parental introgression lines differed significantly with each other

for glossiness and trichome density scores, and among the F_2 population and derived F_3 progenies, glossiness scores ranged from 0.09 to 4.95 and trichome density scores ranged from 0.00 to 5.00. For both the seedling leaf blade glossiness and trichome density scores, CV values were low due to the qualitative nature of these traits. In both the F_2 population and its derived F_3 progenies, glossy score and trichome density score were highly correlated with each other (Table 4), indicating that a high trichome density score was associated with a low glossiness score and therefore that high trichome density is associated with a high degree of glossiness. The statistical Z test results showed (significant $P<0.05$) genetic variation for glossiness score (Gls) and trichome density (Td) indicating that the data are suitable for QTL mapping.

4.5. F_4 stay-green traits mean performances

The stay-green values were estimated for two environments (stgENV). StgENV1 is post-rainy season of 2012-2013 (Summer/*rabi* 2013) and StgENV2 is post-rainy season of 2013-2014 (Summer/*rabi* 2014) grown under water limited conditions (Table 5 and Fig.7).

4.5.1. Percent green leaf area 7 days after flowering (% GL 7 DAF)

Stay-green scores were recorded on weekly basis and % GL 7 DAF mean values for parent RSG04008-6 (88-99) which has high scores in comparison with J2614-11(84-95). Mean F_4 progeny values were 88% GL and 96 % GL during summer 2013 and 2014 respectively. For both the parents % GL 7 DAF did not show much variation but the female parent showed higher values when compared to the male parent. Across season mean values are intermittent to both the seasons. Skewing RSG04008-6 (93), J2614-11(89) and F_4 progeny (92), it is observed that the values leaned more towards female parent (Fig .7a).

4.5.2. Percent green leaf area 14 days after flowering (% GL 14 DAF)

RSG04008-6 female parental introgression line showed 79% GL 14 DAF during *rabi* 2012-2013 and 84% green leaf area during *rabi*2013-2014 and the male

parent J2614-11 showed 83% GL 2012-2013 *rabi* and 85% GL during 2013-2014. For both the parents, the rate of senescence did not vary much and in case of progeny 79-84, for both the *rabi* 2012-2013 and 2013-2014 and the exhibited progeny mean values were slightly towards the female parent. Across season mean values, much variation was not observed between female parent (82) and F_4 progeny (82) but have slight higher values than J2614-11 (78) male parent (Fig .7b).

4.5.3.Percent green leaf area 21 days after flowering (% GL 21 DAF)

Stay-green introgression line female parent derived from E36-1, RSG04008-6 has 62-74% GL 21 DAF and shoot fly introgression line J2614-11has 61-75% GL 21 DAF during both the *rabi* 2012-2013 and 2013-2014. Mean F_4 progeny values showed 65-73% GL 21 DAF during both the seasons. The male parent J2614-11 showed slight higher values than the RSG04008-6 due to reduced plant height of J2614-11. The mean value for across season did not vary for parents and progeny (Fig .7c).

4.5.4.Percent green leaf area 28 days after flowering (% GL 28 DAF)

Female stay-green parent RSG04008-6 showed 47-66% GL 28 DAF and 47-64% GL 28 DAF during both the seasons *rabi*2012-2013 and 2013-2014. The mean F_4 readings ranging from 50-65% GL 28 DAF showed higher values than parents. As the days to flowering increased, the percent green leaf area decreased gradually (Fig .7d).

4.5.5.Percent green leaf area 35 days after flowering (% GL 35 DAF)

Mean values of RSG04008-6 have 23 and 25% GL 35 DAF and mean values of J2614-11 have 37-50% GL 35 DAF for both the seasons of *rabi* 2012-2013 and *rabi* 2013-2014. F_4 mean values ranged 39-51% GL 35 DAF for both the seasons. The across season mean values for parents (44) and progeny (45) did not significantly differ (Fig .7e).

4.5.6.Percent green leaf area 42 days after flowering (% GL42 DAF)

RSG04008-6 parent recorded 23-25% GL42 DAF and 30-40% GL42 DAF during *rabi* 2012-2013 and 2013-2014. In F_4, recombinant mean values ranged from 29-39% GL exhibiting higher % GL after 42 DAF. Retaining green leaf area after seed set formation may reduce the yield also. % GL correlation with yield totally gives the meaningful output for stay-green. The male parent J2614-11 (35) and F_4 progeny exhibited higher mean values than female parent RSG04008-6 (2) surprisingly (Fig .7f).

4.5.7.Percent green leaf area 49 days after flowering (% GL 49 DAF)

RSG04008-6 mean values ranged from 17-11% GL49 DAF, while J2614-11 mean values ranged from 30 - 40 during both the screens. F_4 recombinant progeny displayed higher stay-green values of 29-39% GL49 DAF for both *rabi* 2012-2013 and 2013-2014 than RSG04008-6 and showed similar values as J2614-11. As both the parents are introgression lines, their trait expression pattern was influenced by the background genome as well as environment. But, the progeny mean values are interesting. The across season mean value distribution was similar to previous week scores and the male parent J2614-11 (24), F_4 progeny (24) recorded higher values than the RSG04008-6 (14) (Fig .7g).

4.6. Mean performances of agronomic and yield related traits

During *rabi* 2012-2013 (stgENV1), plants were exposed to severe stress as the sowing was done on 31st December 2012 and at the time of flowering plants were exposed to severe heat stress when compared to the plants grown in *rabi* 2013-2014 (stgENV2) which were sown during 20th November 2013. In *rabi* 2012-2013 plants were exposed to high temperature stress, but not in 2013/stgENV1 and 2013-2014 summer 2014/stgENV2 (Table 6 and Fig.8).

4.6.1. Time to 50% flowering (days): The lineRSG04008-6 flowered in 61 days, i.e., earlier than usual in 2013 summer season when compared to 87 days in 2014 summer. On the other hand, J2614-11 has taken 77-82 days to flower

i.e.,earlier in 2014 summer season when compared to RSG04008-6. The entries significantly differed for this trait across the two environments, the flowering of F_2-derived F_4 progenies ranged from 68 to 82 days during 2013 and 2014 summer season respectively. During across season mean values, the parent RSG04008-6 (75) and F_4 progeny (74) flowered 4 days earlier to J2614-11 (79) (Fig .8a).

4.6.2. Plant height (cm) (PlHt): RSG04008-6 was significantly taller (132-194 cm) than J2614-11 (89-120 cm) across the 2013 and 2014 summer seasons, respectively. The recombinant F_4 lines recorded population means of 117 cm and 175 cm for this trait during the 2013 and 2014 summer seasons, respectively. The mean values for plant height were higher among the F_4 plants and parents during the 2014 summer season when compared to the 2013 summer season, indicating a large influence of environment on this trait. Across season mean values for RSG04008-6, it is higher (164 cm) than J2614-11 (103 cm) and the F_4 progeny (146 cm) also showed higher mean values than J2614-11 (Fig .8b).

4.6.3. Panicle dry weight in grams/plot (PnDW/plot): Panicle dry weight in the parent RSG04008-6 was 598 g during summer 2013 but 945 g during summer 2014 and thus the variation was significant across two seasons due to two different sowing dates and their exposure to the severity of stress. For J2614-11, the panicle dry weight ranged from 361-1103 g during summer 2013 and summer 2014 respectively. The mean values of F_4 progeny ranging from 598-1109 g showed high yield when compared to the parents during summer 2014. Significant variation between parents and progeny was not observed for across season means (Fig .8c).

4.6.4. Panicle harvest index (PHI): The mean value of RSG04008-6 (66-68) did not show significant difference with J2614-11 (62-71) during summer 2013 and 2014. The F_4 progeny (68-70) mean values were similar to the parent RSG04008-6. For across season mean values, significant variation was not noticed (Fig .8d).

4.6.5. Grain dry weight grams/plot (GDW/plot): The mean value of grain weight per plot of RSG04008-6 parent was 392 g during summer 2013 and 632 g during summer 2014. Mean value of J2614-11 dry weight was 233 g during summer 2013 but 795 g during summer 2014. Mean values for F_4 progeny were 412 g during summer 2013 and 773 g during summer 2014. No significant variation was recorded between parents and progeny for across season mean values (Fig .8e).

4.6.6. Mean 100-grain mass (g) (HGM): Mean 100 grain mass values of RSG04008-6 were 2.14 g and 3.28 g during summer 2013, 2014 respectively. The male parent J2614-11 recorded 2.06 and 2.71 g during summer 2013, 2014 respectively. But, RSG04008 values were higher when compared to J2614-11 during both the seasons. The progeny mean values ranging from 1.94 (summer 2013) to 2.95 (summer 2014) indicated that these values are similar to RSG04008-6 female parent. RSG04008-6 showed higher mean values when compared to male parent J2614-11 and F_4 progeny (Fig .8f).

4.6.7. Grain number per plot (GNP/plot): The parent RSG04008-6 mean values (20456 during summer 2013 and 2012 during summer 2014) were higher when compared to J2614-11 (17807 during summer 2013 and 2689 during summer 2014). The mean values of F_4 progeny were 21232 during summer 2013 and 22191 during summer 2014. These results indicate that the values are intermittent to parents. For the parent J2614-11, the magnitude of difference, during both the seasons was high when compared to RSG04008-6 and F_4 progeny mean values. For across season, F_4 lines recorded higher mean values than the parents (Fig .8g).

4.6.8. Grain number per panicle (GNPP): The mean grain number values of RSG04008-6 ranging from 786-777 during summer 2013 and 2014 respectively did not exhibit significant variation. The mean value of J2614-11 (684) was lower during summer 2013 than the value recorded during summer 2014 (1034).

For F_4 progeny, mean values ranging from 816-853 did not record significant variation between two seasons. But, across season mean values of F_4 progeny displayed higher values than the parents (Fig.8h).

4.7. F_4 mean performances of shoot fly resistance component traits: During *kharif* 2013 and *rabi* 2013, plants were exposed to severe shoot fly stress in the field with fish meal distributed in the random locations of the field and data were recorded for F_4 progenies for both the environments (Table 7 and Fig .9). F_4 Phenotyping for glossy, non glossy and trichome density presence and absence images are depicted in Fig 10.

4.7.1 Seedling leaf blade glossiness (1-5 score): Mean parental values differed significantly from each other as female parent RSG04008-6 is a non-glossy parent. The values for glossiness were 3.5 and 4.6 during *kharif* 2013 and *rabi* 2013 respectively. The mean values of parent J2614-11 showed 2.6 during both the seasons. The F_4 progeny mean value recorded was 3 during both the rainy and post-rainy seasons of 2013. Across season means varied for the parent RSG04008-6 (4), J2614-11 (2.5), but F_4 progeny recorded 3.1 mean values. So, the values thus represent non-glossy, glossy and intermittent respectively (Fig .9a).

4.7.2. Percent shoot fly dead heart (% SFDH): The percent shoot fly dead heart was high during rainy season when compared to the postrainy season across parents and population. when compared between parents, % SFDH was high for female parent RSG04008-6 as it is susceptible to shoot fly (Fig .9b).

4.7.3. Leaf blade trichome density upper (adaxial) (numbers/microscopic field): The RSG04008-6 parent trichome density mean values were 28 no/microscopic field, on upper leaf surface during rainy season 2013 and 5 no/microscopic field during post-rainy 2013. The mean values of trichome count on upper leaf surface of parent J2614-11 are 93 and 46 no/microscopic field, on upper leaf surface during rainy and post-rainy 2013 respectively. F_4 progeny exhibited 64 and 28 mean values respectively across rainy and post-rainy 2013.

The progeny leaned towards male parent J2614-11. For across season means, J2614-11 exhibited higher trichome count than F_4 progeny and RSG04008-6 (Fig .9c).

4.7.4 Leaf blade trichome density lower (abaxial) (numbers/microscopic field): RSG04008-6 mean values were2 and 10 no/microscopic field on lower leaf surface field during rainy, post-rainy seasons of 2013 respectively. Mean values of J2614-11 were 57 and 91 no/microscopic field during both the seasons. The F_4 progeny mean values were 14 and 58 no/microscopic fields on lower leaf surface during both the seasons. This shows an increased trichome density than the female parent RSG04008-6. For across season means, J2614-11 displayed higher trichome counts on lower leaf surface than F_4 progeny and RSG04008-6 (Fig .9d).

4.7.5. Leaf sheath pigmentation (1-3 score) and seedling vigour (1-3 score): The leaf sheath pigmentation was higher in the parent RSG04008-6 (1-1.5) than J2614-11 (2.9) and F_4 progeny (2) exhibitted intermittent values in both the seasons (Fig .9e). Mean values of the parent RSG04008-6 are 2.56 and 1.1 during rainy and post-rainyseasons respectively during 2013. Mean values of J2614-11 for leaf sheath pigmentation were 2.65 and 1.88 and F_4 progeny mean values were 2.44, and 1.3 respectivley for *kharif* 2013 and *rabi* 2013. Significant variation was noticed between two seasons and during post-rainy season, but high seedling vigour was observed in *rabi* compared to rainy season (Fig .9f).

4.8. Analysis of variance (ANOVA): Analysis of variance for stay-green scores was highly significant for individual environments as well as across season environments (Table 8).In case of agronomicdata scores, the effective tiller per plot and panicle harvest index for both the seasons was not significant. As these traits did not have much economic importance, they were not studied extensively and both the traits appeared to be independent of environment. For across season data, both ETNP and PHI showed significantly different values. All other agronomic traits showed highly significant values. Interestingly, in summer

2013, grain dry weight/plot (GDW/plot) and grain number per panicle (GNPP) values were not significant. In summer 2014, GDW/plot and GNPP values were significant but for across season values, they were not significant. This may be due to diverse sowing dates of summer 2013 (31st Dec 2012) and summer 2014 (20th Nov 2013) and their stress exposure levels. In summer 2014, G×E values for both the traits GDW/plot and GNPP were higher than the estimated genotypic variance. Except for the FT, PlHt, all others recorded high genotypic variance values than G×E values. From ANOVA table mean sum of square values for genotype and G×E from ANOVA table were compared (Table 9). In case of shoot fly component traits, much genetic variance was observed for all the traits and genotypic values were highly significant for all the traits (Table 10). G×E were significant for all the agronomic except GNP/plot and GNPP. For stay-green weekly scores and shoot fly resistance component traits, G×E values were highly significant except seedling leaf blade glossiness (in case of shoot fly resistance).

4.9. Frequency distribution for F_4 progeny: Various traits distribution among the F_4 progeny and their frequency disribution for stay-green and agronomic traits for summer 2013 (*rabi* 2013-2013) and 2014 (*rabi* 2013-2014) are represented in Figures 11, 12 and 13 (Tables 5, 6 and 7).

4.9.1. Stay-green frequency distribution: Percent green leaf area in 7, 14, 21, 28, 35, 42 and 49 days after flowering: The frequency distribution for % GL 7 DAF ranged from 72-97 in summer 2013 and 79-99 for summer 2014. The F_4 progeny was highly skewed towards RSG04008-6 female parent and variations in the seasonal data were less. During summer 2013, the F_4 progenydata showed senecence and % GL was reduced upto 72 due to delayed *rabi* sowing (Fig .11a). The frequency distribution of % GL 14 DAF in summer 2013 data showed maximum number of individuals skewed towards female parent RSG04008-6 with delayed senescence. The mean values ranged from 58-96, 68-96 for summer 2013, 2014 respectively (Fig .11b). The frequency distribution of summer 2013 was uniform and for summer 2014 it is near to normal distribution. The data

ranged from 43-93, and 63-84 during summer 2013, 2014 respectively (Fig .11c). The frequency distribution graphs recorded discontinuous distribution for summer 2013 and 2014, and the graph skewed towards left (Fig .11d). The frequency graph for summer 2013 showed near to a binomial graph and for summer 2014, the graph resembled near to normal distribution. The means ranged from 22-62, 19-66 for summer 2013, 2014 respectively (Fig .11e). The frequency distribution graphs for both the seasons exhibited two different pictures near to normal distribution graphs due to environmental influence (Fig .11f). Normal distribution was observed for summer 2013 and F_4 progeny skewed towards male parent J2614-11 during summer 2014 (Fig .11g).

4.9.2. Frequency distribution for agronomic traits:

4.9.2.1. Days to 50% flowering time (FT): The frequency distribution of flowering time for both the seasons vary significantly. The mean range values of flowering for summer 2013 and 2014 varied from 57-80 and 75-90. This clearly shows the influence of environment on flowering time. Thus, the frequency distribution graphs were clearly dipected for the flowering time disribution for both the environments (Fig .12a).

4.9.2.2. Plant height(PlHt): The frequency distribution of plant height was clearly different from summer 2013 to that of plants grown in summer 2014. For summer 2014 a near normal distribution was observed. The range varied from 84-147 and 105-225 for summer 2013 and 2014 respectiveely showing clear differentiation for both the environments (Fig .12b).

4.9.2.3. Panicle dry weight per plot (PnDW/pot): The frequency distribution for PnDW/plot was greatly influenced by the environment. The PnDW/plot ranged from 416-779 and 763-1102 of summer 2013 and 2014 respectively showing clear variation for both the seasons (Fig .12c).

4.9.2.4. Grain dry weight per plot (GDW/plot): The grain dry weight per plot frequency distribution clearly showed the influence of environment on the grain yeild for summer 2013 and the grain yeild varied from267-539 and 531-777 for summer 2014 respectively. The clear variation observed for both the seasons and exposed stress levels reduced the grain yeild per plot (Fig .12d). The reduction in yield is mainly because of late sowing in *rabi*.

4.9.2.5. Grain number per plot (GNP/plot): Thefrequency distribution graph showing less variation across both the seasons which ranged from 18453-43294,17204-27250 for summer 2013, 2014 respectively. Maximum number of F_4 genotypes skewed towards the male parent (Fig .12e).

4.9.2.6. Grain number per panicle (GNPP): The frequency distribution graph of GNPP exhibited overlaying of both the seasons data. The frequency ranged from 709-1665 , 661-1048 during summer 2013, 2014 respectively. F_4 progeny mostly skewed towards the J2614-11 male parent (Fig .12f).

4.9.2.7. Hundred grain mass (HGM): The frequency distribution of hundred grain mass dipicted differently for two different environments. The frequency ranged from 1.22-2.24 and 2.27-3.73 for summer 2013 and 2014. The data clearly showed huge environmental influence on grain filling, grain mass quality and grain weight too. The frequency distribution showing two different near to normal distribution graphs for the hundred grain mass. Maximum F_4 progeny in summer 2013 displayed 1.8 g HGM and maximum F_4 progeny for summer 2014 was 3 g HGM (Fig .12g).

4.9.2.8. Panicle harvest index (PHI): The frequency distribution graph of panicle harvest index (PHI) was near to normal distribution for both the seasons. The frequency ranged from 63-78, 66-74 for summer 2013 and 2014 respectively (Fig .12h).

4.10. Frequency distribution for shoot fly resistance component traits

The data recorded for two different seasons i.e.,*kharif*2013 and *rabi* 2013 and their frequency distributions are shown in Fig 13 and Table 7.

4.10.1. Seedling leaf blade glossiness (1-5 score): Normal frequency distribution was observed for *kharif*2013 while binomial distribution for *rabi* 2013. The mean values ranged from 1.8-4.4, 1.8-4.7 for *kharif* 2013, and *rabi* 2013 respectively and much varition across seasons was not observed (Fig .13a).

4.10.2. Leaf blade trichome density lower (abaxial) (numbers/microscopic field): The frequency graphs indicated a clear environmental influence on trichome density. During *rabi* 2013, trichome density increased when compared to *kharif* season 2013. Discontinous distribution observed for both the seasons. Mean values ranges from 0-58 and 2-68 for *kharif*2013,*rabi* 2014 respectively (Fig .13b).

4.10.3. Leaf blade trichome density on the upper (adaxial) side (numbers/microscopic field): The frequency distribution for trichome density on the upper side of the leaves displayed binomial distribution and trichomes were more in *rabi* than *kharif* 2013. The means ranged from 9-127, 9-122 for both the seasons. The frequency graphs showed the environmental influence on the F_4 progeny (Fig .13c).

4.10.4. Leaf sheath pigmentation (1-3 score): The frequency distribution was discontinous for both the seasons and the influence of environment appeared less. The distribution for both the years was mostly identical (Fig .13d).

4.10.5. Seedling vigour (1-3 score): Seedling vigour was high during *rabi* when compared to *kharif*2013. The seedling vigour ranged from 1.6-2.7, 1.1-1.7 for *kharif*2013 and *rabi* 2013 respectively. These results and graphs clearly indicated the environmental influence (Fig .13e).

4.10.6. Percent shoot fly dead heart (% SFDH): The frequency distribution graph displayed that maximum dead hearts were observed during *kharif*2013 and *rabi*2013. The graphs displayed the environmental influence of SFDH percentage and the means ranged from 85-97, 15-60 during *kharif* 2013 and *rabi* 2013 respectively (Fig .13f).

4.11. Frequency distribution for across season analysis: The BLUPs calculated across seasons were used for across season frequency distribution graphs and showed in Figures 15, 16, 17 and Table 5, 6 and 7.

4.11.1. Across season frequency distribution of % GL: %GL 7 DAF showed a left skewed graph and % GL 21 DAF exhibited right skewed distribution. Binomial distribution was observed for% GL14 DAF and % GL 28 DAF. The frequency distribution graphs for across season percent green leaf area showed normal distribution curves for % GL 35 DAF, % GL 42 DAF and % GL 49 DAF (Fig .14 and Table 5).

4.11.2. Across season frequency distribution of agronomic traits : Frequency distribution graph for across season flowering time, panicle dry weight per plot, grain number per plot, grain dry weight, grain number per panicle and panicle harvest index recorded normal and near to normal distribution. The value means varied according to season and trait. Hundred grain mass and plant height showed binominal distribution irrespective of season (Fig .15 and Table 6).

4.11.3. Across season frequency distribution of shoot fly component traits: seedling leaf blade glossiness recorded normal distribution for across season data. Bimodal/double peaked distribution was observed for trichome density on the upper and right skewed distribution graph for trichome density on the lower leaves. Binomial distribution was noticed for seedling vigour and discontinous

distribution was recorded for leaf sheath pigmentation for across season data (Fig .16 and Table 7).

4.12. Heritabilities

Operative heritability was observed for all the agronomic and stay green traits of selected F_4 recombiant population for both summer 2013 and 2014 environments. Similarly for shoot fly component traits also heritability values were estimated for rainy 2013 and post-rainy 2013. Based on heritability estimates the genotypes were selected for breeding programs. Heritability values were calculated from anova table and percentage heritability below 20 were noted as less heritable,>30 – 50 % heritable are moderatly heritable,>60 % heritable are noted as highly heritable values. Heritability estimates are represented in tables 8, 9 and 10.

4.12.1. Percent green leaf area (% GL) heritabilities (Table 8)

4.12.1.1. Percent green leaf area 7, 14, 21, 28, 35, 42, and 49 days after flowering: The heritability values for F_4 progeny are high for % GL 7 DAF and ranged from 69-81%. For summer 2013 and 2014 heritabilities were 75 and 69 respectively. On the other hand, across season heritability was 81%. The heritability values for % GL 14 DAF was similar with % GL 7 DAF. The F_4 progeny recorded high heritability for both the seasons i.e., summer 2013 (74) and 2014 (68). For across environment analysis also (81), high heritability was noticed. The F_4 progeny for % GL 21 DAF recorded high and moderate heritability for summer 2013 (75) and 2014 (54). For across season analysis (80), high heritability was recorded. High heritability was observed for % GL 28 DAF for summer 2013 (74) and 2014 (62). High heritability (80) was recorded for across season analysis. The F_4 progeny exhibited high heritability values for % GL 35 DAF during summer 2013 (71) and 2014 (67). Across season analysis, high heritability (77) was recorded. % GL 42 DAF of F_4 progeny exhibited moderate heritability values (57) for summer 2013 but high heritability values (80) for summer 2014. In case of across season analysis, high heritability (78)

was noticed. The F_4 progeny for % GL 49 DAF exhibited moderate heritability (52) during summer 2013 and high heritability (75) for summer 2014. Across seasons also, high heritability (72) values were noticed.

4.12.2. Agronomic data heritabilities (Table 9)

4.12.2.1. Flowering time (FT): The F_4 progeny heritabilities for flowering time showed high values (92) for summer 2013 (81) and 2014. Across season values also showed high heritability (91).

4.12.2.2. Plant height(PlHt): F_4 progeny values for plant height exhibited high heritability values (66) for summer 2013 (88) and 2014. Across envirnment analysis also showed high hertability (89).

4.12.2.3. Panicle dry weight per plot (PnDW/pot): The F_4 progeny values for panicle dry weight per plot heritability were moderate (57) for summer 2013 but low for 2014 (35). Across environment analysis, the heritability was moderate (46).

4.12.2.4. Grain dry weight per plot (GDW/plot): The F_4 progeny heritability values for grain dry weight per plot were almost similar to panicle dry weight per plot values. For across seasons, heritability was moderate (45) and for individual seasons moderate (56) and low (37) heritabilities were noticed for summer 2013 and 2014 respectively.

4.12.2.5. Grain number per plot (GNP/plot): The F_4 progeny heritability for grain number per plot showed low heritability (18) for summer 2013 and moderate (40) for summer 2014. The across season heritability displayed moderatly low (27) values inferring the influence of environment on GNP/plot.

4.12.2.5. Grain number per panicle (GNPP): The F_4 progeny heritability values for GNPP were identical to GNP/plot. Heritability values ranged from moderate to low however.

4.12.2.6. Hundred grain mass (HGM): The F_4 progeny values for hundred grain mass showed conistantly high heritability values. For summer 2013, 87% was observed and for summer 2014, 75% heritability was recorded. In case of across season analysis also, high heritability (86%) was noticed.

4.12.2.7. Panicle harvest index (PHI): For F_4 progeny, the heritability values for panicle harvest index ranged form low to moderate (7-32). For across season, heritability was moderate (30).

4.12.3. Heritabilities for shoot fly component traits (Table 10)

4.12.3.1. Seedling leaf blade glossiness: The F_4 progeny heritability for seedling leaf blade glossiness recoreded high values (66) for *kharif*(84) and *rabi* seasons in 2013. For across season analysis also, high heritability (83) was recored inferring less influence of environment.

4.12.3.2. Leaf blade trichome density on the upper (adaxial) surface (numbers/microscopic field): The F_4 progeny recorded high estimates of heritability for *kharif* (74) and *rabi* (83) seasons. For across environment anaysis, high heritability (79) was observed for trichome density.

4.12.3.3. Leaf blade trichome density lower (abaxial) (numbers/microscopic field): High heritability (>80) was recorded for both the seasons and across season observations. The heritability values ranged from 87-80.

4.12.3.4. Seedling vigour: The F_4 progeny seedling vigour heritability values were moderate (50) to high (88) for *kharif*2013 and *rabi* 2013. Across

environment analysis, moderate heritability values (48) were observed indicating the environmental influence on trait.

4.12.3.5. Leaf sheath pigmentation (1-3 score): Consistantly very high heritability (>85) was recorded for both the season of F_4 progeny and across season indicating very less influence of environment.

4.12.3.5. Percent shoot fly dead heart (% SFDH): High heritabilty values (>75) were recorded for % SFDH for F_4 progeny in both the seasons. For across seasons, moderate heritability was observed and the values ranged from 38-76.

4.13. Correlation coefficient: Correlation is a statistical component which showed association or relation between two components. Based on phenotypic values, the correlation coefficients were estimated for both the seasons for all the stay-green and agronomic data (Table 11) and shoot fly component traits (Table 12).

4.13.1. % GL correlations :% GL, 7 (week1) days after flowering was positively correlated with all the observed weekly % GL scores for both the seasons and across seasons (Table 11).

4.13.1.1. % GL correlations with agronomic data and yeild related traits: % GL for all the data were negatively correlated with flowering time, plant height and HGM for both the summer 2013 and 2014 and across seasons. PnDW/plot was positively correlated with % GL7 DAF for both the seasons and positive correlation was noticed with % GL 14, 35, 42, 49 DAF for summer 2014 as well as for % GL 7 DAF and % GL 49 DAF across seasons. GDW/plot for summer 2013 was negativley correlated for all the weekly stay-green scores but positively correlated for all the weeks during summer 2014. For across season data, GDW/plot was negatively correlated for % GL 7, 14, 21, 28, 35 DAF but negatively correlated to % GL for 42, 49 DAF. GNP/plot also negatively

correlated to % GL 7, 14, 21, 28, 35, 42, 49 DAF for summer 2013 and positively correlated to summer 2014 for all weekly stay-green scores. For across season, corelation ceofficient analysis was negatively correlated for all the stay-green weekly scores except % GL 49 DAF. The correlation coefficient analysis for GNPP was most similar to GNP/plot. All the stay-green weekly scores were negatively correlated for summer 2013 and positively correlated for summer 2014 as well as for across season except % GL 49 DAF with GNPP (Table 11).

4.13.2. Agronomic traits correlations

Days to 50% flowering (FT) was positively correlated (r = 0.03 and r = 0.1) with plant height for both the seasons and significantly and positively correlated (r = 0.16*) with across season data. But, FT was negatively correlated with PnDW/plot, GDW/plot, GNP/plot, GNPP for summer 2013. Also, for summer 2014, it was negatively correlated with GDW/plot, GNP/plot, GNPP and PHI. On the other hand, FT was positively correlated with PHI and HGM during summer 2013 as well as PnDW/plot and HGM for summer 2014. During across season data, FT was positively correlated with PnDW/plot, GDW/plot and HGM. FT was negatively correlated with GNP/plot, GNPP and PHI for across season. Plant height was positively correlated to PnDW/plot, GDW/plot, HGM and PHI for both the seasons and significantly and positively correlated to across seasons data for PnDW/plot, GDW/plot, HGM and PHI. Plant height was positively correlated to GNP/plot and GNPP for summer 2013 and negatively correlated for summer 2014. Across season data for GNP/plot and GNPP was positively correlated to plant height. PnDW/plot was significantly and positvely correlated with GDW/plot, HGM, GNP/plot and GNPP for both the summer 2013 and 2014 as well as across seasons except for PHI summer 2014. GDW/plot also showed significant positive correlation with GNP/plot, GNPP and PHI for both the seasons and across seasons except HGM. HGM was negatively correlated with GNP/plot and GNPP but, for across seasons, they were negatively correlated. GNP/plot displayed significant positive correlation with GNPP for both the seasons and across seasons. PHI was negatively correlated for both the seasons

and positive correlation for across seasons. PHI showed significant positive correlation with GNP/plot and GNPP for both the seasons and across seasons (Table 11).

4.13.3. Shoot fly component traits correlation: (Table 12)

4.13.1. Glossiness *vs* other traits: Seedling leaf blade glossiness was positively correlated with leaf sheath pigmentation (r= 0.16* and 0.12) and percent shoot fly dead heart (r= 0.03 and 0.16) for *kharif* 2013 and *rabi* 2013. For *kharif* 2013, seedling vigour was negatively correlated with glossiness (r= 0.07). For across seasons, glossiness was correlated positively with leaf sheath pigmentation (r= 0.77), seedling vigour (r= 0.01) and percent shoot fly dead heart (r= 0.16). Glossiness is negatively correlated to trichome density on the upper surface (r= -0.08 and-0.25*), trichome density on the lower (r= -0.02 and -0.17*) for both the seasons as well for across seasons (r= -0.16 and -0.11). Seedling vigour was also negatively correlated (r= -0.1) with glossiness for *rabi* 2013.

4.13.2. Leaf sheath pigmentation (LSP) *vs* other traits : Leaf sheath pigmentation was highly positively correlated (r = 0.11, r = 0) to seedling vigour for both the seasons. A positive correlation (r = 0.01) was observed for trichome density on the upper for *kharif* 2013 and % SFDH (r = 0.09) for *rabi*seasons in the year 2013. For across seasons, it was positively correlated with seedling vigour (r = 0.08) and % SFDH (r = 0.05). LSP was negatively correlated with (r = -0, r = -0.01) trichome density upper for both the seasons, as well as for trichome density upper (r = -0.06) for *rabi*2013 and for % SFDH (r = -0.1) during *kharif*in 2013. For across season data, LSP was negatively correlated to TDU (r = -0.03) and TDL (r = -0.02) and positively correlated to seedling vigour (r = 0.08) and % SFDH (r = 0.05).

4.13.3. Seedling vigour *vs* other traits: Seedling vigour was negatively correlated to trichome density upper (r = -0.01), trichome density lower (r = -

0.04) and % SFDH (r = -0.33) for *kharif* 2013 and also % SFDH negatively correlated with seedling vigour for *rabi* in 2013. But, seedling vigour was positively correlated with trichome density upper (r = 0.18) and trichome density lower (r = 0.11). For across seasons also SV was positively correlated to TDU (r = 0.1), TDL (r = 0.04) and % SFDH (r = 0.01).

4.13.4. Trichome density upper *vs* other traits: TDU was significantly positively correlated (r = 0.67* and r = 0.88*) with TDL, but negatively with (r = -0.07 and r = 0.66*) % SFDH for both the seasons *kharif* 2013 and *rabi*2013. For across seasons, significant positive correlation (r = 0.85) was noticed for TDL and negative correlation (r = -0.06) for % SFDH.

4.13.5. Trichome density lower vs % SFDH: TDL was negatively correlated with % SFDH (r = -0.09 and r = -0.7) for both the seasons as well as significant negative correlation (r = -0.77) to % SFDH across seasons (Table 12).

4.14. Genetic linkage map on total F_2 and selected F_2 population with SSR markers

The entire F_2 population of 1,894 individuals were genotyped with 5 linked SSR markers spanning the introgression target region for SBI-10 shoot fly resistance component trait QTL alleles from IS18551 present in parent J2614-11, resulting in a map distance of 37 cM (Fig .17a). Based on marker arrangement, genotyping data were categorized into different classes having homozygotes of RSG04008-6, homozygotes of J2614-11, heterozygotes and nearly homozygotes with different recombinations. All the possible genotypic recombinations revealed the QTL effect on phenotype expression. Based on genotyping data across this target region 369, informative recombinants F_2 individuals were selected for advancing to the F_3 generation. The selected recombinant F_2s were genotyped at three additional markers (*Xisep0621, Xisp10262 above Xgap001 and Xisep1011 below Xtxp141* on the long-arm of SBI-10). These markers were added to fully encompass the 'Gls' and 'Td' genomic regions and the linkage map constructed

for these selected recombinant F_2 individuals had an artifactully expanded total length of 71 cM. Marker *Xiabt340* was then excluded from the linkage map as it showed a large portion of missing data (Fig .17b). When the marker arrangement on the genetic map was compared with the physical map, marker order was the same (Fig .17b). Based on F_2 genotyping data of 7 co-dominant SSR marker and $F_{2:3}$ phenotyping data, 182 highly informative recombinants were selected and their genotyping data are represented in GGT (graphical genotype representation) (Fig .18) and F_3. These werefurther selfed to produce F_4 seed which were sent to field trials and can be skim-sequenced by GBS for fine mapping the glossy and trichome density regions as well as stay-green and agronomic regions on SBI-10L (Kiranmayee *et al*., 2016).

4.14.1. Genetic linkage map of highly informative F_2 selected recombinants

4.14.1.1. Genotyping by sequencing approach for increasing marker density in the target region:

Selected 182 F_2 recombinant genomic DNA samples were sent for Skim-sequencing by GBS method for increasing the marker density of the targeted genomic region in order to fine map with the help of replicated two season environment data. A total of 32,836 SNPs were identified from 182 selected recombinants of F_2 genotypes from the total genome (Annexure 2). Our aim was to focus on SBI-10L and the cross as well as the population developed based on the recombination events of SBI-10L region of 45-60Mb where 1515 SNPs were identified in the target 15Mb region (~1SNP/kb). SNPeff was utilized for annotation of each SNP based on their location to predict their coding effect. Nearly 24.16% of SNPs were located in exonic regions (18.5% synonymous coding regions + 5.6% non-synonymous coding regions), 47.72% in intergenic and 11.32% SNPs in intronic regions (Fig .19). A linkage map was constructed from 1894 F_2 fine mapping populations derived from an introgression line cross RSG04008-6×J2614-11 using 7 SSR markers and obtained a 72 cM map of SBI-10L of target region. Out of 1894 F_2 populations, 369 $F_{2:3}$ were selected and 152

were selfed and $F_{2:4}$ were utilized for fine mapping in the present study. Out of 1515 SNP markers and their allelic proportion A, B and H was cross checked with the proportion of SSR marker data. Proportion of SNP 'B' score was almost similar to the proportion of 'B' score of SSRs which indicate the SSR and SNP genotyping data could be accepted and utilized (Fig .20). A, B and H classes of SSR marker data were compared with SNP A, B and H proportion and the mismatched markers were excluded from the SNP data set as these markers may report alleles from other than those intended. Accordingly, a total of 624 markers were left and were used as input for THReaD Mapper studio. THReaD mapper studio has excluded 232 markers and constructed a distance matrix with the left out 393 SSR and SNP markers (Fig .21).

4.14.1.2. Principle co-ordinate analysis (PCA): Principle co-ordinate analysis was carried out for the calculated distance matrix from THReaD mapper studio in order to measure the linkage between the markers. PCA produces an arch effect named as Horseshoe Effect. PCA of distance matrix results in a horseshoe shaped curve and the SSR and SNP data points lying on horseshoe line are assumed to be the order of markers of linkage group. Many data points were placed centrally and the markers appeared to have huge missing data that need to be eliminated from the distance matrix. Markers ordered left to right along the blue line traces the order of the markers. Blue data points were threaded through green points in order to create map order by projecting the points of curve threaded an ellipse through the green points (Fig .22). The markers in the horseshoe line were used to project the distance matrix and the graph showed high linkage and tightly packed markers. Finally, 265 highly linked SSR and SNP markers were left after eliminating 127 marker data points from 392 marker data points. The closely linked or less inter-marker distance markers (265) were used for linkage map construction (Fig .23).

4.14.1.3. Linkage map construction of F_2 selected recombinant fine mapping population on SBI-10L

The segregated 265 markers for 152 F_4 double recombinants were used for linkage map construction derived from cross RSG04008-6 × J2614-11. Join map was used for construction of linkage map and inter-marker distance in cM was calculated using Kosambi mapping function. All the 265 SSRs and SNPs were grouped at LOD 3 into a single group with a total distance of 139.7 cM with average inter marker distance of 0.5 cM by using Kosambi mapping function. For all the sets of SSRs and SNPs, probability and chi square values were calculated and represented in Table 13 and Figure 24. All the markers significantly deviated from 1:2:1 Mendilian segregation ratio of F_2 population due to selected double recombinants. Out of 265 markers, 5 are SSRs and remaining 260 are SNPs. The SSR linkage maps constructed are integrated with 260 SNPs. No large gaps were observed between markers. The genetic map distance/the proportion of recombination distances was compared with physical positions of the markers on chromosome SBI-10L (Fig .25). Table 13 represents linkage map with marker distances and the segregation distortion of 262 SNP-SSR markers on 152 F_2 recombinant progeny and their chi square values and significance.

4.15. QTL mapping of seedling leaf blade glossiness and trichome density in total F_2 and $F_{2:3}$ population with initial SSR linkage map

Moderately large F_2 populations (1,894) were screened for seedling leaf blade glossiness and trichome density scores, and genotyped at 5 SSR markers across the SBI-10L target region. The genotyping and phenotyping data sets were used for QTL analysis. The presence of two QTLs, one each for trichome density and glossiness were confirmed in this target region. The glossy QTL was mapped near *Xgap001,* but clear flanking markers were not demarcated; the trichome density QTL was found in marker interval *Xisep630-Xtxp141* with an overhang. QTL analysis can also be affected by the size of the early-generation large

population, and large populations can result in detection of large numbers of QTLs including minor effect QTLs. Based on phenotyping methods used, the effect of QTLs can also be impacted, determining which are the major QTLs and minor QTLs. At LOD 3, QTLs were detected for seedling leaf blade glossiness and trichome density (Table 14) with the large F_2 population and trait scoring methods used.

4.15.1. SFR component trait QTLs detected in 1,894-individual F_2 population with SSR linkage map

Composite interval mapping (CIM) analysis identified three QTLs for shoot fly resistance, one for leaf glossiness and two for trichome density in the F_2 population of 1,894 individuals. The QTL for seedling glossiness score (Q*Gls10)* was mapped at LOD 24 (Fig .26a) between markers *Xgap001* and *Xnhsbm1004* with an R^2 value of 6.23% (indicating it is relatively a minor QTL). Two seedling leaf blade trichome density score QTLs (Q*Td10a* and Q*Td10b)* were mapped between *Xisep630* and *Xtxp141* at LOD 8.01 with an R^2 value of 2.88% (indicating that they too are minor QTLs). The glossy QTL and trichome density QTLs were found with an interval of 0-10 cM, 25-37 cM, respectively, on the map of *Xgap001* to *Xtxp141* interval on SBI-10L in the F_2 high resolution mapping population (Table 14). F_2 QTL mapping resulted in an incomplete confidence interval for both '*Gls*' and '*Td*' with *Xgap001* and *Xtxp141* marker interval based on F_2 genotyping data. In order to locate exact genomic region of variations few more markers were screened for polymorphism between parents and 3 polymorphic markers (*Xisep0621, Xisp10263 and Xisep1011*) were added to the linkage map.

4.15.2. SFR component traitQTLs detected among 369 selected F_2 individuals and their derived F_3 progenieswith SSR linkage map

At LOD 5.95, the leaf blade glossiness score QTL (*QGls10*) was mapped between *Xisp10263* and *Xgap001* with R^2 of 6.60% in the subset of F_3 progenies and in the full F_2 population it was between same flanking markers with R^2

of11.37% and LOD of 9.67. The selected recombinant F_2 population consisted of 369 individuals, and its derived F_3 progenies were analyzed for trichome density QTLs (*QTd10*) and found co-localized for both the F_2 population and its F_3 progenies in the interval between *Xisep630* and *Xtxp141*. The phenotypic variation accounted for the trichome density QTL for the selected informative F_2-derived F_3 progenies was just 2.03% and for the selected recombinant subset of the F_2 population, it was 3.70% with LOD values of 2.32 and 4.40 respectively (Fig .26b and Table 15).

4.15.3. F_2 and $F_{2:3}$ QTL mapping on selected 369 individuals

Consistent QTLs were detected in two different seasons with two different generations, confirming the presence of conserved QTL regions that need to be finely mapped with a larger number of polymorphic molecular markers. Further fine mapping by increasing marker density of these QTLs will improve our understanding of the molecular basis of both seedling glossiness and seedling leaf blade trichome density (morphological component traits contributing to sorghum shoot fly resistance).

4.15.4. F_2 and $F_{2:3}$ generation QTL mapping confirmation for SFR morphological traits

For the selected 369 recombinant F_2 individuals, QTL mapping analysis was conducted and compared with the results obtained using phenotypic data collected from their F_3 progenies. In both the generations, recombinant F_2s and their derived F_3s, QTL mapping results showed similarity with those obtained from the full F_2 population. These results reconfirm that a glossiness QTL tagged with *Xgap001* and at least one trichome density QTL tagged with X*txp141* were localized on sorghum chromosome SBI-10L (Fig .27b and Table 14). This confirms that the glossy and trichome density QTLs are located in the target marker interval.

4.16. GBS SNP-SSR markers integrated into early generation QTL map for shoot fly component traits

4.16.1. Fine mapping of seedling leaf blade glossiness and trichome density with total 1894 F_2 and selected 369 $F_{2:3}$ population

The high density linkage map of GBS-SNP integrated SSR map was used against the total 1894 F_2 seedling leaf blade glossiness and trichome density phenotyping data. Two QTLs were mapped for F_2 Gls QTL with increased marker density at LOD 3 on 34 cM and 70 cM regions of SBI-10L with phenotypic variance of 11 and 3% respectively. In F_3 population, 369 selected recombinants and two QTLs were mapped but little shift was noticed at the locations of LOD 3.8 and 4 near 38 and 42 cM regions respectively explaining 11 and 18% phenotypic variance (Fig .27). Nearly 15 cM region was reduced to 1 cM for the selected QTLs with increased marker density. In case of trichome density, total F_2 QTL was mapped at 103.11 cM at LOD 2.7 explaining 8% phenotypic variance. F_3 phenotyping data of selected individuals was mapped on the high density map and 2 QTLs were identified at 66.01 cM and 99.11 cM at LOD 2.5 indicating combined phenotypic variance of 8% (Table 16 and Fig .27).

4.16.2. F_2 selectedrecombinants and further fine QTL mapping with F_4 replicated field trials

Composite interval mapping (CIM) was performed using QTL cartographer 2.5V software. Thousand permutations test was performed and minimum LOD 2.5 was used for QTL detection. The BLUP mean values of each environment and across environments were used for QTL detection. QTLs were detected for four different seasons. Stay-green QTLs and agronomic QTLs were detected for summer 2013 (*rabi* 2012-2013), summer 2014 (*rabi* 2013-2014) as well as for across environments (Table 17 and 18). Fourteen QTLs were detected for stay-green during summer 2013 (5) and summer 2014 (9) and 6 QTLs were detected for across seasons. For agronomic data, in summer 2013 and 2014, 17 and 16 QTLs were recorded. For across environment analysis, 21 putative QTLs were

detected. For shoot fly resistance, in both *kharif* 2013 and *rabi* 2013, QTLs were detected alongside across season QTLs. QTLs for shoot fly component traits of *kharif* 2013 and*rabi* 2013, 9 and14 QTLs respectively were noticed. For across environment analysis, 19 putative QTLs were detected for shoot fly component traits on sorghum chromosome long arm (SBI-10L). The positions of the QTLs detected are illustrated in Table 19 and Fig .28.

4.16.2.1. Stay-green QTL mapping

The QTLs for stay-green detected for single environment and across environment are listed in Table 17 and Figures 28 and 29. Percent GL7 DAF was mapped between S10-54877607 and S10-54081973 with 1.8 cM inter marker distance. The closest marker was S10_54081973 SNP at a location of 41.41 cM in the SBI-10L arm. Recombinant F_4 progeny map with phenotypic variance of 8.86% was noticed indicating a minor QTL location on SBI-10. Same QTL (41.41 cM) was mapped at LOD 2 during summer 2014, but no phenotypic variation was observed. During summer 2014, two QTLs were observed with combined phenotypic variance that ranged from 16.41%. Q10GL 7a_14 was mapped at S10-58311699 (104.81 cM) near to initial flanking SSR marker *Xtxp141* and the other QGL7b_14 was mapped at SNP marker S10-59020363 (112.11 cM). For across season data, the QTL (Q10GL7a_across) was mapped in the SNP S10_54585199 at 44.41 cM with 6.7% phenotypic variance. LOD graph is represented in the Fig.30a. % GL 14 DAF was also mapped in the same QTL region of % GL 7 DAF during summer 2013 at SNP S10_54081973 (41.41 cM) and across season SNP S10_545199 (44.41 cM). Q10GL14a_r13 QTL was mapped between 40-43 cM interval at the position 41.41 (SNP S10_54081973) with LOD at 2.86 and Q10GL14a_r13 was mapped at SNP S10_56433597 (82.71 cM) at LOD 2.18 with 9%, 4% and 24% of phenotypic variance respectively during summer 2013. For summer 2014, five QTLs were mapped. Q10GL14a_r14 was mapped at 36.91 cM (SNP S10_54269620), Q10GL14b_14 at 45.01 cM (SNP S10_54535306), Q10GL14c_14 at 129.51 (SNP S10_60287963), Q10GL14d_14 at 29.01 cM (S10_52036901), Q10GL14e_14 at

123.61 (SNP S10_60024056) and the combined phenotypic variance was 53.68% for summer 2014. For across season data, the % GL 7 DAF and % GL 14 DAF were mapped at the same region 44.41 cM (SNP S10_54585199) of SBI-10L at LOD 3.7 with phenotypic variance (10.20%), and this appeared as a major QTL (stay-green QTLs mapped between *Xgap001* and *Xtxp141*). LOD graphs are represented in Figure 30b. % GL 21 DAF was mapped at S10_59342820 SNP (115.31 cM) for summer 2013, 2014 and across seasons with phenotypic variance of 9%, 9% and 10.14% respectively indicating that it as a major QTL. Two different minor QTLs were identified for each season at different locations, i.e., Q10GL21b_r13 at 41.41 cM overlapping with % GL 7 and % GL 14 QTLs with 6.91% phenotypic variance. Q10GL21c_13 was mapped at 125.01 cM (SNP S10_60194381). In case of summer 2014, Q10GL21b_r14 was mapped at 79.41 cM (S10_56381721) and Q10GL21c_r14 at 99 cM (S10_57248800). LOD graphs are shown in the Fig.30c. Q10GL28a_r13 was mapped at LOD 2.5 with 1.2% phenotypic variance at 125.01 cM (SNP S10_60194381) region overlapping with Q10GL21c_13. Q10GL28a_r14 QTL was mapped at 41.41 cM (SNP S10_54081973) and is co-localized with the Q10GL7a_13, Q10GL14a_13 and Q10GL21b_13 of summer 2013. Q10GL28a_14 was mapped at 36.41 cM (SNP S10_54269620) at LOD 3.67 with 4.96% phenotypic variance overlapping with % GL 14 QTL (Q10GL14a_r14). For across season QTL analysis, Q10GL28a_across was mapped at 124.91 cM at LOD 2.7 overlapping with % GL 21 QTL (Q10GL21c_r13) and the combined phenotypic variance was found 15.62%. LOD graphs are shown in the Fig.30d. A single QTL (Q10GL35_r13) was detected for this trait for the season *rabi*2013 with 2% phenotypic variance at LOD 2.36 at 121.91 cM (SNP S10_59850910). LOD graphs are represented in the Fig.30e. During summer 2013, Q10GL42a_13 QTL was located at 121.91 cM (S10_59850910) at LOD 2.5 explaining 4.5% of phenotypic variance similar to % GL 35 QTL and Q10GL42b_r13 mapped at 131.91 cM (SNP S10_60701880) at LOD 2. For summer 2014, Q10GL42a_r14 was observed at 102.31 cM (SNP S10_57522978) at LOD 2.6, Q10GL42b_r14 at 38.41 cM (S10_52812930) Q10GL42c_r14 at 107.81 cM (S10_58460662) and

Q10GL42d_r14 at 32.31cM (SNP S10_52781712), identified with a combined phenotypic variance of 22.18%. For across season analysis, no QTL was detected. LOD graphs were plotted and shown in the Fig.30f. During summer 2013, a QTL (Q10GL49a_13) was mapped at 34.7 cM (SNP S10_50890593) and during 2014, QTLs were not detected but for across season analysis, 2 QTLs were identified, Q10GL49a_across at 36.41 cM (SNP S10_ 54269620) overlapping with % GL 14, % GL 28 with 2.5 LOD with 2.4% phenotypic variance and Q10GL49b_across at 45.01 cM (SNP S10_54535306) overlapping with % GL 14 QTL with LOD 3 explaining 4% phenotypic variance. LOD graphs are represented in the Fig.30g.

4.16.2.2. Agronomic data and QTL mapping (Table 18)

4.16.2.2.1. QTLs for days to 50% flowering time (QFT): A single QTL (Q10FT.a_r13) was obtained for flowering time during summer 2013 at LOD 2.5 explaining 4.4% phenotypic variance at S10_51065106 (12.41 cM). In summer 2014, four QTLs (Q10FT.a_14, Q10FT.b_14, Q10FT.c_14, Q10FT.d_14) were detected at 25.91 cM (S10_51071502), 36.91 cM (S10_45646835), 44.41 cM (S10_54585199), 101.31 cM (S10_57522978) at LOD 3, 5, 5, 2.4 and 3, 12, 12, 0.0003 % phenotypic variance respectively. For across season analysis, two QTLs were detected at LOD 3.4 and 2.8 explaining 17% phenotypic variance. Q10FT.c_14 QTL was also detected in across seasons also. LOD graphs were plotted and shown in the Fig.31 a.

4.16.2.2.2. QTLs for plant height (PlHt): Four different QTLs were detected during summer 2013, Q10PlHt.a_13 was mapped at LOD 3 but without PVE and other 4 QTLs were mapped at 40.41 cM (S10_52940776), 49.21 cM (S10_54535502), 70.11 cM (S10_55747741), and 108.61 cM (S10_58683017 at LOD 2, 3, 2.2, 2.1 respectively explaining 8% combined phenotypic variance and Q10PlHt.a_14 at 97.91 cM (S10_58436230) with LOD 3 explaining 7.2% phenotypic variance. For across seasons, two QTLs were detected at 70.61 cM

(S10_55747741) and 97.91 cM (S10_58436230) with LOD 2.5 and 2.7 respectively explaining 6.3% combined across season phenotypic variance. LOD graphs were plotted and are shown in the Fig.31b.

4.16.2.2.3. QTLs for panicle dry weight per plot (PnDW/plot): In summer 2013, two QTLs (Q10PnDw/plot.a&b_13) mapped at 105.61 cM (Xtxp141) and 124.71 cM (S10_60938250) with 4.1% and 9.5% of phenotypic variance respectively were detected. During summer 2014, Q10PnDW/plot.a_14, Q10PnDw/plot.b_14 and Q10PnDW/plot.c_14 QTLs were detected at 20.91 cM (S10_50452521), 109.01 cM (S10_59833299), 117.31 cM (S10_59418734) with LOD 3, 3.3 and 2.1 respectively explaining 10.2, 2.7, 7.3% phenotypic variance. For across environment analysis, QTLs were not found. LOD graphs are shown in the Fig.31c.

4.16.2.2.4. QTLs for grain dry weight per plot (GDW/plot): Data analysis in summer 2013, revealed two QTLs at 105.61 cM (Xtxp141) and 120.11 cM (S10_60240796) at 2.4 and 2.1 LOD with 10.8% of combined phenotypic variance. During summer 2014, three QTLs were mapped at 82.21 cM (S10_56205739), 107.41 cM (S10_58831404), 117.31 cM (S10_59418734) at LOD 2.7, 3.7 and 2.1 explaining total phenotypic variance of 11%. Across season analysis, detected two QTLs Q10GDW/Plot_across (19.41cM) and Q10GDW/plot.b_across (107.21cM) at S10_47939440 and S10_58991881 with LOD 3.6, 2.8 having 18 and 2.4% phenotypic variance respectively. LOD graphs are shown in the Fig.31c.

4.16.2.2.5. QTLs for hundred grain mass (HGM): Four QTL for summer 2013 and three QTLs for summer 2014 were identified. Q10HGM.a_13 QTL was mapped at 0.01 cM (S10_48719070), Q10HGM.b_13 at 99.61 cM (S10_57432493), Q10HGM.c_13 at 108.61 cM (S10_58683017), Q10HGM.d_13 at 117 cM (S10_59418734) with LOD 3.1, 2.5, 2.2, 2.3 explaining 12.8% phenotypic variation and QTLs

Q10HGM.a_14,Q10HGM.b_14 and Q10HGM.c_14 were mapped at 20.11 (SNP S10_50235747), 95.01 cM (S10_57331300) and 126.31 cM (S10_60650722) with 3.3, 2 and 2.5 LOD explaining 11.74% combined phenotypic variance. For across season analysis also, two QTLs at 20.11 cM that overlaps with summer 2013 QTL (Q10HGM.d_13), and 117 cM that overlaps with summer 2014 QTL (Q10HGM.a_14) were identified at LOD 2.7 and 2.9 explaining 7.2% total phenotypic variance. LOD graphs are shown in the Fig.31d.

4.16.2.2.5. QTLs for grain number per plot (GNP/plot) and grain number per panicle (GNPP): Six QTLs (Q10GNP/plot.a_13, Q10GNP/plot.b_13, Q10GNP/plot.c_13, Q10GNP/plot.d_13, Q10GNP/plot.e_13, Q10GNP/plot.f_13) were mapped during summer 2013 for GNP/plot and GNPP at 31.01 cM (S10_51919897), 98.41 cM (S10_57552719), 106.7 cM (S10_58490384), 113.7 cM (S10_59419567), 129.8 cM (S10_60349808), 133.7 cM (S10_60354221) with LOD 2.5, 3.8, 32.7, 36.1, 34.1, 35 respectively explaining a total of 12% phenotypic variance. During summer 2014, Q10GNP/plot.a_14 QTL was detected at 77.61 cM (S10_56249651) with LOD 2.5 with 1.27% phenotypic variance. Across season analysis also, six QTLs were detected for GNP/plot of which 3 QTLs were common from summer 2013. Q10GNP/plot a, b, c, d, e, f were located at 103.1 (S10_58356424), 107.4 (S10_58831404), 113.7 (S10_59419567), 122.8 (S10_59419567), 129.8 cM (S10_60349808), 133.7 cM (S10_60354221) at LOD 4, 7, 17, 5, 17, 19 respectively, explaining 27% combined phenotypic variance. LOD graphs are shown in the Fig.31e.

4.16.2.2.6. QTLs for panicle harvest index (PHI): During summer 2013, three QTLs were identified at 113.71 cM (S10_59419567), 122.51 cM (S10_60324251), 125.71 cM (S10_60308140) with 3, 3.6, 2 LOD having 2, 12, 4.5% of phenotypic variance respectively. During summer 2014, two QTLs were identified with a major QTL (Q10PHI.a_14) explaining 15% PVE at 35.31 cM (S10_50140543) and the other QTL (Q10PHI.b_14) explaining 9% phenotypic

variance at LOD 3.4. A single QTL was observed for across season analysis at 18.51 cM (S10_51263932) with LOD 2.8 having 10.9% PVE (Fig .31f).

4.16.2.3. QTLs for shoot fly morphological traits (Table 19):

Integrated GBS SNP genotyping data along with SSR map were utilized for QTL mapping of shoot resistance (morphological traits) in both *kharif* and *rabi* seasons of 2013.

4.16.2.3.1. QTLsfor seedling leaf blade glossiness: During *kharif* 2013, two QTLs were detected at 36.41 cM (S10_54269620) and 42.41 cM (54507175; Xgap001) with LOD 4 and 4.5 respectively explaining 7.8% combined phenotypic variance. Single QTLs were detected during *rabi* 2013 for seedling leaf blade glossiness on SBI-10L at 36.41 with 4.6 LOD explaining 3.5% phenotypic variance. In case of across season analysis, a single consistent glossy QTL was observed at 36.41 cM at 3.7 LOD. LOD graphs are shown in the Fig.32a.

4.16.2.3.2. QTLs for trichome density upper (TDU): During*kharif* 2013, single QTL was detected at 107.81 cM (S10_58460662) at LOD 7.4 with 13% phenotypic variance indicating it as a major QTL. During *rabi* 2013, two QTLs were mapped at 97.31 cM (S10_57400347) and 106.71 cM (S10_58490384) with LOD 4.5 and 3.6 explaining 7% phenotypic variance for each QTL. For across seasons, four trichome upper density QTLs were detected at 34.71 (S10_50890593), 97.31 (S10_57400347), 99.61 (S10_57432493) and 109.51 cM (S10_58839857) at LOD 2.8, 7.5, 8.3, 6.1 having 5, 14, 14 and 12% phenotypic variance. LOD graphs are shown in the Fig.32b.

4.16.2.3.3. QTLs for trichome density lower (TDL): During *kharif* 2013, three different QTLs were detected at 90.51 (S10_57403166), 99.61 (S10_57432493) and 108.01 cM (S10_58357039) at LOD 5.6, 23.6, 16 explaining 6.5, 48.5 and 38.3% phenotypic variance respectively. During *rabi* 2013, one QTL was

observed at 99.61 cM (S10_57432493) at LOD 7 with 8.2% phenotypic variance. In case of across seasons, 4 different QTLs were identified at 96.11 cM (S10_57088032), 99.61 cM (S10_57432493), 103.61 cM (S10_58022779) and 108 cM (S10_58357039) at LOD 12, 15, 12, 10 explaining 19, 19, 22, 19 % R^2 values respectively. LOD graphs are shown in the Fig.32c.

4.16.2.3.4. QTLs for percent shoot fly dead heart (% SFDH): Single QTL was detected during *kharif* at 25.91 (S10_51071502) with LOD 3 having 7% phenotypic variance. Five different QTLs were detected during *rabi* 2013 at 82.71 cM (S10_56433597) 90.51 cM (S10_57403166), 99.61 cM (S10_57432493), 103.11 cM (S10_58356424) and 108.61 cM (S10_58683017) having LOD 3, 9, 12, 11, 9 explaining 0 (no value), 10, 12, 11, 12% phenotypic variance. Across season analysis, five QTLs were detected, of which 3 QTLs were common between *rabi* 2013 and two are new at 90.51 (S10_57403166), 99.61 (S10_57432493), 103.11 (S10_58356424), 105.61 (*Xtxp141*), and 109.51 cM (S10_58839857) at LOD 8, 8, 11, 10 and 8 explaining 6, 3, 10, 10 and 9.8 % phenotypic variance respectively (Fig .32d).

4.16.2.3.5. QTLs for seedling vigour (SV): During *kharif* 2013, SV QTL was not detected but in case of *rabi* 2013, two different QTLs were mapped at 56.5 (S10_55071264) and 60.6 cM (S10_55370553) at LOD 3 with combined phenotypic variance of 14%. During across season, for single seedling vigour, one QTL was detected on SBI-10L at 62.11 cM (S10_55387439) with LOD 2.5 explaining 7% phenotypic variance (Fig .32e).

4.16.2.3.6. QTLs for leaf sheath pigmentation (LSP): Two LSP QTLs were detected during *kharif*2013 at 63.61 (S10_55747507) and 108 cM (S10_58357039) with LOD 3.8 and 2.5 respectively. The total phenotypic variance explained was 9.8%. In case of *rabi* 2013, single QTL was detected at 40.41 cM (S10_52940776) at LOD 3 explaining 8% phenotypic variance. Across season analysis, three different QTLs were detected at 32.61 cM

(S10_52784725), 39.41 cM (S10_53544398) and 42.41 cM (*Xgap001*) at LOD 2.7, 3.2, 2.8 explaining 12, 11 and 14% phenotypic variance respectively (Fig .32f).

4.17. Fine mapping based on physical map positions of the mapped SSR and SNP markers

Fine mapping was carried out using GBS-SNPs and SSR marker genotyping data. F_2 Initial map distance was 37.71 cM interval for the target region (*Xgap001 – Xtxp141*), later increasing the marker interval (*Xisep0621–Xisep1011*) enhanced the map distance to 72 cM and after integrating the GBS markers, the distance has been found at 132 cM interval. The map distance increased due to selective recombinants heterozygosity and recombination frequency. Small variations were observed between SSR SNP genetic map and physical map position of SNPs but, they are nearly similar. In order to locate the variant SNPs associated with the trait of interest, the BLUP means for the individual environment and across environments were aligned along with the GBS - SNP data and SSR data, observed for marker genotype variations.

4.17.1. Fine mapping of stay-green traits: QTL cluster analysis and fine mapping

Many QTL were co-localised for stay-green QTL mapping studies. Out of 33 detected stay-green QTLs, maximum QTLs were co-localised and clustered also. Different % GL stay-green QTLs were identified for different scores but QTLs with common positions sharing common confidence intervals were integrated as QTL clusters. After looking at the QTL mapping results, most of the QTLs were co-localized and cluster of QTLs were observed. So, it was further analyzed and 19 stg QTLs were identified. These were co-localized into 7 QTL clusters based on the present results (Table 20). Cluster QTL cQstg10.1 having 3stg QTLs were mapped at the same position 54.26 (36.41cM) where AP2 transcription factor was associated with the identified location (Q10GL14a_14, Q10GL28a_14, Q1049a_across) (Fig .33).

The region cQstg10.2 contained 4 QTLs (Q10GL7a_13,Q10GL14a_13, Q10GL21b_13, Q10GL28b_14) of combined PVE and 31% located at 54.08 Mb (41.41 cM) position and uncharacterized protein was also associated (Fig .34). cQstg10.3 (Q10GL14b_14, Q10GL49b_across) contained 2 QTLs and were mapped at the position 54.58 Mb (44.41 cM) with a combined phenotypic variance of 10.12%. When the physical map positions were observed, the region was of (54585199 - 54593246Mb) 8 kb region encoding two ankyrin repeat protein (Sb10g025310) and WD40 repeat family protein (Sb10g025320) (Fig .35). cQstg10.4 (Q10GL7a_across, Q10GL14a_across) contained 2 QTLs located at the position 54.53 Mb (45.01 cM) with 16.95% of combined phenotypic variance encoding NBS-LRR protein (Fig .36). Region cQstg10.5 contained 3 QTLs (Q10GL21a_13, Q10GL21a_14,and Q10GL21a_across) at 59.34 Mb (115.31 cM) position with 28% of combined phenotypic variance and an uncharacterized protein was found associated in this region which was identified to be a late embryogenesis abundant (LEA 2) encoding protein homolog (Sb10g029570) of rice involved in drought stress tolerance (Fig .37). In cQstg10.6, 2 QTLs (Q10GL35a_13,Q10GL42a_13) were mapped at 121.91 cM in SNP 59.85 Mb, but, at SNP 59.77 Mb position, an intergenic region showed variation with phenotypic values and found to be associated with calcium/calmodulin protein kinase (Sb10g030040) (Fig .38). In cQstg10.7, 3 QTLs (Q10GL21c_13,Q10GL28a_13,Q10GL28a_across) were mapped at 60.19 Mb (125.01 cM) position and fell in the senescence associated protein encoding region (SAP) of 5.5% of combined phenotypic variance (Fig .39).

4.17.1.1. Fine mapping-haplotype analysis of QTL clusters with GBS SNPs and candidate gene identification

All the identified QTL clusters were arranged for haplotype analysis. GBS SNPs present around the peak position were examined for their variation and delimited with single gene for each QTL clusters (Fig.40). It was narrowed down to single SNPs for all the QTL clusters except cQstg10.4 which was reduced to 8 kb interval having two candidate genes (Sb0g025310-ankyrin repeat protein and

Sb10g025320-Transducin family protein/WD40, a synonymous coding region) showed variation when compared to their phenotypic values. In case of cQstg10.6, SNP variation was observed at S10_59775456 (120.6 cM) which encodes for calcium/calmodulin protein kinase (Sb10g030040) in other than the QTL mapped region. Altogether stay-green fine mapping is a complex process and from our data sets, we can interpret that transcriptional factors play major roles in drought stress as QTL clusters are near AP2 (Sb10g025053), WRKY (Sb10g025600) and NAC (Sb10g030770) transcriptional factors.

4.17.2. Agronomic traits fine mapping: Few of the important agronomic traits mapped in the target region were also fine mapped with the help of SSR SNP map and the two season's phenotyping data.

4.17.2.1. Fine mapping of flowering time: Nearly, 3 SNPs showed similar genotypic variation with the variation in phenotype. S10_58050693, S10_58069749, and S10_58079690 are the three SNPs which showed association with flowering time. 'A' allele showed early flowering when compared to 'B' and 'H' alleles (Fig .41).

4.17.2.2. Fine mapping of plant height: S10_59020363 SNP showed genotypic variation along with the phenotypic data and the J2614-11 parental allele 'B' showed dwarf phenotype when compared to 'A' RSG04008-6 allele and 'H' (Fig .42).

4.17.2.3. Fine mapping of hundred grains mass: SNP S10_59525199 showed variation with the phenotype 100 grain mass. Increased 'A' allele showed increased grain weight (Fig .43).

4.17.2.4. Fine mapping of grain number per plot: The grain number also increased with increased 'A' allele from RSG04008-6 parent.S10_59419567 SNP showed genotypic variation with variation in phenotype (Fig .44).

4.18. Shoot fly component traits fine mapping: Seedling leaf blade glossiness fine mapping: The QTL mapping itself is a clear fine mapping as single SNP was clearly identified in the QTL region. In case of manual observation, a region from 54223864 to 54507426 of 283 kb showed variation for glossiness trait. The SNP S10_54269620 showed variation for glossy and non-glossy phenotypes in maximum of the individuals but due to missing data points, we confined to 283 kb region. Allele 'B' of the SNP S10_54269620 showed glossy phenotype and vice versa as B alleles were from J2614-11, a shoot fly donor parent. Unfortunately, less number of polymorphic SNPs was detected in this region and the nearest SSR marker *Xgap001* was previously used as flaking marker for glossiness (Fig .45).

4.18.1. Trichome density lower fine mapping: Most important phenotype for antixenosis is the trichome density lower. Alignment of genotype and phenotype BLUPs showed variation in window between 57331385-57552719 of 221 kb region. A single SNP S10_57432493 was clearly identified for trichome density lower phenotypic variation showed much variation along with the genotype allele cals. Maximum individuals with 'B' allele showed higher number of lower trichome density but vice versa for 'A' allele cal (Fig .46).

4.18.2. Trichome density upper fine mapping: SNPs S10_57400347 and S10_57403166 recorded 2819 base pair difference, presence of 'B' alleles in both the SNPs with more trichome density. These SNPs are also fell in the trichome density lower window of 221 kb region and the nearest SSR marker is *Xtxp141* used as flanking marker for the trichome density for QTL identification (Fig .47).

4.19. GWAS for the mapped traits

In order to identify the marker trait associations (MTAs) between the detected SNPs and the trait of interest, BLUPs were calculated for each phenotype of 3 replicated field data of 152 genotypes. The R package GAPITwas used to perform association mapping and to find out significant MTAs of the stay-green

weekly scores, agronomic and yield related traits and shoot fly component traits represented in tables 20, 21 and 22 respectively.

4.19.1. GWAS for stay-green weekly scores

The MTAs in the target region on sorghum chromosome SBI-10 for the stay-green weekly observations with P value less than 10-3 were preferred and shown in Table 21 and Figure 48. During summer 2013, nearly 11 loci were found associated with % GL 7 with P value ranging from 2.1×10^{-3} - 8.7×10^{-3} and with r^2 value of 25-27% in the target region of SBI-10. In case of summer 2014, fourteen SNPs were discovered with % GL 7 and P values ranging from 1.1×10^{-4} to 8.3×10^{-3} and the r^2 value ranging from 14-17%. In case of across season data, 12 SNP were observed with % GL 7, and P-value ranging from 3.8×10-3 to 9.6 ×10-3 having r^2 values ranging from 14-15% (Fig .48a). During summer 2013, nearly 14 SNPs were found associated with % GL 14 with P values ranging from 9.6×10-4 to 6.3×10-3 having r^2 values ranging from 28-30%. In case of summer 2014, 10 SNPs were noticed having P values recorded in the range from 1.1×10-3 to 4.7×10-3 with r^2 value range from 21-23%. For across season, 11 SNP were found associated with P values ranging from 4×10-4 to 9.5×10-3 having 23-26% r^2 values (Fig .48b). During summer 2013, 4 SNPs were observed and the P values ranged from 4.8×10-3 to 3.4×10-3 and r^2 values ranged from 31-33%. In case of summer 2014, 12 loci were associated with % GL 21 with P values 1.1×10-3 to 6.6×10-3 and r^2 values ranging from 21-23%. In across season data, 5 SNPs were observed with % GL 21 and P values ranging from 5.6×10-3 to 7.7×10-3 with r^2 value of 26% (Fig .48c). During summer 2013, 4 SNPs were found associated with % GL 28 with P value ranging 4×10-3 to 8.8×10-3 and r^2 value of 30%. During summer 2014, 6 SNPs were recorded with % GL 28 having P values that ranged from 2.3×10-3 to 5.4×10-3 with r^2 value of 15-16%. For across season analysis, P values ranged from 3.6×10-3 to 9.9×10-3 with r^2 value of 22-23% (Fig .48d). During summer 2013, 7 loci were found associated with % GL 35 with P values having 2.4×10-3 to 8.3×10-3 and r^2 values that exhibited 23-24%. During summer 2014, 2 SNPs were noticed with P values

ranging from 3.1×10-3 to 9.9×10-3 with r^2 value of 20-22%. For across season analysis, 6 MTAs were found with P values ranging from 1.1×10-3 to 8.3×10-3 and r^2 values from 17-19% (Fig .48e). During summer 2013, 5 loci were found associated with the trait % GL 42 having P values 2×10-3 to 6×10-3 bearing r^2 19% values. In case of summer 2014, 2.9×10-4 to 9.5×10-3 with r^2 values ranging from 11-16%. For across season data, 9 MTAs were observed with P values ranging from 6.6×10-4 to 9.8×10-3 with r^2 values of 9-13% (Fig .48f). During summer 2013, 8 MTAs were recorded with P values ranging from 3.7×10-4 to 7.9×10-3 and with 27-30% r^2 values. In case of summer 2014, 10 MTAs were observed with P values 3.1×10-3 to 9.5×10-3 and r^2 values ranging 11-13%. For across season analysis, 12 MTAs were noticed having P values ranging from 3.3×10-3 to 9.8×10-3 and r^2 values 11-13% (Fig .48g).

4.19.2. GWAS for agronomic data

The MTAs in the target region on sorghum chromosome SBI-10 for the observed agronomic traits with P value less than 10-3 was preferred and shown in Table 22 (Fig .49).

4.19.2.1. GWAS for FT: During summer 2013, 4 MTA s were observed with P value 5×10-3 to 8.9×10-3 and with 37% r^2 value. In case of summer 2014, nearly 5 MTAs were noticed with values ranging from 2.8×10-3 to 7.2×10-3 and with 22% r^2 value. Across season analysis resulted in the identification of 16 MTAs having P value of 1×10^{-3} to 9.9×10^{-3} and with r^2 values 25-27% (Fig .49a).

4.19.2.2. GWAS for PlHt: During summer 2013, 12 MTAs were found associated having P values ranging from 1.6×10-3 to 7.4×10-3 with an average of 18% r^2 values. In summer 2014, GWAS analysis showed 5 MTAs having P value 1.9×10-3 to 8.4×10-3 with r^2 value ranging 28-30%. For across season analysis, 24 MTAs were observed having 1.6×10-4 to 9.7×10-3 P value range and with r^2 values of 19-23% (Fig .49b).

4.19.2.3. GWAS for PnDW/plot: During summer 2013, 2 SNPs having P values ranging from 5.9×10-4 to 1.1×10-3 with average r^2 value of 18% were found associated with PnDW/plot. Eight MTAs were found during summer 2014, with P values ranging from 3.1×10-3 to 7.4×10-3 and average r^2 value of 9%. Across season analysis identified 13 SNPs falling in the range of 4.1×10-3 to 9.7×10-3 with an average r^2 value of 10% (Fig .49c).

4.19.2.4. GWAS for GDW/plot: During summer 2013, 5 SNP were found associated with GDW/plot with P value ranging from 2.1×10-3 to 4.6×10-3 with average 9% r^2 values. In summer 2014, GWAS analysis identified 11 SNPs associated with GDW/plot having P value ranging from 4.9×10-3 to 7.4×10-3 with an average r^2 value of 7%. For across season analysis, 13 MTAs were observed having P value ranging from 4. ×10-3 to 9.7×10-3 with an average of 10% phenotypic variance (Fig .49d).

4.19.2.5. GWAS for HGM: During summer 2013, 34 MTAs were found associated with HGM having P values ranging from 2.6×10-5 to 9.9×10-3 with r^2 value ranging from 16-24%. GWAS analysis in summer 2014 resulted in the identification of 12 MTAs having 1.6×10-3 to 9.2×10-3 with r^2 values ranging from 8-10%. Across season analysis resulted in the identification of 82 SNPs associated with HGM having P value 4×10-4 to 9.4×10-3 and with r^2 value of 4-8% (Fig .49e).

4.19.2.6. GWAS for GNP/plot and GNPP: During summer 2013, 7 SNPs were found with P values ranging from 9.9×10-4 to 9.2×10-3 and with average r^2 value of 19%. In case of summer 2014, 2 SNPs were seen associated with similar P value of 8.3×10-3 having similar r^2 value of 5%. Across season analysis showed 5 MTAs with P value of 1×10-3 to 7.2×10-3 with an average r^2 value of 12% for both the traits as both the traits are interrelated (Fig .49f).

4.19.3. GWAS for shoot fly component traits

The MTAs identified in the target region of sorghum chromosome SBI-10 with P value above 10-3 were considered as significant marker associations with the trait. The results of important fine mapped component traits analysed for genome wide association studies are represented in Table 23 and Figure 50.

4.19.3.1. GWAS for seedling leaf blade glossiness: During *kharif*2013, 6 SNP were found associated with seedling leaf blade glossiness with P values ranging from 6×10-4 to 9.9×10-3 and with average r^2 value of 20%. In case of *rabi* 2013, 17 SNPs were found having P values that ranged from 6.3×10-4 to 9.2×10-3 with r^2 values of 22-25%. For across season analysis, 35 SNPs were found associated with P values ranging from 6.7×10-5 to 8.9×10-3 bearing r^2 values 19-25% (Fig .50a).

4.19.3.2. GWAS for seedling leaf blade trichome density lower: During *kharif* 2013, 73 significant SNPs were observed with P values ranging from 5.3×10-8 to 9.3×10-4 having r^2 values 34-45%. In case of *rabi,* 70 SNPs were noticed with TDL having P values that ranged from 9.7×10-7 to 9.7×10-4 bearing r^2 values 44-50%. Across season analysis, identified 8 highly significant SNPs with P values ranging from 3.6×10-10 to 9.4×10-5 and with r^2 value 42-54% (Fig .50b).

4.19.3.3. GWAS for seedling leaf blade trichome density upper: During *kharif* 2013, 55 SNPs were found associated with 1.3×10-4 to 9.7×10-3 P value range bearing r^2 values 23-28%. In case of *rabi* 2013, 75 MTAs were seen with P values ranging from 2.5×10-6 to 9.7×10-3 and with r^2 values 43-50%. Across season analysis resulted in the identification of 45 MTAs with P values having 2.6×10-7 to 9.3×10-5 and with r^2 values ranging from 38-42%. Several agronomic traits and stay-green traits did not show significant associations. Several stay-green traits and agronomic traits did not show significant associations, may be due to less variant genotypes evaluated for GWAS which affect the levels of significance (Fig .50c).

4.20. Candidate genes present in the target region: Nearly 200 unique putative candidate genes are present in the target region of 15 Mb which encodes different functions in different metabolic pathways. Due to increased SNP number, synonymous and non-synonymous SNPs were identified in the total SNPs. Putative candidate genes are present in the target region of which the polymorphic SNPs located within the candidate gene and SSRs close to candidate genes were selected. All the possible candidate genes in the target region are tabulated in Tables 24, 25 and 26.

4.20.1. Candidate genes for stay-green trait

Based on the QTL map intervals, stay green related probable candidate genes are listed in the Table 24. Nearly 20 genes were found in the % GL7 mapped region, out of which eight genes are putative uncharacterized proteins and others are SPB proteins (Sb10g029190), hAT dimerization proteins (Sb10g029230), MADS box transcriptional factors (Sb10g029810), mitogen activated protein kinases (Sb10g028270), translation initiation factors (Sb10g029245), meiotic serine threonine protein kinases (Sb10g028870), UDP glycosyl transferases (Sb10g028810), peroxidases (Sb10g028500), pentatricopeptide repeat proteins (Sb10g029530), putative receptor protein kinases (Sb10g030270) and aspartic proteinase nepnthesin 2 (Sb10g030330) which have been reported earlier in different drought stress tolerance studies. Mapped QTL intervals of QGL14 possessed 19 candidate genes of which (Sb10g024110) helix-loop-helix DNA-binding, (Sb10g030520, Sb10g030260) similar to senescence-associated protein, AGO1 (argounate1-Sb10g031030) and no apical meristem (NAM) proteins (Sb10g030770) were reported in different plants and during different studies under drought stress conditions. % GL21 DAF mapped QTL region contained 10 genes which are mostly the putative uncharacterized proteins, and others include mitogen activated proteins (MAP) (Sb10g028780), mate efflux family proteins (Sb10g029392), anthocyanin1 (Sb10g029660) and Zf-FLZ type plant specific zinc finger domains (Sb10g030090). In the % GL28 DAF QTL region, 16 genes

were identified; two of them being senescence associated proteins (Sb10g030260, Sb10g030520). APETALA2 (AP2-Sb10g025053) type and ankyrin repeat protein (Sb10g030330) was strongly up-regulated during senescence. In % GL35 DAF QTL region, 11 genes were senescence associated (Sb10g030260) and Argonaute proteins (Sb10g031030). QGL42 mapped region contained 25 genes of which mostly the putative uncharacterized proteins, senescence associated proteins (Sb10g030260,Sb10g030520), APETALA2 (Sb10g025053), Squamosa binding promoter domains (Sb10g026200) and NAM (Sb10g030770) which is a NAC transcription factor protein were noticed. QGL49 mapped region contained 10 genes; out of which 3 are putative uncharacterized proteins, helix-loop-helix (Sb10g024110), ankyrin repeat protein (Sb10g025310) and a NAM protein (Sb10g030770). These genes could be probable candidate genes involved in delaying the senescence.

4.20.2. Candidate genes for agronomic traits

Probable candidate genes for agronomic traits are listed in the Table 25.

4.20.2.1. Candidate genes for flowering time: On the basics of available annotated sorghum genome sequence version 2.4V, probable candidate genes for the mapped traits were predicted based on GBS-SNPs. Nearly 25 genes were located in the target region that included AP2 transcription factors (Sb10g025053), leucine zipper family proteins (Sb10g024190) and squamosa-promoter binding protein (SBP-Sb10g029190). These genes are supposed to be involved in flower development.

4.20.2.2. Candidate genes for plant height: QPlHt mapped region contained 18 candidate genes of which 6 are putative uncharacterized proteins. O-methyl transferase (Sb10g027340, Sb10g027640), meiotic serine proteinase (Sb10g028870), pentatricopeptide (PPR) repeat-containing protein (Sb10g029530) and Squamosa promoter-binding-like protein 12 (Sb10g029190) are perhaps the probable candidate genes.

4.20.2.3. PnDW/plot and GDW/plot: Panicle dry weight and grain dry weight QTLs were mapped in the same region having 6 candidate genes. Out of them, 3 are putative uncharacterized proteins, one is meiotic serine proteinase (Sb10g028870), and the third is a glycosyl family transferase protein (Sb10g028810).

4.20.2.4. HGM: Nearly 16 candidate genes are present of which 9 are putative uncharacterized proteins, one MATE efflux family protein (Sb10g029392), and two calcium dependant protein kinase proteins (Sb10g030040, Sb10g030150). S10_59525199 SNP near to MADS box transcription factor was closely linked to hundred grain mass.

4.20.2.5. GNP/plot and GNPP: Nearly 11 genes wereidentified of which 4 are putative uncharacterized proteins, 2 are predicted proteins, one is a mitogen activated protein kinase (Sb10g028780), and 3 senescence associated proteins (Sb10g030260) like aspartic protein nepenthesin-II and similar to chloroplast precursor (Sb10g030720).

4.20.3. Candidate genes for shoot fly resistance

The QTL mapped and fine mapped glossiness and trichome density QTL regions were searched for the candidate gene and the probable candidate genes are shown in the Table 26.

4.20.3.1. Candidate genes for glossiness: Glossines QTL (QGls) region has 15 candidate genes of which 4 are putative uncharacterized proteins, and others include signal transduction receptor-regulator domain (Sb10g024170), cal lipid binding domain (Sb10g025040) near to glossy QTL region, glossy15/AP2 transcription family protein (Sb10g025053), Myb type of transcription factor family protein (Sb10g024180), NBS-LRR disease resistance protein (Sb10g025283) and ankyrin repeat-containing protein (Sb10g025310).

4.20.3.2. Candidate genes for trichome density (upper and lower): Nearly 20 candidate genes have been identified in the QTDL,QTDU mapped region where 4 are putative uncharacterized proteins, and others include WRKY type of transcription factor (Sb10g025600), Specikle type POZ protein (Sb10g026730), MYB transcription factor (Sb10g027280), ethylene zinc finger protein (Sb10g027550), two F-box protein domains (Sb10g027730, Sb10g027760), O-methyl transferase (Sb10g027340, Sb10g027640), putative thaumatin-protein (Sb10g028130), meiotic serine protein (Sb10g028870) and Armidillo repeat protein (Sb10g027680).

4.21. Selection of final double recombinant plants: Based on the phenotyping data obtained in the present study, nearly 20 sorghum double recombinants with a delay in senescence response as well as shoot fly resistance traits with high agronomic performance are listed in the Table 27. These selected recombinants were further selfed until homozygosity for the desired traits and an introgression line with high yield having both stay-green trait and shoot fly resistance was obtained. Therefore, the pyramided traits can be utilized in breeding programs that are aimed at improving sorghum lines with better resistance characters.

TABLES

Table 2: Parents and grand parents variation and their genotype confirmation

Sample name	Xiabtp476	Xnhsbm1044	Xiabtp488	Xiabtp410	Xisep0643	Xnhsbm1013	Xiabtp466	Xisep0630	Xiabtp340	Xnhsbm1008	Xiabtp389	Xnhsbm1011	Xtxp141	Xgap001
Comment for SFR	Poly	poly	poly	poly	mono	poly	poly	poly	poly	poly	poly	poly	poly	poly
Is18551_B10PL1	176.08	254.03	138.47	132.62	233.04	167.01	224.03	196.49	140.37	210.01	201.6	188.79	175.9	273.31
BTX623_D12	175.06	196.05	140.75	140.82	233.39	163.38	235.99	197.47	147.08	214.3	203.75	163.71	175.15	271.46
E36_1_F12pl3	176.19	224.77	138.64	132.4	235.28	159.15	234.35	198.46	142.5	239.5	203.62	163.57	170.03	260.91
R16	175.96	276.25	132.51	132.65	232.87	169.26	219.43	196.22	140.56	205.78	203.76	192.27	154.7	282.72
Comment for STN	Poly	poly	poly	mono	poly	poly	poly	poly	poly	poly	mono	poly	poly	poly
Comment for fine-mapping	Mono	Poly	Mono	Mono	Poly	Poly	Poly	Poly	poly	poly	poly	poly	poly	Poly
J2614_1	176.2	253.97	138.63	132.51	233.24	166.59	224.43	196.32	140.34	209.21	201.64	190.62	176.14	273.62
J2614_10	176.54	254.95	138.63	132.51	233.12	167.46	224.32	196.27	140.53	210.61	201.47	190.62	176.09	273.27
J2614_11	**176.03**	**254.62**	**138.62**	**132.53**	**233.17**	**167.26**	**224.36**	**196.37**	**140.34**	**210.39**	**201.48**	**190.73**	**176.09**	**273.52**
J2614_12	176.55	255.47	138.62	132.56	233	167.26	224.41	196.23	140.34	210.29	201.29	190.66	176.24	273.7
J2614_2	176.32	254.94	138.63	132.56	233.35	166.72	224.42	196.45	140.53	209.9	201.65	191.13	176.2	273.62
J2614_3	177.01	254.44	138.63	132.56	233.16	166.87	224.31	196.27	140.34	209.86	201.66	192.87	176.14	273.62
J2614_5	176.77	254.2	138.62	132.45	233.41	165.97	224.22	196.43	140.34	208.2	201.47	191.13	176.2	273.88
J2614_6	176.54	254.82	138.62	132.66	233.36	166.66	224.33	196.41	140.5	209.38	201.47	190.48	176.14	273.71
J2614_7	176.72	253.85	138.63	132.49	233.6	166.26	224.32	196.49	140.53	209.02	201.83	190.37	176.93	273.73
J2614_8	176.6	254.74	138.63	132.41	233.6	165.49	224.52	196.49	140.31	207.18	201.64	189.91	176.31	273.92
J2614_9	175.73	254.32	138.63	132.52	233.17	167.32	224.42	196.47	140.34	210.66	201.63	190.38	176.2	273.59
Rsg04008	175.61	225.13	138.64	132.41	235.04	159.3	234.47	198.28	142.49	239.63	203.77	163.56	170.17	260.83
Rsg04008	175.44	224.68	138.46	132.38	235.02	159.48	234.59	198.28	142.49	239.59	203.61	163.39	170.31	260.81
Rsg04008_1	176.08	223.81	138.63	132.56	235.3	159.31	234.43	198.39	142.7	240.35	203.7	164.55	170.4	261.11
Rsg04008_10	176.73	224.43	138.44	132.5	235.33	159.12	234.49	198.4	142.57	239.49	203.65	163.78	170.08	260.97
Rsg04008_2	175.85	223.72	138.63	132.59	235.36	159.31	234.29	199.29	142.63	240.03	203.67	164.35	170.43	261.21
Rsg04008_3	176.03	223.64	138.63	132.39	235.54	159.32	234.37	198.41	142.62	240.2	203.84	163.61	170.35	261.33
Rsg04008_4	176.83	223.91	138.63	132.55	235.62	158.99	234.37	198.59	142.56	240.14	203.84	163.46	170.09	261.33
Rsg04008_5	175.73	223.91	138.63	132.48	235.22	158.99	235.81	198.42	142.49	240.16	203.64	163.6	170.32	261
Rsg04008_6	**175.78**	**224.88**	**138.63**	**132.49**	**235.33**	**159.83**	**235.57**	**198.42**	**142.5**	**239.75**	**203.84**	**164.33**	**170.17**	**261.01**
Rsg04008_7	176.03	224.6	138.63	132.68	235.26	159.65	235.57	198.41	142.7	239.52	203.66	164.47	170.13	260.95
Rsg04008_8	175.49	225.4	138.63	132.44	235.26	159.83	235.58	198.57	142.5	239.85	203.67	164.16	170.41	261.01
Rsg04008_9	175.85	224.62	138.63	132.65	235.33	159.49	234.37	198.4	142.5	239.72	203.64	164.13	170.09	260.96

Table 3: F_1 Progeny confirmation

Sample	pedigree	Xgap001	Xgap001	Xnhsbm1008	Xnhsbm1008	Xnhsbm1011	Xnhsbm1011	Xisep0643	Xisep0643	Xiabtp389	Xiabtp389	Xnhsbm1044	Xnhsbm1044	Xiabtp340	Xiabtp340	Xtxp141	Xtxp141	Xiabtp466	Xiabtp466	percentage heterozygocity
BTx623	BTx623	260	260	212	212	163	163	222	222	193	193	195	195	146	146	182	182	234	234	0.00
E36-1	E36-1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.00
IS18551	IS18551	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	0.00
U110049	J2614_11	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	0.00
U110054	RSG04008-6_5	A	A	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	0.00
U110055	RSG04008-6 x J 2614-11	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	100.00
U110056	RSG04008-6 x J 2614-11	NA	NA	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	88.00
U110057	RSG04008-6 x J 2614-11	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	100.00
U110058	RSG04008-6 x J 2614-11	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	100.00
U110059	RSG04008-6 x J 2614-11	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	100.00
U110060	RSG04008-6 x J 2614-11	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	88.00
U110061	RSG04008-6 x J 2614-11	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	100.00
U110062	RSG04008-6 x J 2614-11	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	100.00
U110063	RSG04008-6 x J 2614-11	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	0.00
U110064	RSG04008-6 x J 2614-11	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	100.00
U110065	RSG04008-6 x J 2614-11	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	100.00
U110066	RSG04008-6 x J 2614-11	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	100.00

Table 4: Descriptive statistics and correlations of seedling leaf blade glossiness score and trichome density score in the full F_2 population and F_3 progenies derived from 369 selected informative recombinant F_2 individuals

S .NO.	Trait	P1 (RSG)	P2 (J2614)	Min	Max	Mean ± SE	CV (%)	Correlations *Gls*	*Td*
1	*F_2Gls*	5	1	0.99	4.96	1.94 ± 0.09	13.66	1	
2	*F_2Td*	2	4	0	5.08	2.66 ± 0.11	11.89	-0.0097*	1
3	*F_3Gls*	5	1	1.02	4.94	2.10 ± 0.24	11.91	1	
4	*F_3Td*	2	5	0.27	4.9	3.63 ± 0.25	11.68	-0.0065*	1

* Correlation significant at $P<0.05$, F_2 =1,894 individuals, F_3=selfed progeny of 369 selected informative recombinant F_2 individuals. CV= coefficient of varience, *Gls* Glossiness,*Td* Trichome density

Table 5 : Mean values of parents, F2:4 progeny, and their range for individual seasons and across seasons for stay-green traits derived from cross RSG04008-6 × J2614-11.

		Post rainy 2012-2013				Post rainy 2013 - 2014				Across season			
		Mean			Range	Mean			Range	Mean			Range
S. No.	Trait	RSG04008-6	F_4 progeny	J2614-11	F_4 progeny	RSG04008-6	F_4 progeny	J2614-11	F_4 progeny	RSG04008-6	F_4 progeny	J2614-11	F_4 progeny
1	%GLA 7 DAF	88.38	88.67	83.68	72.33 - 97.78	99.03	96.05	95.24	79.14 - 99.26	93.86	92.36	89.56	76.31 - 98.65
2	%GLA 14 DAF	79.18	79.58	72.06	58.73 - 96.64	84.22	84.29	85.26	68.60 - 96.93	81.71	81.94	78.65	63.34 - 96.69
3	%GLA 21 DAF	62.52	65.96	61.88	43.29 - 93.77	74.39	73.73	75.05	63.34 - 84.91	68.43	69.85	68.48	54.12 - 90.37
4	%GLA 28 DAF	47.00	50.72	47.68	29.92 - 78.11	66.49	65.03	64.04	51.36 - 77.49	56.83	57.88	55.78	42.44 - 77.36
5	%GLA 35 DAF	34.46	39.49	37.72	22.90 - 62.25	54.17	51.57	50.54	19.89 - 66.05	44.39	45.53	44.19	19.74 - 61.07
6	%GLA 42 DAF	23.44	29.41	30.73	17.19 - 45.88	25.96	39.73	40.1	6.09 - 53.89	25.28	34.71	35.25	13.73 - 48.77
7	%GLA 49 DAF	17.12	18.56	20.33	9.90 - 31.90	11.68	29.07	28.4	4.73 - 44.61	14.67	24.01	24.94	8.72 - 38.12

Table 6: Mean values of parents, F4 progeny, and their range for individual seasons and across seasons for Agronomic traits derived from cross RSG04008-6 × J2614-11.

		Post rainy 2012-2013				Post rainy 2013 - 2014				Across season			
		Mean			Range	Mean			Range	Mean			Range
S. No.	Trait	RSG04008-6	F_4 progeny	J2614-11	F_4 progeny	RSG04008-6	F_4 progeny	J2614-11	F_4 progeny	RSG04008-6	F_4 progeny	J2614-11	F_4 progeny
1	FT	61.33	68.14	76.83	57.97 - 79.94	86.95	82.91	82.30	75.33 - 90.29	75.00	75.52	79.21	67.11 - 83.95
2	PlHt	131.89	116.67	89.07	84.78 - 147.09	193.96	175.14	120.60	105.19 - 225.34	163.99	145.93	103.20	92.65 - 181.09
3	ETNP	0.97	0.96	0.92	0.90 - 1.00	1.55	1.47	1.40	1.34 - 1.79	1.27	1.22	1.14	1.14 - 1.38
4	PnDW/plot	598.1	577.49	372.53	416.17 - 779.52	945.95	935.88	1016.03	763.31 - 1102.78	768.65	756.15	738.23	668.70 - 887.70
5	GDW/plot	392.72	397.96	233.42	267.61 - 539.88	632.53	650.60	732.21	531.50 - 777.47	513.47	523.70	518.19	461.19 - 599.05
6	PHI	66.9	68.4	62.88	63.08 - 78.21	68.63	70.14	71.69	66.35 - 74.21	67.48	69.27	67.72	65.00 - 73.43
7	HGM	2.14	1.94	2.06	1.28 - 2.44	3.28	2.95	2.71	2.27 - 3.73	2.70	2.45	2.38	1.79 - 3.07
8	GNP/plot	20456.12	21232.45	17807.9	18453.82-43294.30	20212.25	22191.42	26891.54	17204.13 - 27250.23	20746.04	21720.92	21254.12	19611.06 - 33652.47
9	GNPP	786.78	816.64	684.92	709.77-1665.17	777.40	853.52	1034.29	661.7 - 1048.09	797.92	835.42	817.47	754.27 - 1294.33

Table 7: Mean values of parents, F4 progeny, and their range for individual seasons and across seasons for shoot fly component traits derived from cross RSG04008-6 × J2614-11.

		Rainy 2013				Post-rainy 2013				Across season			
		Mean			Range	Mean			Range	Mean			Range
S. No.	Trait	RSG04008-6	F_4 progeny	J2614-11	F_4 progeny	RSG04008-6	F_4 progeny	J2614-11	F_4 progeny	RSG04008-6	F_4 progeny	J2614-11	F_4 progeny
1	Gls	3.48	3.04	2.66	1.82 - 4.41	4.62	3.18	2.61	1.87 - 4.78	4.09	3.11	2.59	1.87 - 4.77
2	Tdu	28.04	64.13	93.3	9.69 - 127.57	9.97	58.1	91.83	8.59 - 122.00	17.64	61.11	93.17	8.13 - 120.29
3	Tdl	2	14.37	57.95	0.11 -58.93	5.32	28.5	46.9	1.80 - 68.45	2.56	21.45	53.62	1.43 - 61.77
4	SV	2.56	2.44	2.65	1.65 - 2.77	1.1	1.3	1.88	1.10 - 1.77	1.29	1.87	2.29	1.30 - 2.30
5	LSP	1.09	1.86	2.58	1.08 - 2.89	1.54	2.05	2.91	1.06 - 2.97	1.84	1.96	2.79	1.04 - 2.95
6	%SFDH	97.17	94.21	91.69	85.13 - 97.89	62.29	38.79	22.53	15.33 - 60.62	78.32	66.49	58.16	52.42 - 76.24

Table 8: Genotype variance, genotype X environment, standard error, and heritability estimates (on mean basis) for stay-green scores derived from cross RSG04008-6 × J2614-11

STG ANOVA tables

		Post-rainy 2012-2013			Post-rainy 2013 – 2014			Across season			
S. No.	Trait	σ^2g	SE±	h2	σ^2g	SE±	h2	σ^2g	Gx E	SE±	h2
1	%GLA 7 DAF	64.34	7.941	75.38	26.42	5.916	69.37	73.44	100.99 **	7.00	81.80
2	%GLA 14 DAF	183.30	13.8	74.27	42.63	7.644	68.64	181.90	256.5 **	11.16	81.42
3	%GLA 21 DAF	198.33	13.85	75.63	17.13	6.538	54.59	160.13	283.3 **	10.83	80.39
4	%GLA 28 DAF	121.70	11.13	74.65	30.21	7.308	62.93	120.54	182.82 **	9.42	80.31
5	%GLA 35 DAF	63.26	8.775	71.14	62.33	9.484	67.52	98.61	164.42 **	9.14	77.99
6	%GLA 42 DAF	29.74	8.04	57.98	97.99	8.385	80.70	80.03	210.67 **	8.21	78.08
7	%GLA 49 DAF	28.20	8.719	52.67	104.33	10.13	75.31	79.26	249.26 **	9.44	72.73

Table 9 : Genotype variance, genotype × environment, standard error, and heritability estimates (on mean basis) for agronomic traits derived from the cross RSG04008-6 × J2614-11

		Post-rainy 2012-2013			Post-rainy 2013 – 2014			Across season			
S. No.	Trait	σ^2g	SE±	h^2	σ^2g	SE±	h^2	σ^2g	Gx E	SE±	h^2
1	ETNP	**0.00**	0.18	3.6072144 -	**0.01**	0.35	23.72	**0.01**	0.09068 *	0.28	22.12444
2	FT	**26.86**	2.55	92.536662	**10.85**	2.73	81.31	**26.77**	26.653 **	2.65	91.94385
3	PlHt	**170.87**	15.08	69.260911	**550.07**	14.38	88.86	**634.07**	431.5 **	14.79	89.69257
4	PHI	**9.48**	7.61	32.954809	**1.73**	8.02	7.45	**8.75**	69.66 **	7.65	30.98808
5	PnDW/plot	**4307.00**	4562.00	57.941704	**8033.33**	208.50	35.66	**7717.67**	40344 **	162.40	46.73597
6	HGM	**0.04**	0.12	87.951618	**0.07**	0.26	75.91	**0.08**	0.10271 **	0.19	86.91109
7	GNP/plot	**12233333**	12822	18.2496	**67,100,00**	5443.00	40.45	**12,460,000**	11,640,0000 ns	9850	27.81
8	GDW/plot	**3341.67**	88.51	56.131019	**3906.67**	139.50	37.59	**3610.33**	21672 **	113.40	45.69849
9	GNPP	**18072.00**	493.20	18.2284	**9924.00**	209.40	40.45	**18417.00**	172259 ns	378.80	27.79617

Table 10: Genotype varience,GX E interactions,respective Standard Error, and Heritability estimates (on mean basis) for component traits of shoot fly resistance derived from cross RSG04008-6 × J2614-11

		Rainy 2013				Post-rainy 2013				Across season		MSS		
S. No.	Trait	σ^2g	Genotype MSS	SE±	h2	σ^2g	Genotype	SE±	h2	σ^2g	Genotype	Gx E	SE±	h2
1	%SFDH	24.13	95.14 **	4.771	76.08	174.73	692.5 **	12.97	75.70	61.67	486.32 **	95.53 **	9.77	38.04
2	GS	0.50	2.24 **	0.8668	66.46	0.50	1.7797 **	0.52	84.96	0.96	3.4569 **	0.5095 ns	0.71	83.72
3	LSP	0.54	1.8835 **	0.4994	86.76	0.49	1.6666 **	0.43	88.85	0.96	3.2202 **	0.2176 **	0.47	89.76
4	SV	0.11	0.659 **	0.5735	50.09	0.49	1.6666 **	0.43	88.85	0.10	0.651 **	0.2336 *	0.48	48.03
5	TDL	203.46	699.7 **	9.451	87.24	278.00	1031.8 **	14.06	80.83	397.83	1462.5 **	143.6 **	11.98	81.61
6	TDU	985.33	3977 **	31.96	74.33	725.00	2601.6 **	20.65	83.60	1433.60	5439.9 **	724 **	26.91	79.06

* significant at 0.05 ** significant at 0.001 ^{ns} non significant non significant Rainy-*kharif* Post-rainy-*rabi*

Table 11: Correlation between stay-green weekly scores with agronomic traits and yield related traits for summer 2013, summer 2014, across season data.

Summer 2013

	FT_13	PlHt_13	ETNP_13	PnDW/plot_13	GDW/plot_13	PHI_13	HGM_13	GNP/plot_13	GNPP_13	%GL7_13	%GL14_13	%GL21_13	%GL28_13	%GL35_13	%GL42_13	%GL49_13
FT_13	1	0.03	-0.36*	-0.05	0	0.11	0.19*	-0.02	-0.02	-0.83*	-0.87*	-0.87*	-0.81*	-0.71*	-0.56*	-0.73*
PlHt_13	0.03	1	0.1	0.3*	0.32*	0.19*	0.35*	0.1	0.1	-0.07	-0.07	-0.1	-0.09	-0.1	-0.07	-0.05
ETNP_13	-0.36*	0.1	1	0	-0.03	-0.06	-0.1	-0.02	-0.02	0.24*	0.28*	0.26*	0.23*	0.18*	0.06	0.13
PnDW/plot_13	-0.05	0.3*	0	1	0.94*	0.27*	0.25*	0.38*	0.38*	0.01	-0.01	-0.05	-0.06	-0.07	-0.07	-0.04
GDW/plot_13	0	0.32*	-0.03	0.94*	1	0.58*	0.18*	0.45*	0.45*	-0.02	-0.04	-0.09	-0.09	-0.1	-0.1	-0.05
PHI_13	0.11	0.19*	-0.06	0.27*	0.58*	1	-0.12	0.37*	0.37*	-0.08	-0.09	-0.14	-0.14	-0.14	-0.14	-0.06
HGM_13	0.19*	0.35*	-0.1	0.25*	0.18*	-0.12	1	-0.26*	-0.26*	-0.15	-0.17*	-0.16*	-0.1	-0.06	0.06	0
GNP/plot_13	-0.02	0.1	-0.02	0.38*	0.45*	0.37*	-0.26*	1	1*	-0.1	-0.07	-0.1	-0.13	-0.14	-0.21*	-0.1
GNPP_13	-0.02	0.1	-0.02	0.38*	0.45*	0.37*	-0.26*	1	1	-0.1	-0.07	-0.1	-0.13	-0.14	-0.21*	-0.1
%GL7_13	-0.83*	-0.07	0.24*	0.01	-0.02	-0.08	-0.15	-0.1	-0.1	1	0.98*	0.92*	0.89*	0.82*	0.69*	0.81*
%GL14_13	-0.87*	-0.07	0.28*	-0.01	-0.04	-0.09	-0.17*	-0.07	-0.07	0.98*	1	0.93*	0.9*	0.81*	0.67*	0.83*
%GL21_13	-0.87*	-0.1	0.26*	-0.05	-0.09	-0.14	-0.16*	-0.1	-0.1	0.92*	0.93*	1	0.98*	0.93*	0.8*	0.85*
%GL28_13	-0.81*	-0.09	0.23*	-0.06	-0.09	-0.14	-0.1	-0.13	-0.13	0.89*	0.9*	0.98*	1	0.97*	0.87*	0.87*
%GL35_13	-0.71*	-0.1	0.18*	-0.07	-0.1	-0.14	-0.06	-0.14	-0.14	0.82*	0.81*	0.93*	0.97*	1	0.94*	0.87*
%GL42_13	-0.56*	-0.07	0.06	-0.07	-0.1	-0.14	0.06	-0.21*	-0.21*	0.69*	0.67*	0.8*	0.87*	0.94*	1	0.85*
%GL49_13	-0.73*	-0.05	0.13	-0.04	-0.05	-0.06	0	-0.1	-0.1	0.81*	0.83*	0.85*	0.87*	0.87*	0.85*	1

Table 11 (Contd..)

Summer 2014

	FT_14	PlHt_14	ETNP_14	PnDW/plot_14	GDW/plot_14	PHI_14	HGM_14	GNP/plot_14	GNPP_14	%GL7_14	%GL14_14	%GL21_14	%GL28_14	%GL35_14	%GL42_14	%GL49_14
FT_14	1*	0.1	0.06	0.02	0	-0.1*	0.05	0	0	-0.6*	-0.9*	-0.7*	-0.6*	-0.7*	-0.5*	-0.6*
PlHt_14	0.1*	1	-0.2	0.15	0.2	0.06*	0.39	-0.1	-0.1	-0.2*	-0.2*	-0.3*	-0.3*	-0.1*	-0*	0*
ETNP_14	0.06*	-0.2	1	-0.2	-0.2	0.05*	-0.1	-0.1	-0.1	0.08*	0.1*	0.27*	0.29*	0*	0.03*	-0*
PnDW/plot_14	0.02*	0.15	-0.2	1	0.87	-0.3*	0.18	0.6	0.6	0.07*	0.02*	-0*	-0*	0.03*	0.06*	0.04*
GDW/plot_14	-0*	0.2	-0.2	0.87	1	0.18*	0.09	0.77	0.77	0.09*	0.04*	0*	0*	0.05*	0.09*	0.08*
PHI_14	-0.1*	0.06	0.05	-0.3	0.18	1*	-0.2	0.3	0.3	0*	0.05*	0.02*	0.04*	0.05*	0.05*	0.06*
HGM_14	0.05*	0.39	-0.1	0.18	0.09	-0.2*	1	-0.5	-0.5	-0.1*	-0*	-0.1*	-0.1*	0*	0.02*	-0.1*
GNP/plot_14	-0*	-0.1	-0.1	0.6	0.77	0.3*	-0.5	1	1	0.1*	0.02*	0.03*	0.06*	0.03*	0.05*	0.05*
GNPP_14	-0*	-0.1	-0.1	0.6	0.77	0.3*	-0.5	1	1	0.1*	0.02*	0.03*	0.06*	0.03*	0.05*	0.05*
%GL7_14	-0.6*	-0.2	0.08	0.07	0.09	0*	-0.1	0.1	0.1	1*	0.68*	0.64*	0.71*	0.66*	0.52*	0.66*
%GL14_14	-0.9*	-0.2	0.1	0.02	0.04	0.05*	0	0.02	0.02	0.68*	1*	0.87*	0.82*	0.81*	0.61*	0.62*
%GL21_14	-0.7*	-0.3	0.27	0	0	0.02*	-0.1	0.03	0.03	0.64*	0.87*	1*	0.88*	0.76*	0.58*	0.55*
%GL28_14	-0.6*	-0.3	0.29	0	0	0.04*	-0.1	0.06	0.06	0.71*	0.82*	0.88*	1*	0.74*	0.55*	0.6*
%GL35_14	-0.7*	-0.1	0	0.03	0.05	0.05*	0	0.03	0.03	0.66*	0.81*	0.76*	0.74*	1*	0.79*	0.71*
%GL42_14	-0.5*	0	0.03	0.06	0.09	0.05*	0.02	0.05	0.05	0.52*	0.61*	0.58*	0.55*	0.79*	1*	0.82*
%GL49_14	-0.6*	0	0	0.04	0.08	0.06*	-0.1	0.05	0.05	0.66*	0.62*	0.55*	0.6*	0.71*	0.82*	1*

Across season data

Table 11 (Contd..)

	FT	PlHt	ETNP	PnDW/ plot	GDW/ plot	PHI	HGM	GNP /plot	GNP P	GLA1 3_W1	GLA13_ W2	GLA13 _W3	GLA1 3_W4	GLA13 _W5	GLA1 3_W6	GLA13 _W7
FT	1	0.16*	-0.19*	0.01	0.04	-0.08	0.01	-0.01	-0.01	-0.85*	-0.91*	-0.87*	-0.83*	-0.77*	-0.6*	-0.72**
PlHt	0.16*	1	-0.13	0.31*	0.16*	0.18*	0.24*	0.04	0.04	-0.25*	-0.25*	-0.32*	-0.29*	-0.2*	-0.11	-0.06
ETNP	-0.19*	-0.13	1	-0.22*	-0.08	-0.14	-0.18*	0.08	0.08	0.16	0.18*	0.25*	0.27*	0.14	0.09	0.06
PnDW/plot	0.01	0.31*	-0.22*	1	0.29*	0.54*	0.88*	0.5*	0.5*	0	-0.07	-0.12	-0.11	-0.11	-0.07	0.03
GDW/plot	0.04	0.16*	-0.08	0.29*	1	0.04	-0.17*	0.35*	0.35*	-0.01	-0.05	-0.08	-0.06	-0.02	0.02	0.07
PHI	-0.08	0.18	-0.14	0.54*	0.04	1	0.53*	0.29*	0.29*	0.03	-0.02	-0.12	-0.14	-0.1	-0.11	0.04
HGM	0.01	0.24*	-0.18*	0.88*	-0.17*	0.53*	1	0.35*	0.35*	0	-0.06	-0.1	-0.1	-0.11	-0.09	-0.02
GNP/plot	-0.01	0.04	0.08	0.5*	0.35*	0.29*	0.35*	1	1*	-0.02	-0.08	-0.1	-0.09	-0.1	-0.1	0.02
GNPP	-0.01	0.04	0.08	0.5*	0.35*	0.29*	0.35*	1*	1	-0.02	-0.08	-0.1	-0.09	-0.1	-0.1	0.02
GLA13_W1	-0.85*	-0.25*	0.16	0	-0.01	0.03	0	-0.02	-0.02	1	0.93*	0.87*	0.86*	0.79*	0.63*	0.77*
GLA13_W2	-0.91*	-0.25*	0.18*	-0.07	-0.05	-0.02	-0.06	-0.08	-0.08	0.93*	1	0.94*	0.91*	0.85*	0.67*	0.75*
GLA13_W3	-0.87*	-0.32*	0.25*	-0.12	-0.08	-0.12	-0.1	-0.1	-0.1	0.87*	0.94*	1	0.98*	0.9*	0.72*	0.73*
GLA13_W4	-0.83*	-0.29*	0.27*	-0.11	-0.06	-0.14	-0.1	-0.09	-0.09	0.86*	0.91*	0.98*	1	0.91*	0.75*	0.76*
GLA13_W5	-0.77*	-0.2*	0.14	-0.11	-0.02	-0.1	-0.11	-0.1	-0.1	0.79*	0.8*5	0.9*	0.91*	1	0.86*	0.8*
GLA13_W6	-0.6*	-0.11	0.09	-0.07	0.02	-0.11	-0.09	-0.1	-0.1	0.63*	0.67*	0.72*	0.75*	0.86*	1	0.84*
GLA13_W7	-0.72*	-0.06	0.06	0.03	0.07	0.04	-0.02	0.02	0.02	0.77*	0.75*	0.73*	0.76*	0.8*	0.84*	1

Table12: Shoot fly resistance component traits correlation for*kharif* (rainy) 2013,*rabi*(Post-rainy) 2013, across seasons.

SFR_K13

	Gls_K13	LSP_K13	SV_K13	Tdu_K13	Tdl_K13	% SFDH_K1
Glossy_K13	**1**	**0.16***	**0.07**	**-0.08**	**-0.02**	**0.03**
Leaf sheath pigmentation_K13	0.16*	1	0.11	0.01	-0.01	-0.06
PLANT VIGOUR_K13	0.07	0.11	1	-0.01	-0.04	-0.33*
TRICHOME UP_K13	-0.08	0.01	-0.01	1	0.67*	-0.07
TRICHOME LOW_K13	-0.02	-0.01	-0.04	0.67*	1	-0.09
%SFDH_K13	0.03	-0.06	-0.33*	-0.07	-0.09	1

SFR_R13

	Gls_R13	LSP_R13	SV_R13	Tdu_R13	Tdl_R13	% SFDH_R1
Glossy_R13	1	0.12	-0.06	-0.25*	-0.17*	0.16
Leaf sheath pigmentation_R13	0.12	1	0	-0.06	-0.01	0.09
Seedling Vigour_R13	-0.06	0	1	0.18	0.11	-0.04
TRICHOME UP_R13	-0.25*	-0.06	0.18*	1	0.88*	-0.66*
TRICHOME LOW_R13	-0.17*	-0.01	0.11	0.88*	1	-0.72*
%SFDH_R13	0.16	0.09	-0.04	-0.66*	-0.72*	1

Across season data

	Gls	LSP	SV	Tdu	Tdl	%SFDH
Glossy score (Gls)	1	0.17*	0.01	-0.16*	-0.11	0.16
Leaf sheath pigmentation (LSP)	0.17*	1	0.08	-0.03	-0.02	0.05
Seedling vigor (SV)	0.01	0.08	1	0.1	0.04	0.01
Trichome density up (Tdu)	-0.16*	-0.03	0.1	1	0.85*	-0.66*
Trichome density low (Tdl)	-0.11	-0.02	0.04	0.85*	1	-0.77*
Percent shoot fly dead heart (% SFDH)	0.16	0.05	0.01	-0.66*	-0.77*	1

Table 13:Linkage map with marker distances and the segregation distortion of 262 SNP-SSR markers on 152 F_2 recombinant progeny and their chi-square values and significance

			Alleles %				
S.No.	Locus	Position (cM)	RSG04008-6	Hetero zygote	J2614-11	$\chi 2$	Significance
1	S10_48719070	0	19	24	75	94.7	********
2	S10_45832539	0.575	31	19	81	104.2	********
3	S10_48255426	1.374	28	17	59	65.6	********
4	S10_45924600	2.047	32	14	46	48.8	********
5	S10_49764198	2.691	24	7	37	47.9	********
6	S10_48780999	4.118	40	30	41	23.4	********
7	S10_48781003	4.815	40	30	39	22.1	********
8	S10_48098678	5.77	23	6	43	61.1	********
9	S10_45991208	6.247	27	8	59	86.5	********
10	S10_48098791	7.457	32	18	76	95	********
11	S10_50809263	8.036	15	5	52	91.4	********
12	S10_48087353	8.206	22	23	61	62.7	********
13	S10_51699119	9.373	24	13	57	72.4	********
14	S10_48098807	10.331	32	24	69	69.3	********
15	S10_48098802	10.366	32	23	70	73	********
16	S10_49085931	11.638	27	19	72	88.6	********
17	S10_51065106	12.358	31	29	73	68.8	********
18	S10_49095753	12.877	26	22	61	61.2	********
19	S10_50809289	13.419	17	13	79	133.7	********
20	S10_45235333	13.877	17	5	47	76.5	********
21	S10_46553315	14.172	18	6	50	79.6	********
22	S10_45695389	14.434	21	8	48	67.3	********
23	S10_51934952	15.06	15	18	75	114.7	********
24	S10_48076362	15.53	20	40	86	89.5	********
25	S10_47137453	16.084	23	22	77	97.7	********
26	S10_50809328	16.653	10	2	58	128.1	********
27	S10_48076342	16.881	22	16	85	131.9	********
28	S10_48014895	17.657	18	57	76	53.6	********
29	S10_51263932	18.488	23	24	65	68.1	********
30	S10_47939440	19.403	28	35	76	67.4	********
31	S10_50235747	20.076	27	23	66	68.5	********
32	S10_50452521	20.887	23	18	75	101.8	********
33	S10_50093128	21.352	25	13	72	104.3	********

Table 13: (Contd..)

			Alleles %				
S.No.	Locus	Position (cM)	RSG04008-6	Heterozygote	J2614-11	$\chi 2$	Significance
34	S10_50093129	21.44	25	14	71	99.6	*******
35	S10_49632608	22.034	27	19	70	84.3	*******
36	S10_50734011	22.76	21	19	64	77.4	*******
37	IS10263_49672890	23.029	21	51	79	60.5	*******
38	S10_48938062	23.962	24	26	76	86.4	*******
39	S10_49288320	24.664	23	9	51	69.8	*******
40	S10_48069066	24.963	28	14	64	81.8	*******
41	S10_51071502	25.88	30	33	65	49.2	*******
42	S10_51228412	26.918	28	17	62	71.4	*******
43	S10_52042465	27.524	24	18	62	72.2	*******
44	S10_52676228	27.785	32	19	69	78.8	*******
45	S10_51224387	28.41	26	20	83	111.8	*******
46	S10_52036901	29.023	24	8	78	133.3	*******
47	S10_52677221	29.365	15	3	51	95.1	*******
48	S10_52676281	29.715	23	4	48	76.5	*******
49	S10_53262363	30.175	33	9	61	85.4	*******
50	S10_50972176	30.462	36	12	67	88.7	*******
51	S10_51919897	30.959	30	10	57	76.2	*******
52	S10_52853071	31.827	37	20	75	86	*******
53	S10_52781712	32.291	23	20	84	118.2	*******
54	S10_52784725	32.558	22	43	84	78.2	*******
55	S10_46230564	33.08	23	16	63	79.4	*******
56	S10_50121434	33.837	26	19	70	85.2	*******
57	S10_54859431	33.902	7	1	74	187.5	*******
58	S10_54859409	33.948	7	1	75	190.5	*******
59	S10_50890593	34.686	21	2	45	77.2	*******
60	S10_53682073	34.972	29	19	65	72.7	*******
61	S10_50140543	35.288	9	6	56	111.3	*******
62	S10_53160198	35.678	16	17	102	185.1	*******
63	S10_53834366	35.758	20	3	43	70.6	*******
64	S10_52675727	35.997	18	3	48	83.6	*******
65	S10_53058516	36.145	26	5	42	61.4	*******
66	S10_54269620	36.401	15	11	90	173.2	*******
67	S10_45646835	36.843	21	4	44	69.3	*******
68	S10_52673863	37.229	32	22	66	67.4	*******
69	S10_49366136	37.73	15	6	53	91	*******
70	S10_53714284	38.079	22	5	48	74.4	*******
71	S10_54185069	38.342	20	7	49	72.7	*******
72	S10_52812930	38.346	14	2	49	94.9	*******
73	S10_53855394	38.66	28	33	84	86.3	*******

Table 13: (Contd..)

			Alleles %				
S.No.	Locus	Position (cM)	RSG04008-6	Heterozygote	J2614-11	$\chi 2$	Significance
74	S10_50232568	39.38	18	7	50	76.9	*******
75	S10_50232566	39.38	18	7	50	76.9	*******
76	S10_53544398	39.394	14	4	63	125.1	*******
77	S10_52700141	39.782	24	9	60	88.3	*******
78	S10_52940776	40.377	24	5	44	65.3	*******
79	S10_54877607	40.677	20	2	43	73.5	*******
80	S10_53576112	40.76	24	7	55	82.6	*******
81	S10_54081973	41.371	28	13	59	74	*******
82	xgap001_54507175	42.833	32	44	74	49.1	*******
83	S10_54532995	43.958	41	16	70	84.3	*******
84	S10_54585199	44.416	30	10	59	80	*******
85	S10_54535306	44.945	30	14	65	82.7	*******
86	S10_54185546	45.857	30	30	81	83.4	*******
87	S10_54185539	45.985	30	30	80	81.4	*******
88	S10_54185186	46.461	23	3	53	90.2	*******
89	S10_54966382	47.424	32	19	76	92.9	*******
90	S10_54974701	48.021	17	7	52	82.8	*******
91	S10_54247479	48.532	29	5	52	79.5	*******
92	S10_54535502	49.165	28	7	46	63.4	*******
93	S10_53681243	49.555	15	5	62	117.1	*******
94	S10_54527903	49.678	27	5	58	92.5	*******
95	S10_54535745	50.491	45	18	58	62.5	*******
96	S10_54535807	51.036	34	24	71	72.1	*******
97	S10_55051409	51.386	31	14	66	84.1	*******
98	S10_54584527	52.221	33	14	64	79.4	*******
99	S10_54877733	53.005	19	5	47	74.5	*******
100	S10_55016723	53.54	34	27	72	68.6	*******
101	S10_54223864	54.416	29	9	60	84.9	*******
102	S10_54185408	55.076	14	6	53	92.6	*******
103	S10_54184947	55.529	24	4	41	62.3	*******
104	S10_54185417	55.779	16	6	56	96.9	*******
105	S10_55071264	56.465	33	9	60	83.5	*******
106	S10_54632304	57.098	30	3	39	62.8	*******
107	S10_54187184	57.606	27	10	55	73.4	*******
108	S10_54187185	57.743	27	9	53	71.8	*******
109	S10_55283533	58.521	34	9	66	94.8	*******
110	S10_55370553	60.56	36	24	78	84.3	*******
111	S10_55387439	62.051	28	3	41	65.2	*******
112	S10_55177860	62.658	30	0	42	76	*******
113	S10_55747507	63.545	35	4	52	82	*******

Table 13: (Contd..)

			Alleles %				
S.No.	Locus	Position (cM)	RSG04008-6	Heterozygote	J2614-11	$\chi 2$	Significance
114	S10_55599913	64.305	43	15	59	69.1	*******
115	S10_55600708	65.957	44	25	73	71.5	*******
116	S10_55376713	66.472	40	8	32	52.8	*******
117	S10_56158623	67.237	24	8	40	50.7	*******
118	S10_55669377	68.043	38	8	48	66.8	*******
119	S10_55649047	69.328	45	8	38	62.9	*******
120	S10_56155818	70.055	37	8	44	61	*******
121	S10_55747741	70.634	30	3	40	64.2	*******
122	S10_55473978	71.482	42	9	44	62.5	*******
123	S10_56050653	72.248	40	15	62	73	*******
124	S10_56252649	73.641	38	8	56	78.9	*******
125	S10_56350371	74.202	32	19	62	65.7	*******
126	S10_56093654	75.152	51	13	55	73	*******
127	S10_56393810	76.646	55	20	67	75.3	*******
128	S10_56381792	77.204	41	8	42	61.8	*******
129	S10_56249651	77.614	35	1	45	79.5	*******
130	S10_56207658	78.719	40	7	45	66.7	*******
131	S10_56207637	78.725	41	7	45	67.5	*******
132	S10_56381721	79.367	35	6	37	56	*******
133	S10_56490707	80.008	39	11	55	70.5	*******
134	S10_56217191	80.752	26	8	52	72.7	*******
135	S10_56216248	81.702	45	15	52	60.9	*******
136	S10_56205739	82.18	52	12	58	79.3	*******
137	S10_56433597	82.729	35	4	39	63.2	*******
138	S10_56730378	83.885	53	33	58	42.6	*******
139	S10_56730380	84.793	49	17	51	59	*******
140	S10_56730384	84.947	51	17	51	60.7	*******
141	S10_56659463	86.428	55	14	59	78.4	*******
142	S10_56834308	86.987	31	2	44	73.6	*******
143	S10_56249757	87.779	37	4	29	56.7	*******
144	S10_56047792	88.617	39	7	32	53.8	*******
145	S10_56595416	89.482	43	5	34	65.2	*******
146	S10_57403166	90.684	18	4	77	154	*******
147	S10_57341007	91.295	45	7	51	77.6	*******
148	S10_57331278	92.115	64	17	64	85	*******
149	S10_57145296	93.122	59	31	62	53.4	*******
150	S10_57552456	94.409	42	2	37	73.8	*******
151	S10_57331300	94.987	61	4	48	100.6	*******
152	S10_57331385	95.618	59	5	49	95.7	*******
153	S10_57122482	95.877	37	1	39	73.2	*******

Table 13: (Contd..)

			Alleles %				
S.No.	Locus	Position (cM)	RSG04008-6	Heterozygote	J2614-11	$\chi 2$	Significance
154	S10_57088032	96.055	37	3	35	63.6	*******
155	S10_57547037	96.424	42	6	32	60.3	*******
156	S10_57547066	96.622	43	6	31	61.4	*******
157	S10_57400347	97.265	70	17	62	89.6	*******
158	S10_58436230	97.876	15	2	63	129.8	*******
159	S10_57552719	98.396	35	2	33	62.3	*******
160	S10_57549720	98.701	58	6	51	93.1	*******
161	S10_57248800	99.044	50	2	46	90.5	*******
162	S10_57432493	99.608	65	21	56	71.6	*******
163	S10_57453669	100.398	71	15	55	91	*******
164	S10_57522978	101.244	62	8	57	97.4	*******
165	S10_58640688	102.44	39	2	21	64.7	*******
166	S10_58356424	103.078	55	8	49	82.9	*******
167	S10_58022779	103.623	47	5	36	71.9	*******
168	S10_58342553	104.412	46	3	35	75.3	*******
169	S10_58311699	104.802	42	3	42	75.4	*******
170	Xtxp141_58245122	105.596	71	19	62	86.6	*******
171	xiabt466_58310536	105.94	69	17	49	81.5	*******
172	S10_58490384	106.676	55	2	28	94.3	*******
173	S10_58991881	107.172	63	6	30	98.5	*******
174	S10_58831404	107.396	35	0	41	77	*******
175	S10_58460662	107.796	63	11	39	83.5	*******
176	S10_58357039	108.032	28	2	31	53.6	*******
177	S10_58489833	108.357	57	0	38	102.6	*******
178	S10_58683017	108.615	62	7	33	92.4	*******
179	S10_59833299	109.008	50	4	32	78.3	*******
180	S10_59148610	109.224	40	4	42	70.8	*******
181	S10_58839857	109.466	58	6	41	87.9	*******
182	S10_58425747	109.749	40	2	27	66.1	*******
183	S10_58652548	109.901	49	2	20	86.9	*******
184	S10_59316155	110.125	42	6	38	64	*******
185	S10_59024190	110.337	50	10	46	70.1	*******
186	S10_59069052	110.528	41	3	30	65.8	*******
187	S10_58380486	110.886	46	3	30	73.9	*******
188	S10_59833250	111.191	46	1	33	80.3	*******
189	S10_58839711	111.359	72	13	49	94.9	*******
190	S10_59020155	111.735	49	3	35	79.9	*******
191	S10_59020363	112.082	75	19	54	87.7	*******
192	S10_59062420	112.3	69	14	49	88	*******
193	S10_59554262	112.633	32	5	43	64.3	*******

Table 13: (Contd..)

			Alleles %				
S.No.	Locus	Position (cM)	RSG04008-6	Heterozygote	J2614-11	$\chi 2$	Significance
194	S10_59223134	113.022	57	9	45	80.5	*******
195	S10_59223139	113.057	57	10	44	77.7	*******
196	S10_58665801	113.284	57	6	41	86.3	*******
197	S10_58634237	113.523	50	6	37	74.2	*******
198	S10_59419567	113.698	72	2	35	126.3	*******
199	S10_59547620	113.992	66	8	43	96.2	*******
200	S10_59206763	114.325	69	11	46	94.2	*******
201	S10_59342804	114.593	39	5	36	61.5	*******
202	S10_59564696	114.809	69	13	48	90	*******
203	S10_59215385	115.101	38	8	29	48.6	*******
204	S10_59342820	115.321	69	11	46	94.2	*******
205	S10_59566700	115.589	70	7	46	106	*******
206	S10_59566699	115.632	69	7	47	104.5	*******
207	S10_59565625	116.207	59	8	37	83.8	*******
208	S10_59565627	116.207	59	8	37	83.8	*******
209	S10_59808039	116.518	68	7	43	102.3	*******
210	S10_59418734	116.702	46	6	33	66.7	*******
211	S10_59525199	116.941	61	4	43	98.6	*******
212	S10_59691336	117.319	58	4	19	103.3	*******
213	S10_59476045	117.456	77	17	44	94.2	*******
214	S10_59608554	117.745	61	10	34	82.7	*******
215	S10_59413371	117.994	39	0	23	70.3	*******
216	S10_59821802	118.156	51	7	42	75.6	*******
217	S10_59571506	118.271	63	6	40	96	*******
218	S10_59835807	118.575	52	6	34	76.6	*******
219	S10_59775260	118.728	49	4	29	76.5	*******
220	S10_59808110	119.005	71	15	36	89.5	*******
221	S10_59866581	119.287	38	5	22	54.4	*******
222	S10_59609220	119.541	64	18	40	70.1	*******
223	S10_59826585	119.868	42	5	33	63.3	*******
224	S10_60240796	120.044	51	9	30	67.4	*******
225	S10_59560739	120.325	70	11	39	96	*******
226	S10_59775456	120.618	36	3	29	58	*******
227	S10_60900987	120.867	62	13	41	77.4	*******
228	S10_60900986	121.02	63	12	41	81.3	*******
229	S10_60900982	121.034	63	13	41	79	*******
230	S10_60344348	121.363	33	3	20	50.7	*******
231	S10_59889374	121.68	68	15	40	83.1	*******
232	S10_59850910	121.911	38	3	32	62.5	*******
233	S10_60282257	122.169	38	4	31	59.2	*******

Table 13: (Contd..)

			Alleles %				
S.No.	Locus	Position (cM)	RSG0 4008-6	Hetero zygote	J2614 -11	$\chi 2$	Significance
234	S10_60324251	122.46	73	23	40	75.6	*******
235	S10_59946988	122.798	80	3	9	190	*******
236	S10_60324214	122.951	62	14	32	75.9	*******
237	S10_60024056	123.601	78	30	41	71.5	*******
238	S10_60333532	124.099	42	7	27	56.5	*******
239	S10_60423900	124.349	77	31	38	69.2	*******
240	S10_60938250	124.648	73	29	46	64.6	*******
241	S10_60194381	124.892	48	6	29	69.4	*******
242	S10_60194379	124.978	47	6	29	67.7	*******
243	S10_60631094	125.224	36	6	35	54.9	*******
244	S10_60240729	125.463	41	8	36	56.6	*******
245	S10_60308140	125.703	60	13	46	76	*******
246	S10_60650722	126.247	36	4	30	55.9	*******
247	S10_60302611	126.61	71	7	35	109.7	*******
248	S10_60324265	127.043	63	10	33	86.8	*******
249	S10_60756251	127.635	69	16	43	82.6	*******
250	S10_60297335	128.102	73	13	42	96.3	*******
251	S10_60380079	128.373	65	11	43	87.2	*******
252	S10_60342880	128.942	77	12	46	105.5	*******
253	S10_60287963	129.512	49	7	46	76.1	*******
254	S10_60349808	129.798	51	3	34	83	*******
255	S10_60048878	130.063	39	3	35	65.9	*******
256	S10_60499001	130.34	58	9	38	79.7	*******
257	S10_59946860	130.887	56	13	47	71.2	*******
258	S10_59946877	131.167	62	7	39	91.6	*******
259	S10_60701880	131.9	16	0	16	32	*******
260	Xisep1011_60746656	132.224	71	34	42	53.9	*******
261	S10_59930523	132.579	45	6	24	64.7	*******
262	S10_60245187	133.343	38	5	25	54.4	*******
263	S10_60354221	133.711	75	0	3	210.9	*******
264	S10_60601449	133.934	41	4	37	67.2	*******
265	S10_60384475	135.692	5	0	42	105.3	*******

*** highly significantly deviated from 1:2:1 F_2 segregation ratio.

Table 14: Shoot fly resistance component trait QTLs detected on SBI-10 using QTL Cartographer with data from a large F_2 population of 1,894 individuals derived from cross RSG04008-6 × J2614-11

QTL	Pos (cM)	Marker interval	Supp.IV (cM)	LOD	R^2%	Add	Dom
QGls10	1	*Xgap001-Xnhsbm1044*	0-10	24.13	6.23	-0.69	0.01
QTd10	31.6	*Xisep0630-Xtxp141*	25-37	8.11	2.88	0.31	-0.09

QTL- Quantitative Trait Loci, Pos- Position of QTL in cM, LOD- logarithm of odds, R^2%- Percentage of Phenotypic variance, Add- Additive, Dom- Dominant

Table 15: F2 and F3 QTL mapping on selected 369 individuals

QTL	Pos (cM)	Marker interval	Supp.IV (cM)	LOD	R^2%	Add	Dom
F_2QGls10	14	*Xisp10263-Xgap001*	20-Jun	9.67	11.37	0.69	-0.01
F_3QGls10	12	*Xisp10263-Xgap001*	20-Jun	5.95	6.6	0.7	0.36
F_2QTd10	58	*Xtxp141-Xisep1011*	48-70	4.4	3.7	-0.32`	0.56
F_3QTd10	48	*Xisep0630-Xtxp141*	46-54	2.32	2.29	0.031	0.01

QTL- Quantitative Trait Loci, Pos- Position of QTL in cM, LOD- logarithm of odds, R^2%- Percentage of Phenotypic variance, Add- Additive, Dom- Dominant

Table 16: GBS SNP map for fine mapping F_2,$F_{2:3}$ population of seedling leaf blade glossiness and trichome density

Trait	pos cM	Marker pos	LOD	Additive	Dominant	R^2%	left marker pos	Right marker Pos	Marker interval
F2_Gls	34.71	S10_53682073	3.35	0.6495	-0.6036	11.16	34.00	35.00	S10_54859409 - S10_53682073
F2_Gls	70.61	S10_55747741	3.44	-0.1555	1.7831	3.63	70.10	71.10	S10_56155818 - S10_55473978
F3_Gls	38.41	S10_54185069	3.79	0.7721	-0.3206	11.78	37.80	38.70	S10_49366136 - S10_53855394
F3_Gls	42.41	Xgap001	4.08	0.9051	-0.6975	18.68	41.50	43.20	S10_54081973 - S10_54532995
F2_Td	103.1	S10_58356242	2.74	-0.4069	0.6389	8.39	102.50	103.60	S10_58640688 - S10_58346424
F3_Td	66.01	S10_55600708	2.51	-0.3112	0.8769	7.17	63.80	69.30	S10_55747507 - S10_55649047
F3_Td	92.11	S10_57331278	3.04	0.5359	0.8627	1.72	91.00	94.10	S10_57341007 - S10_57552456

Table 17: Stay-green QTL mapping during summer 2013, summer 2014 and across season analysis

S No.	QTLs on SBI-10L	Pos cM	Closest marker	Marker Interval	Support Interval	LOD	%R^2	Additive	Dominant
1	*Q10GL7a_across*	44.41	S10_54585199	*Xgap001*_54507175 – S10_54535306	42.8 – 44.9	2.76	6.75	2.2331	0.7273
2	*Q10GL7a_r13*	41.41	S10_54081973	S10_54877607 – S10_54081973	40.6 – 42.4	2.93	8.86	2.8201	-2.6178
3	*Q10GL7a_r14*	104.81	S10_58311699	S10_58022779 – S10_58311699	103.7 – 105.9	3.40	6.98	-1.5675	-3.095
4	*Q10GL7b_r14*	112.11	S10_59020363	S10_58380486 – S10_59223134	110.9 – 113.1	3.11	9.43	-1.5537	1.6478
	Q10GL7combined r^2						**32.02**		
5	*Q10GL14d_r14*	29.01	S10_52036901	S10_51224387 – S10_52677221	28.4 – 29.4	2.21	0.09	0.5767	5.886
6	*Q10GL14a_r14*	36.41	S10_54269620	S10_54269620 – S10_52673863	36.3 – 37.2	2.59	5.03	2.0097	2.8007
7	*Q10GL14b_r14*	45.01 S10_54535306		*Xgap001*_54507175 – S10_54966382	42.8 – 47.4	2.55	6.49	2.7621	0.5624
8	*Q10GL14e_r14*	123.61	S10_60024056	S10_59946988 – S10_60333532	122.9 – 124.1	2.37	9.70	-2.3214	3.225
9	*Q10GL14c_r14*	129.51	S10_60287963	S10_60342880 – S10_60048878	129 – 130.0	3.38	8.94	-2.7106	6.1702
10	*Q10GL14a_r13*	41.41	S10_54081973	S10_54877607 – *Xgap001*_54507175	40.7 – 42.8	2.86	9.00	4.9036	-4.2892
11	*Q10GL14a_across*	44.41	S10_54585199	*Xgap001*_54507175 – S10_54185546	42.8 – 45.6	3.73	10.20	4.5978	0.0381
12	*Q10GL14b_r13*	82.71	S10_56433597	S10_56217191 – S10_56730384	82.6 – 85	2.18	4.24	-5.2408	-4.2484
	Q10GL14combined r^2						**53.68**		
13	*Q10GL21a_across*	115.31	S10_59342820	S10_59342804 – S10_59566700	114.5 – 115.6	2.91	10.14	-5.0744	2.4482
14	*Q10GL21b_r13*	41.41	S10_54081973	S10_54877607 – *Xgap001*_54507175	40.5 – 42.8	2.18	6.91	4.9353	-3.5171
15	*Q10GL21a_r13*	115.31	S10_59342820	S10_59342804 – S10_59565625	114.5 – 116.2	2.76	9.07	-6.0311	6.0466
16	*Q10GL21c_r13*	125.01	S10_60194381	S10_60024056 – S10_60240729	123.6 – 125.5	2.33	1.41	-0.3211	13.2821
17	*Q10GL21b_r14*	79.41	S10_56381721	S10_56350371 – S10_56433597	74.3 – 82.7	2.12	7.04	-1.4956	2.3253
18	*Q10GL21c_r14*	99.01	S10_57248800	S10_58436230 – S10_57453669	97.9 – 100.4	2.16	5.31	1.055	-4.1377
19	*Q10GL21a_r14*	115.31	S10_59342820	S10_59342804 – S10_59566700	114.5 – 115.6	2.74	9.20	-2.2148	1.2957
	Q10GL21combined r^2						**49.08**		
20	*Q10GL28a_r14*	36.41	S10_54269620	S10_54269620 – S10_52673863	36.3 – 37.2	3.67	4.96	2.044	3.4449
21	*Q10GL28b_r14*	41.41	S10_54081973	S10_52700141 – *Xgap001*_54507175	39.8 – 42.8	2.77	6.62	2.1001	0.7883

Table 17: (Contd..)

S No.	QTLs on SBI-10L	Pos cM	Closest marker	Marker Interval	Support Interval	LOD	%R^2	Additive	Dominant
22	*Q10GL28a_across*	124.91	S10_60194381	S10_60024056 – S10_60240729	123.6 – 125.5	2.74	2.85	-0.6203	7.2213
23	*Q10GL28a_r13*	125.01	S10_60194381	S10_60024056 – S10_60240729	123.6 – 125.5	2.52	1.20	-0.1975	11.1276
	Q10GL28combined r^2						**15.62**		
24	*Q10GL35a_r13*	121.91	S10_59850910	S10_60344348 – S10_60324251	121.4 – 122.5	2.36	2.06	-0.6135	8.0863
25	*Q10GL42a_r13*	121.91	S10_59850910	S10_59889374 – S10_60324251	121.7 – 122.5	2.59	4.54	-0.5686	5.187
26	*Q10GL42b_r13*	131.91	S10_60701880	S10_59946877 – *Xisep1011*_60746656	131.2 – 132	2.01	0.52	2.4162	3.149
27	*Q10GL42d_r14*	32.31	S10_52781712	S10_51919897 – S10_54859431	30.8 – 33.9	2.47	6.66	2.0509	-6.9547
28	*Q10GL42b_r14*	38.41	S10_52812930	S10_52673863 – S10_53544398	37.7 – 39.6	2.33	2.28	3.5114	4.965
29	*Q10GL42a_r14*	102.31	S10_57522978	S10_57453669 – S10_58356424	100.4 – 103.6	2.60	2.43	1.7652	-29.678
30	*Q10GL42c_r14*	107.81	S10_58460662	S10_58490384 – S10_58489833	106.7 – 108.5	2.44	5.75	2.0457	-7.1224
	Q10GL42combined r^2						**22.18**		
31	*Q10GL49a_r13*	34.71	S10_50890593	S10_50121434 – S10_53834366	33.9 – 35.8	2.37	2.17	1.4224	3.2659
32	*Q10GL49a_across*	36.41	S10_54269620	S10_54269620 – S10_52673868	36.4 – 37.2	2.54	2.45	2.1417	3.2671
33	*Q10GL49b_across*	45.01	S10_54535306	xgap001_54507175 – S10_54185546	42.8 – 45.9	3.04	4.08	2.6273	2.1788
	Q10GL49combined r^2						**8.71**		

Table 18: Agronomic traits and yield related traits QTL mapping for summer 2013, summer 2014 and across season QTL analysis

S No.	QTLs on SBI-10L	Position cM	Closest marker	Marker interval	Support interval	LOD	%R^2	Additive	Dominant
1	*Q10FT.a_13*	12.41	S10_51065106	S10_51699119 – S10_49095753	9.4 – 12.9	2.55	4.4443	-1.8558	-1.0923
2	*Q10FT.a_14*	25.91	S10_51071502	S10_51071502 – S10_52676228	25.8 – 27.8	2.9	3.2923	-1.1211	-1.0466
3	*Q10FT.b_14*	36.91	S10_45646835	S10_54269620 – S10_52673863	36.4 – 37.2	4.88	12.5	-1.6449	-0.6713
4	*Q10FT.c_14*	44.41	S10_54585199	*Xgap001*_54507175 – S10_54185546	42.8 – 45.9	4.937	12.012	-1.6713	-0.1611
5	*Q10FT.d_14*	101.31	S10_57522978	S10_57453669–S10_58640688	100.4–102.4	2.376	0.0003	0.4642	3.5948
6	*Q10FT.a_across*	44.41	S10_54585199	S10_54532995 – S10_54535306	43.1 – 45	3.422	8.7257	-1.7486	0.2071
7	*Q10FT.b_across*	67.31	S10_56158623	S10_55600708 – S10_55669377	65.7 – 68	2.848	8.4726	1.4153	-2.1831
	Combined r^2 FT						**49.447**		
8	*Q10PlHt.a_13*	40.41	S10_52940776	S10_52812930–S10_53576112	38.4–40.7	2.026	2.454	1.7923	-16.1104
9	*Q10PlHt.b_13*	49.21	S10_54535502	S10_54247479 – S10_53681243	48.7 – 49.6	3.028	0.0003	-2.3595	-14.3612
10	*Q10PlHt.c_13*	70.11	S10_55747741	S10_56158623–S10_55473978	67.4–71.5	2.259	0.1064	-1.0644	-12.5499
11	*Q10PlHt.d_13*	108.61	S10_58683017	S10_58460662–S10_59316155	107.8–110.1	2.12	5.144	4.838	3.0909
12	*Q10PlHt.a_14*	97.91	S10_58436230	S10_57400347 – S10_57549720	97.3 – 98.6	2.959	7.263	9.3318	8.72
13	*Q10meanPlHt.a_across*	70.61	S10_55747741	S10_55649047 – S10_55473978	69.3 – 71.5	2.563	0.7411	-4.5037	-16.9514
14	*Q10meanPlHt.b_across*	97.91	S10_58436230	S10_57400347 – S10_57549720	97.6 – 98.7	2.704	5.5711	6.2766	9.2171
	Combined r^2PlHt						**21.28**		
15	*Q10PnDW/plot.a_13*	105.61	*Xtxp141*_58245122	S10_58342553–*Xiabt466*_58310536	104.4–106	2.059	4.1155	-27.9697	-10.89
16	*Q10PnDW/plot.b_13*	124.71	S10_60938250	S10_60423900–S10_60240729	124.4–125.5	2.028	9.537	29.9879	-22.2823
17	*Q10PnDW/plot.a_14*	20.91	S10_50452521	S10_50235747 – S10_50093128	20.1 – 21.3	3.033	10.572	-28.3753	15.6606
18	*Q10PnDW/plot.b_14*	109.01	S10_59833299	S10_58683017 – S10_59833299	108.6 – 109.9	3.326	2.7359	14.527	64.5807
19	*Q10PnDW/plot.c_14*	117.31	S10_59418734	S10_59525199–S10_59476045	117–117.5	2.109	7.3994	24.9129	-18.1471
	Combined r^2 PnDW/Plot						**34.359**		
20	*Q10GDW/plot.a_13*	105.61	*Xtxp141*_58245122	S10_58342553–*Xiabt466*_58310536	104.4–106	2.413	3.3423	-23.3356	-15.5574
21	*Q10GDW/plot.b_13*	120.11	S10_60240796	S10_59609220–S10_60900987	119.6–120.8	2.185	7.4706	19.5374	-17.9613
22	*Q10GDW/plot.a_14*	82.21	S10_56205739	S10_56216248 – S10_56433597	81.7 – 82.6	2.798	1.1532	-0.2253	41.091
23	*Q10GDW/plot.b_14*	107.41	S10_58831404	S10_58490384 – S10_58460662	106.8 – 107.8	3.691	3.0014	9.6131	62.5842
24	*Q10GDW/plot.c_14*	117.31	S10_59418734	S10_59525199–S10_59476045	117–117.5	2.189	6.4672	18.2301	1.414
25	*Q10GDW/plot.a_across*	19.41	S10_47939440	S10_51263932 – S10_50235747	18.2 – 20.1	3.628	18.34	-15.4436	10.2061

Table 18 : (Contd..)

S No.	QTLs on SBI-10L	Position cM	Closest marker	Marker interval	Support interval	LOD	%R^2	Additive	Dominant
26	*Q10GDW/plot.b_across*	107.21	S10_58991881	S10_58490384 – S10_58460662	106.5 – 107.8	2.8	2.4018	5.9777	23.7169
	Combined r^2 GDW/Plot						**42.177**		
27	*Q10HGM.a_13*	0.01	S10_48719070	S10_48719070 – S10_45832539	0 – 0.4	3.183	9.619	-0.0723	0.1238
28	*Q10HGM.b_13*	99.61	S10_57432493	S10_57549720–S10_57522978	99.5–101.3	2.485	0.0333	0.041	0.1471
29	*Q10HGM.c_13*	108.61	S10_58683017	S10_58683017–S10_59833299	108–109	2.257	1.6035	0.0916	0.1445
30	*Q10HGM.d_13*	117.01	S10_59418734	S10_59565625–S10_59476045	116.2–117.5	2.326	1.6187	0.0705	0.1834
32	*Q10HGM.a_14*	20.11	S10_50235747	S10_50093128 – S10_50093129	19.4 – 21.6	3.365	6.8156	-0.0579	0.2301
31	*Q10HGM.b_14*	95.01	S10_57331300	S10_57145296–S10_57547037	93.1–96.1	2.05	4.4703	0.0744	-0.2653
33	*Q10HGM.c_14*	126.31	S10_60650722	S10_60308140 – S10_60302611	125.7 – 126.8	2.552	0.4563	0.0136	0.3525
34	*Q10HGM.a_across*	20.11	S10_50235747	S10_47939440 – S10_50093128	19.4 – 21.4	2.689	3.1292	-0.0299	0.1549
35	*Q10HGM.b_across*	117.01	S10_59525199	S10_59566700 – S10_59691336	115.6 – 117.3	2.883	4.197	0.0586	0.1583
	Combined r^2 HGM						**31.943**		
36	*Q10GNP/plot.a_13/*	31.01	S10_51919897	S10_50972176 – S10_52784725	30.5 – 32.6	2.53	0.6387	-74.7478	0
37	*Q10GNP/plot.b_13*	98.41	S10_57552719	S10_57552719 – S10_57549720	98.1 – 98.8	3.83	0.3558	-70.5453	0
38	*Q10GNP/plot.c_13*	106.71	S10_58490384	*Xiabt466*_58310536 – S10_58490384	106 – 106.7	32.74	3.9514	-282.9358	0
39	*Q10GNP/plot.d_13*	113.71	S10_59419567	S10_58665801 – S10_59547620	113.5 – 113.8	36.34	1.7422	-155.5585	0
40	*Q10GNP/plot.e_13*	129.81	S10_60349808	S10_60287963 – S10_60499001	129.5 – 130.2	34.11	0.5811	107.9076	0
41	*Q10GNP/plot.f_13*	133.71	S10_60354221	S10_60245187 – S10_60354221	133.3 – 133.7	35.08	5.8143	-83.3681	0
42	*Q10GNP/plot.a_14*	77.61	S10_56249651	S10_56393810 – S10_56207658	76.9 – 78.5	2.504	1.2737	-212.3514	0
43	*Q10GNP/plot.a_across*	103.11	S10_58356424	S10_57522978 – S10_58022779	101.7 – 103.5	3.978	9.7799	-392.4407	0
44	*Q10GNP/plot.b_across*	107.41	S10_58831404	S10_58831404 – S10_58460662	107.2 – 107.6	7.624	9.7598	-308.0604	0
45	*Q10GNP/plot.c_across*	113.71	S10_59419567	S10_58665801 – S10_59547620	113.5 – 113.8	17.45	3.4349	-117.9362	0
46	*Q10GNP/plot.d_across*	122.81	S10_59946988	S10_60324251 – S10_60324214	122.5 – 123	5.104	1.1814	397.637	0
47	*Q10GNP/plot.e_across*	129.81	S10_60349808	S10_60349808 – S10_60048878	129.8 – 129.9	17.18	0.1468	65.0339	0
48	*Q10GNP/plot.f_across*	133.71	S10_60354221	S10_60245187 – S10_60354221	133.3 – 133.8	19.35	3.0574	55.471	0
	Combined r^2 GNP/Plot						**41.717**		
49	*Q10GNPP.a_14*	77.61	S10_56249651	S10_56393810 – S10_56207658	76.9 – 78.5	2.504	1.2737	-8.1674	112.1652

Table 18: (Contd..)

S No.	QTLs on SBI-10L	Position cM	Closest marker	Marker interval	Support interval	LOD	%R^2	Additive	Dominant
50	*Q10GNPP.a_13*	31.01	S10_51919897	S10_50972176 – S10_52784725	30.5 – 32.6	2.53	0.6387	-2.8749	74.0566
51	*Q10GNPP.b_13*	98.41	S10_57552719	S10_57552719 – S10_57549720	98.1 – 98.8	3.83	0.3558	-2.7133	169.5069
52	*Q10GNPP.c_13*	106.71	S10_58490384	*Xiabt466*_58310536 – S10_58490384	106 – 106.7	32.74	3.9514	-10.8821	0
53	*Q10GNPP.d_13*	113.71	S10_59419567	S10_58665801 – S10_59547620	113.5 – 113.8	36.34	1.7422	-5.983	0
54	*Q10GNPP.e_13*	129.81	S10_60349808	S10_60287963 – S10_60499001	129.5 – 130.2	34.11	0.5811	4.1503	0
55	*Q10GNPP.f_13*	133.71	S10_60354221	S10_60245187 – S10_60354221	133.3 – 133.7	35.08	5.8143	-3.2065	0
56	*Q10GNPP.a_across*	103.11	S10_58356424	S10_57522978 – S10_58022779	101.7 – 103.5	3.978	9.7799	-15.0939	46.779
57	*Q10GNPP.b_across*	107.41	S10_58831404	S10_58831404 – S10_58460662	107.2 – 107.6	7.624	9.7598	-11.8485	128.7525
58	*Q10GNPP.c_across*	113.71	S10_59419567	S10_58665801 – S10_59547620	113.5 – 113.8	17.45	3.4349	-4.536	468.4428
59	*Q10GNPP.d_across*	122.81	S10_59946988	S10_60324251 – S10_60324214	122.5 – 123	5.104	1.1814	15.2937	78.6598
60	*Q10GNPP.e_across*	129.81	S10_60349808	S10_60349808 – S10_60048878	129.8 – 129.9	17.18	0.1468	2.5013	461.7645
61	*Q10GNPP.f_across*	133.71	S10_60354221	S10_60245187 – S10_60354221	133.3 – 133.8	19.35	3.0574	2.1335	459.1267
	Combined r^2 GNPP						**41.717**		
63	*Q10PHI.a_13*	113.71	S10_59419567	S10_58634237 – S10_59547620	113.5 – 113.9	3.03	2.0491	-0.169	3.4057
64	*Q10PHI.b_13*	122.51	S10_60324251	S10_60282257 – S10_59946988	122.3 – 122.8	3.685	12.033	1.3147	-0.2071
62	*Q10PHI.c_13*	125.71	S10_60308140	S10_60194379 – S10_60650722	125–126.3	2.093	4.5826	0.8854	0.2308
65	*Q10PHI.a_14*	35.31	S10_50140543	S10_53682073 – S10_53160198	35 – 35.6	3.817	15.053	0.6369	-0.702
66	*Q10PHI.b_14*	70.61	S10_56252649	S10_56155818 – S10_56252649	70.1 – 73.4	3.402	9.132	-0.438	1.2338
67	*Q10PHI.a_across*	18.51	S10_51263932	S10_48014895 – S10_47939440	17.7 – 19.4	2.837	10.976	-0.5388	0.5337
	Combined r^2 PHI						**53.826**		

Table 19: QTL mapping results for F_4 field trails during *kharif* (rainy) 2013 and *rabi* (post-rainy) 2013

S No.	QTLs on SBI-10L	Position cM	Closest marker	Marker interval	Support interval	LOD	R^2%	Additive	Dominant
1	*Q10Gls1_K13*	36.41	S10_54269620	S10_53058516 – S10_52673863	36.2 – 37	3.92	1.61	0.21	0.50
2	*Q10Gls2_K13*	42.41	*Xgap001*_54507175	S10_54877607 – S10_54532995	40.7 – 44	4.51	6.26	0.30	0.24
3	*Q10Gls1_R13*	36.41	S10_54269620	S10_53058516 – S10_52673863	36.2 – 37	4.66	3.51	0.27	0.55
4	*Q10Gls.1*	36.41	S10_54269620	S10_53058516 – S10_52673863	36.2 – 36.9	3.7152	0.3502	0.1683	0.6016
	Combined r^2 Gls						**11.73**		
5	***Q10Tdl1_K13***	**90.51**	**S10_57403166**	**S10_56595416 – S10_57331278**	**89.3 – 92.1**	**5.66**	**6.54**	**-6.58**	**-8.51**
6	***Q10Tdl2_K13***	**99.61**	**S10_57432493**	**S10_57248800 – S10_57453669**	**99 – 100.7**	**23.60**	**48.50**	**-11.19**	**-1.60**
7	***Q10Tdl3_K13***	**108.01**	**S10_58357039**	**S10_58831404 – S10_58683017**	**107.5 – 108.6**	**16.26**	**38.34**	**-9.52**	**-2.84**
8	***Q10Tdl1_R13***	**99.61**	**S10_57432493**	**S10_57248800 – S10_57453669**	**99.3 – 100.3**	**7.00**	**8.24**	**-10.28**	**-3.18**
9	*Q10TLow.1_across*	96.11	S10_57088032	S10_57122482 – S10_57547066	95.9 – 96.6	12.602	19.2989	-9.8169	-1.5194
10	*Q10TLow.2_across*	99.61	S10_57432493	S10_57432493 – S10_57432493	99.4 – 100	15.809	19.5457	-12.7806	-2.463
11	*Q10TLlow.3_across*	103.61	S10_58022779	S10_58356424 – S10_58342553	103.3 – 104.5	12.548	22.3855	-10.5308	0.1028
12	*Q10TLlow.4_across*	108.01	S10_58357039	S10_58460662 – S10_58489833	107.6 – 108.5	10.293	19.1029	-9.9778	-1.081
13	*Q10Tdu1_K13*	107.81	S10_58460662	*Xiabt 466*_58310536 – S10_58357039	106 – 108.4	7.45	13.05	-12.95	-16.95
14	*Q10 Tdu1_R13*	97.31	S10_57400347	S10_57547037 – S10_58436230	96.6 – 97.7	4.59	7.21	-12.78	-0.05
15	*Q10 Tdu2_R13*	106.71	S10_58490384	*Xiabt 466*_58310536 – S10_58991881	106 – 107.2	3.65	7.79	-11.13	11.29
16	*Q10Tdu.1_across*	34.71	S10_50890593	S10_54859409 – S10_53682073	34.3 – 35	2.8696	5.9005	-7.8912	9.2437
17	*Q10Tdu.2_across*	97.31	S10_57400347	S10_57547066 – S10_58436230	96.9 – 97.6	7.5935	14.7595	-15.9794	0.8227
18	***Q10Tdu.3_across***	**99.61**	**S10_57432493**	**S10_57248800 – S10_57453669**	**99 – 100.1**	**8.3109**	**14.3854**	**-17.6112**	**-2.4786**
19	*Q10Tdu.4_across*	109.51	S10_58839857	S10_58839857 – S10_58425747	109.4 – 109.7	6.1457	12.2731	-15.9039	-9.2442
	Combined r^2 TDU						**75.36**		
20	*Q10%SFDH1_K13*	25.91	S10_51071502	S10_48069066 – S10_51228412	25.2 – 26.9	2.92	7.34	1.38	0.14
21	*Q10 %SFDH1_R13*	82.71	S10_56433597	S10_56205739 – S10_56730384	82 – 85.2	2.96	0.00	1.55	8.32
22	***Q10 %SFDH2_R13***	**90.51**	**S10_57403166**	**S10_56595416 – S10_57403166**	**89.5 – 90.7**	**9.17**	**10.58**	**6.63**	**9.36**
23	***Q10 %SFDH3_R13***	**99.61**	**S10_57432493**	**S10_57248800 – S10_57453669**	**99 – 100.1**	**12.73**	**12.13**	**8.14**	**4.96**
24	*Q10 %SFDH4_R13*	103.11	S10_58356424	S10_58022779 – S10_58342553	102.6 – 104.4	11.60	11.09	6.22	9.70
25	*Q10 %SFDH5_R13*	108.61	S10_58683017	S10_58489833 – S10_59833299	108.3 – 109	9.08	12.50	5.69	5.99
26	*Q10%SFDH.1_across*	90.51	S10_57403166	S10_56595416 – S10_57403166	89.2 – 90.7	8.2087	6.7381	3.0393	4.2489

Table 19: (Contd..)

S No.	QTLs on SBI-10L	Position cM	Closest marker	Marker interval	Support interval	LOD	R^2%	Additive	Dominant
27	***Q10%SFDH.2_across***	**99.61**	**S10_57432493**	**S10_57248800 – S10_57453669**	**99 – 100.1**	**8.4304**	**3.9912**	**3.6096**	**2.7641**
28	*Q10%SFDH.3_across*	103.11	S10_58356424	S10_58640688 – S10_58022779	102.5 – 103.6	11.159	10.0968	2.8139	4.8031
29	*Q10%SFDH.4_across*	105.61	*Xtxp141*_58245122	S10_58311699 – *Xiabt466*_58310536	105.1 – 106	10.948	10.7927	3.0203	2.7246
30	*Q10%SFDH.5_across*	109.51	S10_58839857	S10_58839857 – S10_58425747	109.5 – 109.6	8.2935	9.8083	2.4822	4.3372
31	*Q10SV1_R13*	56.51	S10_55071264	S10_54185417 – S10_54632304	55.8 – 57.1	2.95	9.51	-0.11	0.03
32	*Q10 SV2_R13*	60.61	S10_55370553	S10_55283533 – S10_55177860	59.3 – 62.6	2.99	4.37	-0.09	-0.08
33	*Q10SV.1_across*	62.11	S10_55387439	S10_55370553 – S10_55177860	60 – 62.7	2.5087	7.2981	-0.08	0.1659
	Combined r^2 SV						**21.18**		
34	*Q10LSP1_K13*	63.61	S10_55747507	S10_55747507 – S10_55600708	63 – 65.3	3.88	4.41	0.28	0.81
35	*Q10LSP2_K13*	108.01	S10_58357039	S10_58490384 – S10_59148610	106.7 – 109.2	2.58	5.48	-0.34	-0.30
36	*Q10LSP1_R13*	6.31	S10_45991208	S10_48098678 – S10_48098791	5.2 – 7.8	2.73	0.94	-0.01	-0.79
37	*Q10LSP2_R13*	40.41	S10_52940776	S10_52700141 – S10_54877607	39.8 – 40.7	2.99	8.01	0.25	-0.80
38	*Q10LSP.1_across*	32.61	S10_52784725	S10_52853071 – S10_50121434	31.9 – 33.9	2.7326	12.2315	0.3171	-0.3218
39	*Q10LSP.2_across*	39.41	S10_53544398	S10_53855394 – S10_52700141	38.7 – 39.8	3.2	11.1242	0.3823	-0.4763
40	*Q10LSP.3_across*	42.41	*Xgap001*_54507175	S10_53576112 – S10_54585199	40.8 – 44.2	2.8109	14.278	0.406	-0.2513
	Combined r^2 LSP						**56.47**		

Gls- Glossiness; Tdl- trichome density lower; Tdu-Teichome density upper; Sv-seedling vigour; LSP-Leaf sheath pigmentation; K13-*kharif* (rainy) 2013; R13- *rabi* (Post-rainy) 2013; Across-Across K13 and R13; Q- QTL

Table 20: Stay-green QTL cluster analysis

cQTL	Nearest marker	position (cM)	marker Intervals	No.of QTLs	Integrated QTLs	Gene ID/MTAs	Combined % R^2	Previous studies of stg QTL maping in sorghum	Candidate genes	SNP effect
cQstg10.1	S10_54269620	36.41	36.41	3	*Q10GLA14a_14,Q10GLA28a_14.Q1049a_across*	**Sb10g025053**	12.4	Haussmann et al. 2002b	AP2/ERF transcryptional factor	Intergenic
cQstg10.2	S10_54081973	41.41	39.8 – 42.8	4	Q10GLA7a_13,Q10GLA14a_13,Q10GL21b_13,Q10GLA28b_14	**Sb10g024920**	31.3	Haussmann et al. 2002b	weakly similar to Putative uncharacterized protein	non synonymous
cQstg10.3	S10_54535306	45.01	42.8 – 47.4	2	Q10GLA7a_across,Q10GLA14a_across	**Sb10g025283**	16.95	Haussmann et al. 2002b	NBS-LRR disease resistance protein	synonimous& non synonimous (Exonic region)
cQstg10.4	S10_54585199	44.41	54.58- 54.59	2	Q10GLA14b_14,Q10GLA49b_across	**Sb10g025310, Sb10g025320**	10.57	Haussmann et al. 2002b	Ankyrin repeat protein and WD40 repeat family protein (trasducin protein)	Intronic region
cQstg10.5	S10_59342820	115.31	114.5 – 115.6	3	Q10GLA21a_13,Q10GLA21a_14,Q10GLA21a_across	**Sb10g029570**	28	Haussmann et al. 2002b	similar to Putative uncharacterized protein P0655A07.24/LEA	synonimous& non synonimous (Exonic region)
cQstg10.6	S10_59775456	120.6	121.4 – 122.5	2	Q10GL35a_13,Q10GLA42a_13	**Sb10g030040**	6.58	Haussmann et al. 2002b	Ca/calmodulin dependant protein kinase	Intron
cQstg10.7	S10_60194379	125.01	124.7 – 125.5	3	Q10GL21c_13,Q10GLA28a_13,Q10GLA28a_across	**Sb10g030520**	5.5	Haussmann et al. 2002b	similar to Senescence-associated protein	3'UTR+Exon

Table 21: GWAS for Stay-green and marker trait associations

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
1	%GL7_R13	S10_56170098	-	-	8.38E-03	0.50	25.87
2	%GL7_R13	S10_57403166	-	-	2.16E-03	0.31	27.35
3	%GL7_R13	S10_57453732	-	-	7.30E-03	0.47	26.02
4	%GL7_R13	S10_58489833	-	-	8.75E-03	0.44	25.83
5	%GL7_R13	S10_49288320	Sb10g022060	leucine-rich repeat family protein / protein kinase family protein	7.25E-03	0.41	26.03
6	%GL7_R13	S10_53022276	Sb10g024130	cytochrome P450, putative, expressed	5.29E-03	0.34	26.37
7	%GL7_R13	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	6.46E-03	0.48	26.15
8	%GL7_R13	S10_56655795	Sb10g027070	putative unchracterised protein	7.69E-03	0.42	25.97
9	%GL7_R13	S10_57145296	Sb10g027370	PBP1_GABAb_receptor	8.14E-03	0.49	25.91
10	%GL7_R13	S10_57251912	Sb10g027450	40S ribosomal protein S14-1	3.00E-03	0.49	26.99
11	%GL7_R14	S10_56249651	-	-	5.51E-03	0.47	15.09
12	%GL7_R14	S10_56249699	-	-	5.51E-03	0.47	15.09
13	%GL7_R14	S10_56525509	-	-	2.23E-03	0.48	16.23
14	%GL7_R14	S10_57770653	-	-	1.90E-03	0.47	16.43
15	%GL7_R14	S10_58345765	-	-	5.94E-03	0.44	15.00
16	%GL7_R14	S10_54632210	Sb10g025350	isoleucyl-tRNA synthetase	7.58E-03	0.47	14.69

Table 21: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
17	%GL7_R14	S10_54632243	Sb10g025350	isoleucyl-tRNA synthetase	7.58E-03	0.47	14.69
18	%GL7_R14	S10_54632244	Sb10g025350	isoleucyl-tRNA synthetase	7.58E-03	0.47	14.69
19	%GL7_R14	S10_54632245	Sb10g025350	isoleucyl-tRNA synthetase	7.58E-03	0.47	14.69
20	%GL7_R14	S10_55201667	Sb10g025880	Putative GDP-L-fucose synthase 2	1.12E-03	0.48	17.12
21	%GL7_R14	S10_58022779	Sb10g028130	Putative thaumatin-protein	2.27E-03	0.46	16.21
22	%GL7_R14	S10_58035226	Sb10g028140	AA-amino acid hydrolase	5.19E-03	0.46	15.17
23	%GL7_R14	S10_59018127	Sb10g029180	Catalytic domain of Protein Kinases	8.30E-03	0.47	14.58
24	%GL7_R14	S10_59018128	Sb10g029180	Catalytic domain of Protein Kinases	8.30E-03	0.47	14.58
25	%GL7_Across	S10_56170095	-	-	6.74E-03	0.50	15.06
26	%GL7_Across	S10_56170098	-	-	6.74E-03	0.50	15.06
27	%GL7_Across	S10_57403166	-	-	3.85E-03	0.31	15.69
28	%GL7_Across	S10_54591109	Sb10g025320	Transducin family protein / WD-40 repeat family protein	4.36E-03	0.48	15.55
29	%GL7_Across	S10_54613971	Sb10g025340	40S ribosomal protein S14 (RPS14C)	7.83E-03	0.35	14.89
30	%GL7_Across	S10_54632210	Sb10g025350	isoleucyl-tRNA synthetase	9.45E-03	0.47	14.68
31	%GL7_Across	S10_54632243	Sb10g025350	isoleucyl-tRNA synthetase	9.45E-03	0.47	14.68
32	%GL7_Across	S10_54632244	Sb10g025350	isoleucyl-tRNA synthetase	9.45E-03	0.47	14.68
33	%GL7_Across	S10_54632245	Sb10g025350	isoleucyl-tRNA synthetase	9.45E-03	0.47	14.68
34	%GL7_Across	S10_58035226	Sb10g028140	AA-amino acid hydrolase	9.63E-03	0.46	14.66

Table 21: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
35	%GL7_Across	S10_59946860	Sb10g030260	Putative senescence-associated protein	7.03E-03	0.47	15.01
36	%GL7_Across	S10_59946900	Sb10g030260	Putative senescence-associated protein	7.03E-03	0.47	15.01
37	%GL7_R13	S10_56170095	-	-	8.38E-03	0.50	25.87
38	%GL14_R13	S10_56170095	-	-	3.35E-03	0.50	29.36
39	%GL14_R13	S10_56170098	-	-	3.35E-03	0.50	29.36
40	%GL14_R13	S10_57403166	-	-	9.64E-04	0.31	30.70
41	%GL14_R13	S10_57770653	-	-	6.25E-03	0.47	28.71
42	%GL14_R13	S10_49288320	Sb10g022060	leucine-rich repeat family protein / protein kinase family protein	3.74E-03	0.41	29.24
43	%GL14_R13	S10_53022276	Sb10g024130	cytochrome P450, putative, expressed	3.64E-03	0.34	29.27
44	%GL14_R13	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	3.29E-03	0.48	29.38
45	%GL14_R13	S10_57251912	Sb10g027450	40S ribosomal protein S14-1	3.01E-03	0.49	29.47
46	%GL14_R13	S10_58312792	Sb10g028500	Peroxidase 16 protein	5.45E-03	0.45	28.85
47	%GL14_R13	S10_58839857	Sb10g029010	DUF617; Protein of unknown function	6.38E-03	0.44	28.69
48	%GL14_R13	S10_58839862	Sb10g029010	DUF617; Protein of unknown function	6.38E-03	0.44	28.69
49	%GL14_R13	S10_58839865	Sb10g029010	DUF617; Protein of unknown function	6.38E-03	0.44	28.69
50	%GL14_R13	S10_58839867	Sb10g029010	DUF617; Protein of unknown function	6.38E-03	0.44	28.69
51	%GL14_R13	S10_58839905	Sb10g029010	DUF617; Protein of unknown function	6.38E-03	0.44	28.69
52	%GL14_R14	S10_50132488	-	-	1.14E-03	0.20	23.38

Table 21: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
53	%GL14_R14	S10_50452521	Sb10g022520	GA3 (GA REQUIRING 3); ent-kaurene oxidase/ oxygen binding	3.26E-03	0.33	22.16
54	%GL14_R14	S10_50592399	Sb10g022580	CGA1 (CYTOKININ-RESPONSIVE GATA FACTOR 1); transcription factor	2.69E-03	0.26	22.38
55	%GL14_R14	S10_50672209	Sb10g022650	unknown protein	4.74E-03	0.43	21.73
56	%GL14_R14	S10_52675727	Sb10g023920	pentatricopeptide (PPR) repeat-containing protein	3.70E-03	0.40	22.01
57	%GL14_R14	S10_52675753	Sb10g023920	pentatricopeptide (PPR) repeat-containing protein	3.70E-03	0.40	22.01
58	%GL14_R14	S10_52675759	Sb10g023920	pentatricopeptide (PPR) repeat-containing protein	3.70E-03	0.40	22.01
59	%GL14_R14	S10_54535306	Sb10g025283	NBS-LRR disease resistance protein	3.27E-03	0.38	22.15
60	%GL14_R14	S10_54535322	Sb10g025283	NBS-LRR disease resistance protein	3.27E-03	0.38	22.15
61	%GL14_R14	S10_54535339	Sb10g025283	NBS-LRR disease resistance protein	3.27E-03	0.38	22.15
62	%GL14_Across	S10_45646835	-	-	7.66E-03	0.42	23.86
63	%GL14_Across	S10_48719070	-	-	5.10E-03	0.32	24.27
64	%GL14_Across	S10_56525509	-	-	3.48E-03	0.48	24.66
65	%GL14_Across	S10_57403166	-	-	8.46E-03	0.31	23.76
66	%GL14_Across	S10_49288320	Sb10g022060	leucine-rich repeat family protein / protein kinase family protein	4.08E-04	0.41	26.92
67	%GL14_Across	S10_53038680	Sb10g024150	Putative uncharacterized protein	8.94E-03	0.38	23.71

Table 21: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
68	%GL14_Across	S10_53038681	Sb10g024150	Putative uncharacterized protein	8.94E-03	0.38	23.71
69	%GL14_Across	S10_53038682	Sb10g024150	Putative uncharacterized protein	8.94E-03	0.38	23.71
70	%GL14_Across	S10_53038691	Sb10g024150	Putative uncharacterized protein	8.94E-03	0.38	23.71
71	%GL14_Across	S10_54081973	Sb10g024920	zinc-binding family protein	9.50E-03	0.40	23.65
72	%GL14_Across	S10_54613971	Sb10g025340	40S ribosomal protein S14 (RPS14C)	1.70E-03	0.35	25.41
73	%GL21_R13	S10_56170095	-	-	3.83E-03	0.50	32.89
74	%GL21_R13	S10_56170098	-	-	3.83E-03	0.50	32.89
75	%GL21_R13	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	3.43E-03	0.48	33.00
76	%GL21_R13	S10_58839857	Sb10g029010	DUF617; Protein of unknown function	1.15E-02	0.44	31.81
77	%GL21_R13	S10_58839862	Sb10g029010	DUF617; Protein of unknown function	1.15E-02	0.44	31.81
78	%GL21_R13	S10_58839865	Sb10g029010	DUF617; Protein of unknown function	1.15E-02	0.44	31.81
79	%GL21_R13	S10_58839867	Sb10g029010	DUF617; Protein of unknown function	1.15E-02	0.44	31.81
80	%GL21_R13	S10_58839905	Sb10g029010	DUF617; Protein of unknown function	1.15E-02	0.44	31.81
81	%GL21_R13	S10_60701880	Sb10g031030	Putative AGO1 homologous protein	4.83E-03	0.50	32.66
82	%GL21_R14	S10_48756049	-	-	1.33E-03	0.37	23.20
83	%GL21_R14	S10_50132488	-	-	1.14E-03	0.20	23.38
84	%GL21_R14	S10_50452521	Sb10g022520	GA3 (GA REQUIRING 3); ent-kaurene oxidase/ oxygen binding	3.26E-03	0.33	22.16

Table 21: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
85	%GL21_R14	S10_50592399	Sb10g022580	CGA1 (CYTOKININ-RESPONSIVE GATA FACTOR 1); transcription factor	2.69E-03	0.26	22.38
86	%GL21_R14	S10_50672209	Sb10g022650	unknown protein	4.74E-03	0.43	21.73
87	%GL21_R14	S10_52675727	Sb10g023920	pentatricopeptide (PPR) repeat-containing protein	3.70E-03	0.40	22.01
88	%GL21_R14	S10_52675753	Sb10g023920	pentatricopeptide (PPR) repeat-containing protein	3.70E-03	0.40	22.01
89	%GL21_R14	S10_52675759	Sb10g023920	pentatricopeptide (PPR) repeat-containing protein	3.70E-03	0.40	22.01
90	%GL21_R14	S10_54535306	Sb10g025283	NBS-LRR disease resistance protein	3.27E-03	0.38	22.15
91	%GL21_R14	S10_54535322	Sb10g025283	NBS-LRR disease resistance protein	3.27E-03	0.38	22.15
92	%GL21_R14	S10_54535339	Sb10g025283	NBS-LRR disease resistance protein	3.27E-03	0.38	22.15
93	%GL21_R14	S10_59024190	Sb10g029190	Squamosa promoter-binding-like protein 12	6.67E-03	0.49	21.34
94	%GL21_Across	S10_56170095	-	-	7.16E-03	0.50	26.53
95	%GL21_Across	S10_56170098	-	-	7.16E-03	0.50	26.53
96	%GL21_Across	S10_49288320	Sb10g022060	leucine-rich repeat family protein / protein kinase family protein	5.61E-03	0.41	26.77
97	%GL21_Across	S10_54591109	Sb10g025320	transducin family protein / WD-40 repeat family protein	6.13E-03	0.48	26.68
98	%GL21_Across	S10_60701880	Sb10g031030	Putative AGO1 homologous protein	7.78E-03	0.50	26.45
99	%GL28_R13	S10_56170095	-	-	4.46E-03	0.50	30.58

Table 21: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
100	%GL28_R13	S10_56170098	-	-	4.46E-03	0.50	30.58
101	%GL28_R13	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	8.90E-03	0.48	29.89
102	%GL28_R13	S10_60701880	Sb10g031030	Putative AGO1 homologous protein	4.03E-03	0.50	30.69
103	%GL28_R14	S10_50132488	-	-	4.35E-03	0.20	15.78
104	%GL28_R14	S10_54609172	-	-	2.35E-03	0.33	16.54
105	%GL28_R14	S10_54609179	-	-	2.35E-03	0.33	16.54
106	%GL28_R14	S10_54609181	-	-	2.35E-03	0.33	16.54
107	%GL28_R14	S10_46444139	Sb10g021077	zinc finger a RING-type-/ E3 ubiquitin ligase involved in grain weight/grain number	5.23E-03	0.41	15.55
108	%GL28_R14	S10_50672209	Sb10g022650	unknown protein	5.42E-03	0.43	15.51
109	%GL28_Across	S10_49288320	Sb10g022060	leucine-rich repeat family protein / protein kinase family protein	3.68E-03	0.41	23.80
110	%GL28_Across	S10_54591109	Sb10g025320	transducin family protein / WD-40 repeat family protein	9.38E-03	0.48	22.85
111	%GL28_Across	S10_54613971	Sb10g025340	40S ribosomal protein S14 (RPS14C)	9.49E-03	0.35	22.84
112	%GL28_Across	S10_60701880	Sb10g031030	Putative AGO1 homologous protein	1.00E-02	0.50	22.79
113	%GL35_R13	S10_53395971	-	-	7.16E-03	0.41	23.45
114	%GL35_R13	S10_56170095	-	-	7.79E-03	0.50	23.36
115	%GL35_R13	S10_56170098	-	-	7.79E-03	0.50	23.36

Table 21: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
116	%GL35_R13	S10_49288320	Sb10g022060	leucine-rich repeat family protein / protein kinase family protein	6.85E-03	0.41	23.50
117	%GL35_R13	S10_53681243	Sb10g024575	zinc-binding family protein	8.05E-03	0.35	23.32
118	%GL35_R13	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	8.36E-03	0.48	23.28
119	%GL35_R13	S10_60701880	Sb10g031030	Putative AGO1 homologous protein	2.48E-03	0.50	24.62
120	%GL35_R14	S10_48756049	-	-	3.20E-03	0.37	21.98
121	%GL35_R14	S10_54591109	Sb10g025320	transducin family protein / WD-40 repeat family protein	9.99E-03	0.48	20.65
122	%GL35_Across	S10_45646835	-	-	8.35E-03	0.42	17.14
123	%GL35_Across	S10_54591109	Sb10g025320	transducin family protein / WD-40 repeat family protein	1.11E-03	0.48	19.39
124	%GL42_R13	S10_53395971	-	-	2.70E-03	0.41	19.59
125	%GL42_R13	S10_53681243	Sb10g024575	zinc-binding family protein	2.66E-03	0.35	19.61
126	%GL42_R13	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	6.06E-03	0.48	18.64
127	%GL42_R13	S10_60384475	Sb10g030760	PLN03210; Resistant to P. syringae 6; Provisional	6.00E-03	0.38	18.65
128	%GL42_R13	S10_60701880	Sb10g031030	Putative AGO1 homologous protein	2.06E-03	0.50	19.92
129	%GL42_R14	S10_48365209	-	-	3.00E-04	0.35	16.38
130	%GL42_R14	S10_48365258	-	-	8.12E-04	0.34	15.01
131	%GL42_R14	S10_57253897	Sb10g027460	OsENODL1_like; Early nodulin-like protein	7.24E-03	0.49	12.12

Table 21: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
132	%GL42_R14	S10_59775260	Sb10g030040	Calcium/calmodulin-dependent protein kinase	9.53E-03	0.43	11.77
133	%GL42_R14	S10_59775288	Sb10g030040	Calcium/calmodulin-dependent protein kinase	9.53E-03	0.43	11.77
134	%GL42_R14	S10_59775290	Sb10g030040	Calcium/calmodulin-dependent protein kinase	9.53E-03	0.43	11.77
135	%GL42_Across	S10_53106278	-	-	7.16E-03	0.41	10.28
136	%GL42_Across	S10_53106289	-	-	7.16E-03	0.41	10.28
137	%GL42_Across	S10_56728368	-	-	8.78E-03	0.50	10.04
138	%GL42_Across	S10_54591109	Sb10g025320	transducin family protein / WD-40 repeat family protein	5.10E-03	0.48	10.68
139	%GL42_Across	S10_57253897	Sb10g027460	OsENODL1_like; Early nodulin-like protein	7.38E-03	0.49	10.24
140	%GL49_R13	S10_49639848	-	-	7.98E-03	0.28	27.24
141	%GL49_R13	S10_49639849	-	-	7.98E-03	0.28	27.24
142	%GL49_R13	S10_49288320	Sb10g022060	leucine-rich repeat family protein / protein kinase family protein	3.78E-04	0.41	30.52
143	%GL49_R13	S10_54535502	Sb10g025283	NBS-LRR disease resistance protein	5.95E-03	0.44	27.54
144	%GL49_R13	S10_54535507	Sb10g025283	NBS-LRR disease resistance protein	5.95E-03	0.44	27.54
145	%GL49_R13	S10_55649047	Sb10g026250	Glutaredoxin-C8 precursor	3.22E-03	0.48	28.19
146	%GL49_R13	S10_55649083	Sb10g026250	Glutaredoxin-C8 precursor	3.22E-03	0.48	28.19
147	%GL49_R13	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	7.80E-03	0.48	27.26

Table 21: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
148	%GL49_R14	S10_59808039	-	-	8.28E-03	0.42	11.97
149	%GL49_R14	S10_59808040	-	-	8.28E-03	0.42	11.97
150	%GL49_R14	S10_59808044	-	-	8.28E-03	0.42	11.97
151	%GL49_R14	S10_59808049	-	-	8.28E-03	0.42	11.97
152	%GL49_R14	S10_46477352	Sb10g021077	zinc finger a RING-type-/ E3 ubiquitin ligase involved in grain weight/grain number	3.13E-03	0.47	13.22
153	%GL49_R14	S10_48059523	Sb10g021730	GTP-binding protein (endocytosis)	9.54E-03	0.47	11.79
154	%GL49_R14	S10_55201667	Sb10g025880	Putative GDP-L-fucose synthase 2	4.73E-03	0.48	12.68
155	%GL49_R14	S10_59775260	Sb10g030040	Calcium/calmodulin-dependent protein kinase	4.25E-03	0.43	12.82
156	%GL49_R14	S10_59775288	Sb10g030040	Calcium/calmodulin-dependent protein kinase	4.25E-03	0.43	12.82
157	%GL49_R14	S10_59775290	Sb10g030040	Calcium/calmodulin-dependent protein kinase	4.25E-03	0.43	12.82
158	%GL49_Across	S10_51466711	-	-	9.14E-03	0.32	11.99
159	%GL49_Across	S10_49288320	Sb10g022060	leucine-rich repeat family protein / protein kinase family protein	4.28E-03	0.41	12.87
160	%GL49_Across	S10_50232566	Sb10g022450	Putative uncharacterized protein	8.70E-03	0.39	12.05
161	%GL49_Across	S10_50232568	Sb10g022450	Putative uncharacterized protein	8.70E-03	0.39	12.05
162	%GL49_Across	S10_50232569	Sb10g022450	Putative uncharacterized protein	8.70E-03	0.39	12.05
163	%GL49_Across	S10_54535502	Sb10g025283	NBS-LRR disease resistance protein	3.39E-03	0.44	13.14

Table 21: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
164	%GL49_Across	S10_54535507	Sb10g025283	NBS-LRR disease resistance protein	3.39E-03	0.44	13.14
165	%GL49_Across	S10_54613971	Sb10g025340	40S ribosomal protein S14 (RPS14C)	8.20E-03	0.35	12.11
166	%GL49_Across	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	9.86E-03	0.48	11.90
167	%GL49_Across	S10_59775260	Sb10g030040	Calcium/calmodulin-dependent protein kinase	6.17E-03	0.43	12.44
168	%GL49_Across	S10_59775288	Sb10g030040	Calcium/calmodulin-dependent protein kinase	6.17E-03	0.43	12.44
169	%GL49_Across	S10_59775290	Sb10g030040	Calcium/calmodulin-dependent protein kinase	6.17E-03	0.43	12.44

maf -minor allele frequency R13-rabi/summer 2013 R14-rabi/summer 2014

Table 22: GWAS for agronomic, yield related traits and their marker trait associations

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
1	FT_R 2013	S10_57275026	-	-	8.13E-03	47.70%	36.65%
2	FT_R 2013	S10_57275060	-	-	8.13E-03	47.70%	36.65%
3	FT_R 2013	S10_55457420	Sb10g026110	Putative (R)-(+)-mandelonitrile lyase isoform MDL3	5.10E-03	42.76%	37.07%
4	FT_R 2013	S10_59346842	-	-	8.90E-03	38.16%	36.57%
5	FT_R14	S10_56487055	-	-	2.83E-03	0.470395	22.32%
6	FT_R14	S10_56487022	-	-	4.45E-03	0.473684	21.80%
7	FT_R14	S10_56525509	-	-	5.26E-03	0.476974	21.61%
8	FT_R14	S10_58357041	Sb10g028550	Xyloglucan endotransglycosylase, member of glycosyl hydrolase family 16	6.84E-03	0.483553	21.31%
9	FT_R14	S10_56386714	-	-	7.29E-03	0.453947	21.24%
10	FT _across	S10_45926714	-	-	1.01E-03	0.391447	27.83%
11	FT _across	S10_45926740	-	-	1.01E-03	0.391447	27.83%
12	FT _across	S10_45926741	-	-	1.01E-03	0.391447	27.83%
13	FT _across	S10_49288320	Sb10g022060	leucine-rich repeat family protein / protein kinase family protein	1.27E-03	0.407895	27.59%
14	FT _across	S10_51466711	-	-	4.11E-03	0.322368	26.40%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
15	FT _across	S10_52675727	Sb10g023920	-	9.99E-03	0.401316	25.53%
16	FT _across	S10_52675753	Sb10g023920	-	9.99E-03	0.401316	25.53%
17	FT _across	S10_52675759	Sb10g023920	-	9.99E-03	0.401316	25.53%
18	FT _across	S10_54535502	Sb10g025283	NBS-LRR disease resistance protein	1.85E-03	0.440789	27.20%
19	FT _across	S10_54535507	Sb10g025283	NBS-LRR disease resistance protein	1.85E-03	0.440789	27.20%
20	FT _across	S10_54585199	Sb10g025310	Ankyrin repeat protein (plant fertility)/protein-cysteine S-palmitoleyltransferase activity	6.42E-03	0.404605	25.96%
21	FT _across	S10_54585201	Sb10g025310	Ankyrin repeat protein (plant fertility)/protein-cysteine S-palmitoleyltransferase activity	6.42E-03	0.404605	25.96%
22	FT _across	S10_54585202	Sb10g025310	Ankyrin repeat protein (plant fertility)/protein-cysteine S-palmitoleyltransferase activity	6.42E-03	0.404605	25.96%
23	FT _across	S10_54613971	Sb10g025340	-	4.04E-03	0.345395	26.42%
24	FT _across	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	9.19E-03	0.480263	25.61%
25	FT _across	S10_56525509	-	-	1.71E-03	0.476974	27.28%
26	PlHt_R13	S10_58663475	-	-	1.96E-03	43.75%	19.05%
27	PlHt_R13	S10_58663474	-	-	3.87E-03	40.13%	18.22%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
28	PlHt_R13	S10_57936622	-	-	4.70E-03	49.01%	17.98%
29	PlHt_R13	S10_53544398	Sb10g024460	Development and cell death domain=-N-rich protein, putative, expressed	1.62E-03	33.88%	19.28%
30	PlHt_R13	S10_53544426	Sb10g024460	Development and cell death domain=-N-rich protein, putative, expressed	1.62E-03	33.88%	19.28%
31	PlHt_R13	S10_59565625	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	6.36E-03	42.76%	17.62%
32	PlHt_R13	S10_59565627	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	6.36E-03	42.76%	17.62%
33	PlHt_R13	S10_59565629	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	6.36E-03	42.76%	17.62%
34	PlHt_R13	S10_59565631	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	6.36E-03	42.76%	17.62%
35	PlHt_R13	S10_59958913	Sb10g030270	" Putative receptor protein kinase"	6.56E-03	40.79%	17.59%
36	PlHt_R13	S10_58991881	-	-	7.29E-03	39.14%	17.46%
37	PlHt_R13	S10_59419148	Sb10g029670	transcription termination factor Rho; Provisional	7.44E-03	46.38%	17.44%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
38	PlHt_R14	S10_55299333	Sb10g025960	hypothetical protein; K03355 anaphase-promoting complex subunit 8	8.44E-03	0.371711	28.65%
39	PlHt_R14	S10_58663475	-	-	1.91E-03	0.4375	30.18%
40	PlHt_R14	S10_57276745	-	-	2.01E-03	0.5	30.13%
41	PlHt_R14	S10_60037942	-	-	6.87E-03	0.453947	28.86%
42	PlHt_R14	S10_57963498	-	-	7.77E-03	0.480263	28.73%
43	PlHt_R14	S10_59565625	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	1.90E-02	0.427632	27.85%
44	PlHt_R14	S10_59565627	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	1.90E-02	0.427632	27.85%
45	PlHt_R14	S10_59565629	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	1.90E-02	0.427632	27.85%
46	PlHt_R14	S10_59565631	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	1.90E-02	0.427632	27.85%
47	PlHt_across	S10_58663475	-	-	1.60E-04	0.4375	23.99%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
48	PlHt_across	S10_58817328	-	-	1.32E-03	0.450658	21.60%
49	PlHt_across	S10_58683017	Sb10g028870	" Putative meiotic serine proteinase"	1.38E-03	0.404605	21.55%
50	PlHt_across	S10_58991881	-	-	1.38E-03	0.391447	21.55%
51	PlHt_across	S10_58663474	-	-	2.31E-03	0.401316	20.98%
52	PlHt_across	S10_57936622	-	-	2.89E-03	0.490132	20.74%
53	PlHt_across	S10_59476435	Sb10g029740	AAI_LTSS: Alpha-Amylase Inhibitors (AAI), Lipid Transfer (LT) and Seed Storage (SS) Protein family;	3.70E-03	0.447368	20.48%
54	PlHt_across	S10_60037942	-	-	3.78E-03	0.453947	20.45%
55	PlHt_across	S10_58652432	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	5.19E-03	0.424342	20.11%
56	PlHt_across	S10_58954853	-	-	5.97E-03	0.421053	19.96%
57	PlHt_across	S10_59770217	-	-	6.66E-03	0.447368	19.85%
58	PlHt_across	S10_59596116	Sb10g029850	" Putative uncharacterized protein P0712G01.1.5	6.77E-03	0.447368	19.83%
59	PlHt_across	S10_59476388	Sb10g029740	AAI_LTSS: Alpha-Amylase Inhibitors (AAI), Lipid Transfer (LT) and Seed Storage (SS) Protein family;	6.95E-03	0.444079	19.80%
60	PlHt_across	S10_59476389	Sb10g029740	AAI_LTSS: Alpha-Amylase Inhibitors (AAI), Lipid Transfer (LT) and Seed Storage (SS) Protein family;	6.95E-03	0.444079	19.80%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
61	PlHt_across	S10_58436224	-	-	8.04E-03	0.345395	19.65%
62	PlHt_across	S10_58079690	Sb10g028200	Catalytic domain of Protein Kinases	8.17E-03	0.447368	19.63%
63	PlHt_across	S10_59018127	Sb10g029180	Catalytic domain of Protein Kinases	8.32E-03	0.470395	19.61%
64	PlHt_across	S10_59018128	Sb10g029180	Catalytic domain of Protein Kinases	8.32E-03	0.470395	19.61%
65	PlHt_across	S10_59202679	-	-	8.37E-03	0.427632	19.60%
66	PlHt_across	S10_59862187	Sb10g030140	" Endoglucanase 18"	8.49E-03	0.421053	19.59%
67	PlHt_across	S10_59558136	Sb10g029810	" MADS box transcription factor"	9.21E-03	0.490132	19.50%
68	PlHt_across	S10_58035226	Sb10g028140	AA-amino acid hydrolase	9.30E-03	0.463816	19.49%
69	PlHt_across	S10_58954854	-	-	9.75E-03	0.417763	19.44%
70	PlHt_across	S10_58954867	-	-	9.75E-03	0.417763	19.44%
71	PnDw/plot_R13	S10_48555177	Sb10g021860	ADP binding	5.93E-04	33.88%	18.40%
72	PnDw/plot_R13	S10_50085077	-	-	1.17E-03	30.26%	17.50%
73	PnDw/plot_R14	S10_51183880	-	-	3.18E-03	0.483553	9.63%
74	PnDw/plot_R14	S10_54608741	-	-	3.99E-03	0.391447	9.33%
75	PnDw/plot_R14	S10_60324265	-	-	4.56E-03	0.401316	9.15%
76	PnDw/plot_R14	S10_60324270	-	-	4.56E-03	0.401316	9.15%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
77	PnDw/plot_R14	S10_60324273	-	-	4.56E-03	0.401316	9.15%
78	PnDw/plot_R14	S10_60282257	Sb10g030610	" Putative uncharacterized protein P0548E04.1.9	7.48E-03	0.476974	8.50%
79	PnDw/plot_R14	S10_60282260	Sb10g030610	" Putative uncharacterized protein P0548E04.1.9	7.48E-03	0.476974	8.50%
80	PnDw/plot_R14	S10_60282261	Sb10g030610	" Putative uncharacterized protein P0548E04.1.9	7.48E-03	0.476974	8.50%
81	PnDw/plot_R14	S10_51753271	-	-	1.12E-02	0.476974	7.98%
82	PnDw/plot_R14	S10_51753276	-	-	1.12E-02	0.476974	7.98%
83	PnDw/plot_R14	S10_51753278	-	-	1.12E-02	0.476974	7.98%
84	PnDw/plot_R14	S10_51753298	-	-	1.12E-02	0.476974	7.98%
85	PnDw/plot_R14	S10_51753261	-	-	1.12E-02	0.476974	7.98%
86	PnDw/plot_R14	S10_51753262	-	-	1.12E-02	0.476974	7.98%
87	PnDw/plot_R14	S10_51753275	-	-	1.12E-02	0.476974	7.98%
88	PnDw/plot_R14	S10_60037971	-	-	1.17E-02	0.430921	7.92%
89	PnDw/plot_R14	S10_60037973	-	-	1.17E-02	0.430921	7.92%
90	PnDw/plot_R14	S10_60037972	-	-	1.17E-02	0.430921	7.92%
91	PnDw/plot_across	S10_60324265	-	-	4.18E-03	0.401316	10.15%
92	PnDw/plot_across	S10_60324270	-	-	4.18E-03	0.401316	10.15%
93	PnDw/plot_across	S10_60324273	-	-	4.18E-03	0.401316	10.15%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
94	PnDw/plot_across	S10_60308229	Sb10g030660	Exo70 exocyst complex subunit	5.98E-03	0.421053	9.72%
95	PnDw/plot_across	S10_60344724	Sb10g030710	Exo70 exocyst complex subunit	6.14E-03	0.407895	9.69%
96	PnDw/plot_across	S10_60344774	Sb10g030710	Exo70 exocyst complex subunit	6.14E-03	0.407895	9.69%
97	PnDw/plot_across	S10_59419148	Sb10g029670	transcription termination factor Rho; Provisional	7.14E-03	0.463816	9.51%
98	PnDw/plot_across	S10_48726890	-	-	7.79E-03	0.302632	9.40%
99	PnDw/plot_across	S10_60363633	Sb10g030730	Leucine rich repaeat domain like	7.82E-03	0.444079	9.40%
100	PnDw/plot_across	S10_60363642	Sb10g030730	Leucine rich repaeat domain like	7.82E-03	0.444079	9.40%
101	PnDw/plot_across	S10_60302803	-	-	8.65E-03	0.398026	9.28%
102	PnDw/plot_across	S10_60342789	-	-	9.27E-03	0.351974	9.20%
103	PnDw/plot_across	S10_54877733	Sb10g025540	Putative prolylcarboxypeptidase isoform 1	9.80E-03	0.407895	9.13%
104	GDW/Plot_R13	S10_54535439	Sb10g025283	NBS-LRR disease resistance protein	2.10E-03	42.11%	9.68%
105	GDW/Plot_R13	S10_54535492	Sb10g025283	NBS-LRR disease resistance protein	2.10E-03	42.11%	9.68%
106	GDW/Plot_R13	S10_48555177	Sb10g021860	ADP binding protein	4.18E-03	33.88%	8.75%
107	GDW/Plot_R13	S10_59866581	Sb10g030150	" Calcium-dependent protein kinase CPK1 adapter protein 2	4.34E-03	44.74%	8.70%
108	GDW/Plot_R13	S10_48726890	-	-	4.64E-03	30.26%	8.61%
109	GDW/Plot_R14	S10_51228412	Sb10g022900	Putative uncharacterized protein	4.99E-03	0.388158	7.99%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
110	GDW/Plot_R14	S10_51458244	-	-	5.70E-03	0.493421	7.82%
111	GDW/Plot_R14	S10_51753261	-	-	6.19E-03	0.476974	7.71%
112	GDW/Plot_R14	S10_51753262	-	-	6.19E-03	0.476974	7.71%
113	GDW/Plot_R14	S10_51753271	-	-	6.19E-03	0.476974	7.71%
114	GDW/Plot_R14	S10_51753275	-	-	6.19E-03	0.476974	7.71%
115	GDW/Plot_R14	S10_51753276	-	-	6.19E-03	0.476974	7.71%
116	GDW/Plot_R14	S10_51753278	-	-	6.19E-03	0.476974	7.71%
117	GDW/Plot_R14	S10_51753298	-	-	6.19E-03	0.476974	7.71%
118	GDW/Plot_R14	S10_56381721	-	-	7.42E-03	0.493421	7.47%
119	GDW/Plot_R14	S10_56381725	-	-	7.42E-03	0.493421	7.47%
120	GDW/plot_across	S10_60324265	-	-	4.18E-03	0.401316	10.15%
121	GDW/plot_across	S10_60324270	-	-	4.18E-03	0.401316	10.15%
122	GDW/plot_across	S10_60324273	-	-	4.18E-03	0.401316	10.15%
123	GDW/plot_across	S10_60308229	Sb10g030660	Exo70 exocyst complex subunit	5.98E-03	0.421053	9.72%
124	GDW/plot_across	S10_60344724	Sb10g030710	Exo70 exocyst complex subunit	6.14E-03	0.407895	9.69%
125	GDW/plot_across	S10_60344774	Sb10g030710	Exo70 exocyst complex subunit	6.14E-03	0.407895	9.69%
126	GDW/plot_across	S10_59419148	Sb10g029670	transcription termination factor Rho; Provisional	7.14E-03	0.463816	9.51%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
127	GDW/plot_across	S10_48726890	-	-	7.79E-03	0.302632	9.40%
128	GDW/plot_across	S10_60363633	Sb10g030730	Leucine rich repaeat domain like	7.82E-03	0.444079	9.40%
129	GDW/plot_across	S10_60363642	Sb10g030730	Leucine rich repaeat domain like	7.82E-03	0.444079	9.40%
130	GDW/plot_across	S10_60302803	-	-	8.65E-03	0.398026	9.28%
131	GDW/plot_across	S10_60342789	-	-	9.27E-03	0.351974	9.20%
132	GDW/plot_across	S10_54877733	Sb10g025540	Putative prolylcarboxypeptidase isoform 1	9.80E-03	0.407895	9.13%
133	HGM_R13	S10_55315031	Sb10g025990	Transducin/WD40 repeat-like superfamily protein	2.63E-05	42.76%	24.38%
134	HGM_R13	S10_55315036	Sb10g025990	Transducin/WD40 repeat-like superfamily protein	2.63E-05	42.76%	24.38%
135	HGM_R13	S10_51183880	-	-	1.73E-03	48.36%	18.91%
136	HGM_R13	S10_58311699	Sb10g028500	" Peroxidase 16 protein"	2.38E-03	50.00%	18.51%
137	HGM_R13	S10_58311702	Sb10g028500	" Peroxidase 16 protein"	2.38E-03	50.00%	18.51%
138	HGM_R13	S10_58311712	Sb10g028500	" Peroxidase 16 protein"	2.38E-03	50.00%	18.51%
139	HGM_R13	S10_58311714	Sb10g028500	" Peroxidase 16 protein"	2.38E-03	50.00%	18.51%
140	HGM_R13	S10_58311715	Sb10g028500	" Peroxidase 16 protein"	2.38E-03	50.00%	18.51%
141	HGM_R13	S10_58311716	Sb10g028500	" Peroxidase 16 protein"	2.38E-03	50.00%	18.51%
142	HGM_R13	S10_58311717	Sb10g028500	" Peroxidase 16 protein"	2.38E-03	50.00%	18.51%
143	HGM_R13	S10_58311719	Sb10g028500	" Peroxidase 16 protein"	2.38E-03	50.00%	18.51%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
144	HGM_R13	S10_58311720	Sb10g028500	" Peroxidase 16 protein"	2.38E-03	50.00%	18.51%
145	HGM_R13	S10_49085931	-	-	2.54E-03	35.20%	18.43%
146	HGM_R13	S10_14567893	-	-	3.35E-03	35.86%	18.09%
147	HGM_R13	S10_59866581	Sb10g030150	" Calcium-dependent protein kinase CPK1 adapter protein 2	3.67E-03	44.74%	17.98%
148	HGM_R13	S10_58683017	Sb10g028870	" Putative meiotic serine proteinase"	4.32E-03	40.46%	17.78%
149	HGM_R13	S10_48938045	-	-	4.47E-03	36.18%	17.74%
150	HGM_R13	S10_58039451	-	-	5.17E-03	47.70%	17.57%
151	HGM_R13	S10_58039503	-	-	5.17E-03	47.70%	17.57%
152	HGM_R13	S10_58039504	-	-	5.17E-03	47.70%	17.57%
153	HGM_R13	S10_58039505	-	-	5.17E-03	47.70%	17.57%
154	HGM_R13	S10_58039507	-	-	5.17E-03	47.70%	17.57%
155	HGM_R13	S10_58039508	-	-	5.17E-03	47.70%	17.57%
156	HGM_R13	S10_57331300	-	-	6.12E-03	45.72%	17.36%
157	HGM_R13	S10_54138397	-	-	6.49E-03	36.18%	17.29%
158	HGM_R13	S10_59419148	Sb10g029670	transcription termination factor Rho; Provisional	6.55E-03	46.38%	17.28%
159	HGM_R13	S10_59476435	Sb10g029740	AAI_LTSS: Alpha-Amylase Inhibitors (AAI), Lipid Transfer (LT) and Seed Storage (SS) Protein family;	6.58E-03	44.74%	17.28%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
160	HGM_R13	S10_54138396	-	-	7.34E-03	35.53%	17.15%
161	HGM_R13	S10_59476388	Sb10g029740	AAI_LTSS: Alpha-Amylase Inhibitors (AAI), Lipid Transfer (LT) and Seed Storage (SS) Protein family;	7.54E-03	44.41%	17.11%
162	HGM_R13	S10_59476389	Sb10g029740	AAI_LTSS: Alpha-Amylase Inhibitors (AAI), Lipid Transfer (LT) and Seed Storage (SS) Protein family;	7.54E-03	44.41%	17.11%
163	HGM_R13	S10_5641743	-	-	8.35E-03	33.55%	16.99%
164	HGM_R13	S10_53670803	Sb10g024570	-	8.66E-03	33.88%	16.95%
165	HGM_R13	S10_17496821	-	-	9.43E-03	35.86%	16.85%
166	HGM_R13	S10_16803414	-	-	9.91E-03	40.13%	16.79%
167	HGM_R14	S10_51183880	-	-	1.69E-03	0.483553	10.14%
168	HGM_R14	S10_49314656	-	-	5.03E-03	0.407895	8.67%
169	HGM_R14	S10_49314673	-	-	5.03E-03	0.407895	8.67%
170	HGM_R14	S10_52902918	-	-	5.38E-03	0.365132	8.59%
171	HGM_R14	S10_52902920	-	-	5.38E-03	0.365132	8.59%
172	HGM_R14	S10_52902923	-	-	5.38E-03	0.365132	8.59%
173	HGM_R14	S10_52902925	-	-	5.38E-03	0.365132	8.59%
174	HGM_R14	S10_52902921	-	-	5.38E-03	0.365132	8.59%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
175	HGM_R14	S10_52902924	-	-	5.38E-03	0.365132	8.59%
176	HGM_R14	S10_52902926	-	-	5.38E-03	0.365132	8.59%
177	HGM_R14	S10_50890593	-	-	7.56E-03	0.421053	8.14%
178	HGM_R14	S10_52241685	Sb10g023600	-	9.22E-03	0.322368	7.88%
179	HGM_across	S10_51183880	-	-	4.04E-04	0.483553	8.26%
180	HGM_across	S10_59476435	Sb10g029740	AAI_LTSS: Alpha-Amylase Inhibitors (AAI), Lipid Transfer (LT) and Seed Storage (SS) Protein family;	7.21E-04	0.447368	7.48%
181	HGM_across	S10_59866581	Sb10g030150	" Calcium-dependent protein kinase CPK1 adapter protein 2	9.68E-04	0.447368	7.09%
182	HGM_across	S10_59476388	Sb10g029740	AAI_LTSS: Alpha-Amylase Inhibitors (AAI), Lipid Transfer (LT) and Seed Storage (SS) Protein family;	1.01E-03	0.444079	7.03%
183	HGM_across	S10_59476389	Sb10g029740	AAI_LTSS: Alpha-Amylase Inhibitors (AAI), Lipid Transfer (LT) and Seed Storage (SS) Protein family;	1.01E-03	0.444079	7.03%
184	HGM_across	S10_58050683	Sb10g028160	N-terminal domain (domain I) of transcription elongation factor S-II (TFIIS)	1.10E-03	0.476974	6.91%
185	HGM_across	S10_58039451	-	-	1.49E-03	0.476974	6.51%
186	HGM_across	S10_58039503	-	-	1.49E-03	0.476974	6.51%
187	HGM_across	S10_58039504	-	-	1.49E-03	0.476974	6.51%
188	HGM_across	S10_58039505	-	-	1.49E-03	0.476974	6.51%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
189	HGM_across	S10_58039507	-	-	1.49E-03	0.476974	6.51%
190	HGM_across	S10_58039508	-	-	1.49E-03	0.476974	6.51%
191	HGM_across	S10_55315031	Sb10g025990	PubMed=19936069	1.63E-03	0.427632	6.40%
192	HGM_across	S10_55315036	Sb10g025990	Transducin/WD40 repeat-like superfamily protein	1.63E-03	0.427632	6.40%
193	HGM_across	S10_58683017	Sb10g028870	" Putative meiotic serine proteinase"	1.97E-03	0.404605	6.15%
194	HGM_across	S10_57254019	Sb10g027460	OsENODL1_like; Early nodulin-like protein	2.29E-03	0.493421	5.95%
195	HGM_across	S10_59609220	-	-	2.42E-03	0.421053	5.88%
196	HGM_across	S10_59419148	Sb10g029670	transcription termination factor Rho; Provisional	2.48E-03	0.463816	5.85%
197	HGM_across	S10_58309478	-	-	2.56E-03	0.381579	5.81%
198	HGM_across	S10_58309481	-	-	2.56E-03	0.381579	5.81%
199	HGM_across	S10_58309482	-	-	2.56E-03	0.381579	5.81%
200	HGM_across	S10_58309484	-	-	2.56E-03	0.381579	5.81%
201	HGM_across	S10_58309486	-	-	2.56E-03	0.381579	5.81%
202	HGM_across	S10_58309487	-	-	2.56E-03	0.381579	5.81%
203	HGM_across	S10_49314656	-	-	2.77E-03	0.407895	5.70%
204	HGM_across	S10_49314673	-	-	2.77E-03	0.407895	5.70%
205	HGM_across	S10_58954853	-	-	2.95E-03	0.421053	5.62%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
206	HGM_across	S10_60363633	Sb10g030730	-	3.00E-03	0.444079	5.61%
207	HGM_across	S10_60363642	Sb10g030730	-	3.00E-03	0.444079	5.61%
208	HGM_across	S10_59020325	Sb10g029190	" Squamosa promoter-binding-like protein 12"	3.55E-03	0.4375	5.39%
209	HGM_across	S10_57808411	Sb10g027950	AT5MAT; O-malonyltransferase/ transferase	3.70E-03	0.486842	5.33%
210	HGM_across	S10_57808412	Sb10g027950	AT5MAT; O-malonyltransferase/ transferase	3.70E-03	0.486842	5.33%
211	HGM_across	S10_58764141	-	-	4.17E-03	0.486842	5.18%
212	HGM_across	S10_58764156	-	-	4.17E-03	0.486842	5.18%
213	HGM_across	S10_58764158	-	-	4.17E-03	0.486842	5.18%
214	HGM_across	S10_58532130	Sb10g028670	Protein of unknown function (DUF1336)	4.55E-03	0.421053	5.07%
215	HGM_across	S10_53670803	Sb10g024570	-	4.65E-03	0.338816	5.04%
216	HGM_across	S10_58652516	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	4.70E-03	0.296053	5.03%
217	HGM_across	S10_58954854	-	-	4.71E-03	0.417763	5.02%
218	HGM_across	S10_58954867	-	-	4.71E-03	0.417763	5.02%
219	HGM_across	S10_57248800	Sb10g027440	Putative uncharacterized protein	4.77E-03	0.486842	5.01%
220	HGM_across	S10_58311699	Sb10g028500	" Peroxidase 16 protein"	5.15E-03	0.5	4.91%
221	HGM_across	S10_58311702	Sb10g028500	" Peroxidase 16 protein"	5.15E-03	0.5	4.91%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
222	HGM_across	S10_58311712	Sb10g028500	" Peroxidase 16 protein"	5.15E-03	0.5	4.91%
223	HGM_across	S10_58311714	Sb10g028500	" Peroxidase 16 protein"	5.15E-03	0.5	4.91%
224	HGM_across	S10_58311715	Sb10g028500	" Peroxidase 16 protein"	5.15E-03	0.5	4.91%
225	HGM_across	S10_58311716	Sb10g028500	" Peroxidase 16 protein"	5.15E-03	0.5	4.91%
226	HGM_across	S10_58311717	Sb10g028500	" Peroxidase 16 protein"	5.15E-03	0.5	4.91%
227	HGM_across	S10_58311719	Sb10g028500	" Peroxidase 16 protein"	5.15E-03	0.5	4.91%
228	HGM_across	S10_58311720	Sb10g028500	" Peroxidase 16 protein"	5.15E-03	0.5	4.91%
229	HGM_across	S10_59554262	Sb10g029810	" MADS box transcription factor"	5.72E-03	0.463816	4.78%
230	HGM_across	S10_59565625	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	5.76E-03	0.427632	4.77%
231	HGM_across	S10_59565627	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	5.76E-03	0.427632	4.77%
232	HGM_across	S10_59565629	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	5.76E-03	0.427632	4.77%
233	HGM_across	S10_59565631	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	5.76E-03	0.427632	4.77%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
234	HGM_across	S10_58425791	-	-	5.86E-03	0.444079	4.75%
235	HGM_across	S10_58426868	-	-	5.98E-03	0.368421	4.72%
236	HGM_across	S10_59018127	Sb10g029180	Catalytic domain of Protein Kinases	6.06E-03	0.470395	4.70%
237	HGM_across	S10_59018128	Sb10g029180	Catalytic domain of Protein Kinases	6.06E-03	0.470395	4.70%
238	HGM_across	S10_56923319	-	-	6.35E-03	0.460526	4.65%
239	HGM_across	S10_58365099	-	-	6.40E-03	0.440789	4.63%
240	HGM_across	S10_58764081	-	-	7.00E-03	0.361842	4.52%
241	HGM_across	S10_58764085	-	-	7.00E-03	0.361842	4.52%
242	HGM_across	S10_58764097	-	-	7.00E-03	0.361842	4.52%
243	HGM_across	S10_57331300	-	-	7.11E-03	0.457237	4.50%
244	HGM_across	S10_58652539	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	7.13E-03	0.404605	4.50%
245	HGM_across	S10_58652548	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	7.13E-03	0.404605	4.50%
246	HGM_across	S10_58652554	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	7.13E-03	0.404605	4.50%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
247	HGM_across	S10_58652555	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	7.13E-03	0.404605	4.50%
248	HGM_across	S10_58652556	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	7.13E-03	0.404605	4.50%
249	HGM_across	S10_56899442	Sb10g027180	Protein prenyltransferase alpha subunit repeat	7.24E-03	0.490132	4.48%
250	HGM_across	S10_59596203	Sb10g029850	" Putative uncharacterized protein P0712G01.1.5	7.26E-03	0.417763	4.48%
251	HGM_across	S10_60333765	Sb10g030700	Exo70 exocyst complex subunit	7.65E-03	0.460526	4.41%
252	HGM_across	S10_59215385	Sb10g029392	Multidrug and toxic compound extrusion family and similar proteins	8.45E-03	0.470395	4.29%
253	HGM_across	S10_60631094	-	-	8.76E-03	0.496711	4.24%
254	HGM_across	S10_58436224	-	-	8.94E-03	0.345395	4.21%
255	HGM_across	S10_60037942	-	-	9.12E-03	0.453947	4.19%
256	HGM_across	S10_56595404	-	-	9.38E-03	0.463816	4.16%
257	HGM_across	S10_59946956	Sb10g030260	" Putative senescence-associated protein"	9.38E-03	0.4375	4.16%
258	HGM_across	S10_59946962	Sb10g030260	" Putative senescence-associated protein"	9.38E-03	0.4375	4.16%
259	HGM_across	S10_59946968	Sb10g030260	" Putative senescence-associated protein"	9.38E-03	0.4375	4.16%
260	HGM_across	S10_56595416	-	-	9.49E-03	0.470395	4.14%
261	GNP/plot_R13	S10_50809289	Sb10g022730	protein serine/threonine kinase activity	9.91E-04	29.61%	20.93%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
262	GNP/plot_R13	S10_53160761	Sb10g024190	Basic-leucine zipper (bZIP) transcription factor	2.82E-03	38.49%	19.77%
263	GNP/plot_R13	S10_53681243	Sb10g024575	zinc-binding family protein	5.47E-03	34.54%	19.04%
264	GNP/plot_R13	S10_54877559	Sb10g025540	Putative prolylcarboxypeptidase isoform 1	5.75E-03	28.95%	18.99%
265	GNP/plot_R13	S10_53191736	Sb10g024220	polyphenol oxidase, putative, expressed	6.57E-03	34.54%	18.85%
266	GNP/plot_R13	S10_53092316	Sb10g024180	ARABIDOPSIS RESPONSE REGULATOR 10-Myb-like DNA-binding domain,(gl3_maize2012)	6.77E-03	22.70%	18.81%
267	GNP/plot_R13	S10_54101884	-	-	9.30E-03	39.47%	18.48%
268	GNP/plot_R14	S10_56381721	-	-	8.36E-03	0.493421	5.73%
269	GNP/plot_R14	S10_56381725	-	-	8.36E-03	0.493421	5.73%
270	GNP/plot_across	S10_56381721	-	-	1.09E-03	0.493421	12.95%
271	GNP/plot_across	S10_56381725	-	-	1.09E-03	0.493421	12.95%
272	GNP/plot_across	S10_48726890	-	-	6.64E-03	0.302632	10.76%
273	GNP/plot_across	S10_54877559	Sb10g025540	Putative prolylcarboxypeptidase isoform 1	7.27E-03	0.289474	10.65%
274	GNPP_R13	S10_50809289	Sb10g022730	protein serine/threonine kinase activity	9.91E-04	29.61%	20.93%
275	GNPP_R13	S10_53160761	Sb10g024190	Basic-leucine zipper (bZIP) transcription factor	2.82E-03	38.49%	19.77%
276	GNPP_R13	S10_53681243	Sb10g024575	zinc-binding family protein	5.47E-03	34.54%	19.04%
277	GNPP_R13	S10_54877559	Sb10g025540	Putative prolylcarboxypeptidase isoform 1	5.75E-03	28.95%	18.99%

Table 22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
278	GNPP_R13	S10_53191736	Sb10g024220	polyphenol oxidase, putative, expressed	6.57E-03	34.54%	18.85%
279	GNPP_R13	S10_53092316	Sb10g024180	ARABIDOPSIS RESPONSE REGULATOR 10-Myb-like DNA-binding domain,(gl3_maize2012)	6.77E-03	22.70%	18.81%
280	GNPP_R13	S10_54101884	-	-	9.30E-03	39.47%	18.48%
281	GNPP_R14	S10_56381721	-	-	8.36E-03	0.493421	5.73%
282	GNPP_R14	S10_56381725	-	-	8.36E-03	0.493421	5.73%
283	GNPP_across	S10_56381721	-	-	1.09E-03	0.493421	12.95%
284	GNPP_across	S10_56381725	-	-	1.09E-03	0.493421	12.95%
285	GNPP_across	S10_48726890	-	-	6.64E-03	0.302632	10.76%
286	GNPP_across	S10_54877559	Sb10g025540	Putative prolylcarboxypeptidase isoform 1	7.27E-03	0.289474	10.65%
287	PHI_R13	S10_56206662	Sb10g026760	Zinc finger POZ domain protein	4.56E-04	0.388158	17.97%
288	PHI_R13	S10_57552456	Sb10g027760	F-box domain	2.78E-03	0.483553	15.64%
289	PHI_R13	S10_59930523	Sb10g030230	TLP-PA; allergenic/antifungal thaumatin-like proteins	9.57E-03	0.430921	14.12%
290	PHI_R14	S10_53160761	Sb10g024190	Basic-leucine zipper (bZIP) transcription factor	5.14E-03	0.384868	14.88%
291	PHI_R14	S10_56206662	Sb10g026760	Zinc finger POZ domain protein	4.56E-04	0.388158	17.97%
292	PHI_R14	S10_57552456	Sb10g027760	F-box domain	2.78E-03	0.483553	15.64%
293	PHI_R14	S10_59930523	Sb10g030230	TLP-PA; allergenic/antifungal thaumatin-like proteins	9.57E-03	0.430921	14.12%

Table22: (Contd..)

S No.	Trait	SNP	Gene Id	Functional annotation	P.value	maf	%R^2
294	PHI_across	S10_50469983			4.43E-03	0.411184	14.38%
295	PHI_across	S10_53746012			6.05E-03	0.375	14.02%
296	PHI_across	S10_53746011			6.62E-03	0.378289	13.92%
297	PHI_across	S10_54877559	Sb10g025540	Putative prolylcarboxypeptidase isoform 1	7.21E-03	0.289474	13.82%
298	PHI_across	S10_53746018			9.76E-03	0.365132	13.48%
299	PHI_across	S10_53746019			9.76E-03	0.365132	13.48%

Table 23: GWAS for Shoot fly resistance and marker trait associations

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
1	Gls _K13	*Xgap001*	SSR	-	6.05E-04	0.3618	22.22%
2	Gls _K13	S10_56730378	Sb10g027090	serine/arginine repetitive matrix protein 2-like	1.75E-03	0.4836	20.93%
3	Gls _K13	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	7.61E-03	0.4803	19.21%
4	Gls _K13	S10_55526794	Sb10g026170	hypothetical protein	8.75E-03	0.4309	19.05%
5	Gls _K13	S10_59889374	Sb10g030175	rod shape-determining protein MreC	9.06E-03	0.4079	19.01%
6	Gls _K13	S10_54185546	Sb10g025010	leucine-rich repeat transmembrane protein kinase(GO:0005575: The part of a cell or its extracellular environment in which a gene product is located)	9.91E-03	0.3322	18.91%
7	Gls _R13	S10_56734981	-	-	6.31E-04	0.4737	25.48%
8	Gls _R13	S10_54593246	Sb10g025320	transducin family protein / WD-40 repeat family protein	6.34E-04	0.3586	25.48%
9	Gls _R13	S10_54138396	-	-	1.46E-03	0.3553	24.51%
10	Gls _R13	S10_54646082	Sb10g025360	SAM (Sterile Alpha Motif)	2.04E-03	0.375	24.12%
11	Gls _R13	S10_54185546	Sb10g025010	leucine-rich repeat transmembrane protein kinase(GO:0005575: The part of a cell or its extracellular environment in which a gene product is located)	2.45E-03	0.3322	23.92%
12	Gls _R13	S10_54138397	-	-	2.73E-03	0.3618	23.79%
13	Gls _R13	*Xgap001*	SSR	-	2.78E-03	0.3618	23.78%
14	Gls _R13	S10_56158409	Sb10g026690	OsMADS30 - MADS-box family gene with MIKCc type-box, expressed	3.35E-03	0.4211	23.56%
15	Gls _R13	S10_56158458	Sb10g026690	OsMADS30 - MADS-box family gene with MIKCc type-box, expressed	3.35E-03	0.4211	23.56%
16	Gls _R13	S10_54185539	Sb10g025010	leucine-rich repeat transmembrane protein kinase(GO:0005575: The part of a cell or its extracellular environment in which a gene product is located)	4.09E-03	0.3355	23.34%
17	Gls _R13	S10_54138399	-	-	5.05E-03	0.3717	23.11%
18	Gls _R13	S10_54527903	Sb10g025280	Vacuolar H+-pyrophosphatase	5.52E-03	0.398	23.01%
19	Gls _R13	S10_55200530	Sb10g025880	Putative GDP-L-fucose synthase 2	5.70E-03	0.4934	22.97%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
20	Gls _R13	S10_54532800	Sb10g025283	NBS-LRR disease resistance protein	6.70E-03	0.3421	22.79%
21	Gls _R13	S10_54907720	-	-	7.45E-03	0.4375	22.68%
22	Gls _R13	S10_55617097	-	-	7.76E-03	0.4375	22.63%
23	Gls _R13	S10_54138395	-	-	9.28E-03	0.3947	22.44%
24	Gls Across	*Xgap001*	SSR	-	6.78E-05	0.3618	25.05%
25	Gls Across	S10_56730378	Sb10g027090	serine/arginine repetitive matrix protein 2-like	3.77E-04	0.4836	23.07%
26	Gls Across	S10_54593246	Sb10g025320	Transducin family protein / WD-40 repeat family protein	5.33E-04	0.3586	22.67%
27	Gls Across	S10_54185546	Sb10g025010	leucine-rich repeat transmembrane protein kinase(GO:0005575: The part of a cell or its extracellular environment in which a gene product is located)	6.13E-04	0.3322	22.51%
28	Gls Across	S10_56158409	Sb10g026690	OsMADS30 - MADS-box family gene with MIKCc type-box, expressed	9.04E-04	0.4211	22.08%
29	Gls Across	S10_56158458	Sb10g026690	OsMADS30 - MADS-box family gene with MIKCc type-box, expressed	9.04E-04	0.4211	22.08%
30	Gls Across	S10_54185539	Sb10g025010	leucine-rich repeat transmembrane protein kinase(GO:0005575: The part of a cell or its extracellular environment in which a gene product is located)	9.50E-04	0.3355	22.02%
31	Gls Across	S10_54646082	Sb10g025360	SAM (Sterile Alpha Motif)	1.03E-03	0.375	21.93%
32	Gls Across	S10_54138396	-	-	1.19E-03	0.3553	21.77%
33	Gls Across	S10_54907720	-	-	1.27E-03	0.4375	21.70%
34	Gls Across	S10_54527903	Sb10g025280	Vacuolar H+-pyrophosphatase	1.55E-03	0.398	21.48%
35	Gls Across	S10_56216953	Sb10g026790	Zinc finger POZ domain protein	1.59E-03	0.4803	21.45%
36	Gls Across	S10_54101884	-	-	2.32E-03	0.3947	21.03%
37	Gls Across	S10_54138397	-	-	3.37E-03	0.3618	20.63%
38	Gls Across	S10_60245187	Sb10g030580	DUF241; Arabidopsis protein of unknown function	4.22E-03	0.4572	20.39%
39	Gls Across	S10_54655292	Sb10g025370	Putative uncharacterized protein	4.26E-03	0.3355	20.38%
40	Gls Across	S10_55200530	Sb10g025880	Putative GDP-L-fucose synthase 2	4.43E-03	0.4934	20.33%
41	Gls Across	S10_54184991	Sb10g025010	leucine-rich repeat transmembrane protein kinase(GO:0005575: The part of a cell or its extracellular environment in which a gene product is located)	4.48E-03	0.3618	20.32%
42	Gls Across	S10_56350371	Sb10g026940	Peptidyl-prolyl cis-trans isomerase	4.62E-03	0.4013	20.29%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
43	Gls Across	S10_54584167	Sb10g025310	Ankyrin repeat protein (plant fertility)/protein-cysteine S-palmitoleyltransferase activity	4.76E-03	0.3618	20.26%
44	Gls Across	S10_56773166	Sb10g027100	NAC domain protein NAC1	4.89E-03	0.4934	20.23%
45	Gls Across	S10_56485883	-	-	5.00E-03	0.4539	20.21%
46	Gls Across	S10_54186806	Sb10g025010	leucine-rich repeat transmembrane protein kinase(GO:0005575: The part of a cell or its extracellular environment in which a gene product is located)	5.20E-03	0.3454	20.16%
47	Gls Across	S10_56738165	-	-	6.12E-03	0.4836	19.99%
48	Gls Across	S10_56738166	-	-	6.12E-03	0.4836	19.99%
49	Gls Across	S10_56738167	-	-	6.12E-03	0.4836	19.99%
50	Gls Across	S10_56728353	-	-	6.56E-03	0.4803	19.92%
51	Gls Across	S10_54532800	Sb10g025283	NBS-LRR disease resistance protein	6.59E-03	0.3421	19.91%
52	Gls Across	S10_54186799	Sb10g025010	leucine-rich repeat transmembrane protein kinase(GO:0005575: The part of a cell or its extracellular environment in which a gene product is located)	6.74E-03	0.3487	19.89%
53	Gls Across	S10_54186809	Sb10g025010	leucine-rich repeat transmembrane protein kinase(GO:0005575: The part of a cell or its extracellular environment in which a gene product is located)	6.74E-03	0.3487	19.89%
54	Gls Across	S10_54186811	Sb10g025010	leucine-rich repeat transmembrane protein kinase(GO:0005575: The part of a cell or its extracellular environment in which a gene product is located)	6.74E-03	0.3487	19.89%
55	Gls Across	S10_54532995	Sb10g025283	NBS-LRR disease resistance protein	7.94E-03	0.4046	19.72%
56	Gls Across	S10_55600708	Sb10g026200	SBP domain protein 4	8.19E-03	0.4046	19.68%
57	Gls Across	S10_55748444	Sb10g026300	auxin efflux carrier component 2	8.22E-03	0.4145	19.68%
58	Gls Across	S10_56376491	Sb10g026970	Auxin responsive protein	8.95E-03	0.4704	19.59%
59	TDL_K13	S10_57432493	Sb10g027640	Omethyl transferase	5.37E-08	0.4704	45.29%
60	TDL_K13	S10_57453669	-	-	3.73E-07	0.4474	42.96%
61	TDL_K13	S10_57427894	-	-	6.99E-07	0.4967	42.22%
62	TDL_K13	S10_57548453	-	-	9.20E-07	0.4901	41.90%
63	TDL_K13	S10_57484592	-	-	1.73E-06	0.5	41.17%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
64	TDL_K13	S10_57484595	-	-	1.73E-06	0.5	41.17%
65	TDL_K13	S10_57484598	-	-	1.73E-06	0.5	41.17%
66	TDL_K13	S10_57484597	-	-	1.73E-06	0.5	41.17%
67	TDL_K13	S10_57963498	-	-	1.85E-06	0.4803	41.10%
68	TDL_K13	S10_58035226	Sb10g028140	AA-amino acid hydrolase	2.75E-06	0.4638	40.64%
69	TDL_K13	S10_57773700	-	-	3.59E-06	0.4737	40.34%
70	TDL_K13	S10_57453732	-	-	4.68E-06	0.4704	40.03%
71	TDL_K13	S10_58356653	-	-	5.46E-06	0.4507	39.86%
72	TDL_K13	S10_57145296	Sb10g027370	PBP1_GABAb_receptor	5.65E-06	0.4901	39.82%
73	TDL_K13	S10_56958200	Sb10g027240	Trans_IPPS_HT; Trans-Isoprenyl Diphosphate Synthases, head-to-tail	7.25E-06	0.5	39.54%
74	TDL_K13	S10_58069749	-	-	8.66E-06	0.4704	39.34%
75	TDL_K13	S10_57623967	Sb10g027790	" Auxin response factor 18"	1.02E-05	0.4638	39.16%
76	TDL_K13	S10_57400347	Sb10g027610	" EF-hand Ca2+-binding protein CCD1"	1.19E-05	0.4737	38.99%
77	TDL_K13	S10_58532130	Sb10g028670	Protein of unknown function (DUF1336)	1.93E-05	0.4211	38.45%
78	TDL_K13	S10_56869829	-	-	1.97E-05	0.5	38.43%
79	TDL_K13	S10_56869835	-	-	1.97E-05	0.5	38.43%
80	TDL_K13	S10_57936436	-	-	2.00E-05	0.4671	38.41%
81	TDL_K13	S10_58022908	Sb10g028130	" Putative thaumatin-protein"	2.02E-05	0.4408	38.40%
82	TDL_K13	S10_57253966	Sb10g027460	OsENODL1_like; Early nodulin-like protein	2.12E-05	0.4967	38.34%
83	TDL_K13	S10_57253967	Sb10g027460	OsENODL1_like; Early nodulin-like protein	2.12E-05	0.4967	38.34%
84	TDL_K13	S10_58048950	Sb10g028160	N-terminal domain (domain I) of transcription elongation factor S-II (TFIIS)	2.29E-05	0.4638	38.26%
85	TDL_K13	S10_58079690	Sb10g028200	Catalytic domain of Protein Kinases	2.36E-05	0.4474	38.23%
86	TDL_K13	S10_57490614	Sb10g027680	" Armadillo/beta-catenin repeat protein-like"	2.38E-05	0.4803	38.22%
87	TDL_K13	S10_57403166	-	-	2.39E-05	0.3059	38.21%
88	TDL_K13	S10_56930141	-	-	2.62E-05	0.4868	38.11%
89	TDL_K13	S10_58022907	Sb10g028130	" Putative thaumatin-protein"	2.71E-05	0.4441	38.07%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
90	TDL_K13	S10_59020325	Sb10g029190	" Squamosa promoter-binding-like protein 12"	3.07E-05	0.4375	37.94%
91	TDL_K13	Xtxp141	-	-	4.14E-05	0.4704	37.61%
92	TDL_K13	S10_57423429	-	-	4.32E-05	0.4737	37.57%
93	TDL_K13	S10_57022696	-	-	4.60E-05	0.4474	37.50%
94	TDL_K13	S10_57331278	-	-	5.95E-05	0.5	37.22%
95	TDL_K13	S10_57403220	-	-	8.29E-05	0.4342	36.86%
96	TDL_K13	S10_57830472	-	-	8.67E-05	0.4704	36.81%
97	TDL_K13	S10_57830492	-	-	8.67E-05	0.4704	36.81%
98	TDL_K13	S10_57830499	-	-	8.67E-05	0.4704	36.81%
99	TDL_K13	S10_57785077	-	-	9.97E-05	0.4243	36.66%
100	TDL_K13	S10_56932767	-	-	1.20E-04	0.5	36.47%
101	TDL_K13	S10_57936524	-	-	1.28E-04	0.4474	36.40%
102	TDL_K13	S10_59082143	Sb10g029270	" Delta-aminolevulinic acid dehydratase"	1.28E-04	0.4178	36.39%
103	TDL_K13	S10_59082132	Sb10g029270	" Delta-aminolevulinic acid dehydratase"	1.28E-04	0.4178	36.39%
104	TDL_K13	S10_56945933	-	-	1.41E-04	0.4934	36.30%
105	TDL_K13	S10_57449076	-	-	1.46E-04	0.4868	36.26%
106	TDL_K13	S10_57251912	Sb10g027450	" 40S ribosomal protein S14-1"	1.56E-04	0.4934	36.19%
107	TDL_K13	S10_58663474	-	-	2.10E-04	0.4013	35.87%
108	TDL_K13	S10_56730378	Sb10g027090	serine/arginine repetitive matrix protein 2-like	2.27E-04	0.4836	35.79%
109	TDL_K13	S10_58954853	-	-	2.35E-04	0.4211	35.75%
110	TDL_K13	S10_57808411	Sb10g027950	AT5MAT; O-malonyltransferase/ transferase	2.66E-04	0.4868	35.62%
111	TDL_K13	S10_57808412	Sb10g027950	AT5MAT; O-malonyltransferase/ transferase	2.66E-04	0.4868	35.62%
112	TDL_K13	S10_59434640	Sb10g029690	" GCIP-interacting family protein-like"	3.08E-04	0.4046	35.47%
113	TDL_K13	S10_57303312	-	-	3.40E-04	0.3618	35.37%
114	TDL_K13	S10_57831107	Sb10g027980	Cysteine protease Mir1	3.41E-04	0.4901	35.36%
115	TDL_K13	S10_58299415	-	-	3.47E-04	0.4474	35.35%
116	TDL_K13	S10_59571447	-	-	3.63E-04	0.4309	35.30%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
117	TDL_K13	S10_58663475	-	-	3.95E-04	0.4375	35.21%
118	TDL_K13	S10_56937708	-	-	4.03E-04	0.4704	35.19%
119	TDL_K13	S10_58954854	-	-	4.07E-04	0.4178	35.18%
120	TDL_K13	S10_58954867	-	-	4.07E-04	0.4178	35.18%
121	TDL_K13	S10_57031008	Sb10g027280	similar to Putative transcription factor GAMyb	4.17E-04	0.4507	35.15%
122	TDL_K13	S10_58310186	Sb10g028500	" Peroxidase 16 protein"	4.64E-04	0.4441	35.04%
123	TDL_K13	S10_56922022	-	-	4.66E-04	0.477	35.04%
124	TDL_K13	S10_57103838	Sb10g027350	p450; Cytochrome P450	5.14E-04	0.4934	34.94%
125	TDL_K13	S10_58345765	-	-	6.07E-04	0.4441	34.77%
126	TDL_K13	S10_58460662	-	-	6.60E-04	0.4211	34.68%
127	TDL_K13	S10_56943507	-	-	8.02E-04	0.4934	34.48%
128	TDL_K13	S10_56773166	Sb10g027100	NAC domain protein NAC1	8.24E-04	0.4934	34.45%
129	TDL_K13	S10_59770217	-	-	8.28E-04	0.4474	34.45%
130	TDL_K13	S10_58652432	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	9.03E-04	0.4243	34.36%
131	TDL_K13	S10_57331385	-	-	9.39E-04	0.4671	34.32%
132	TDL_R13	S10_57484597	-	-	9.76E-07	0.5	50.76%
133	TDL_R13	S10_57484592	-	-	9.76E-07	0.5	50.76%
134	TDL_R13	S10_57484595	-	-	9.76E-07	0.5	50.76%
135	TDL_R13	S10_57484598	-	-	9.76E-07	0.5	50.76%
136	TDL_R13	S10_57253966	Sb10g027460	OsENODL1_like; Early nodulin-like protein	1.81E-06	0.4967	50.15%
137	TDL_R13	S10_57253967	Sb10g027460	OsENODL1_like; Early nodulin-like protein	1.81E-06	0.4967	50.15%
138	TDL_R13	S10_57432493	Sb10g027640	Omethyl transferase/WRKY	3.21E-06	0.4704	49.60%
139	TDL_R13	S10_56958200	Sb10g027240	Trans_IPPS_HT; Trans-Isoprenyl Diphosphate Synthases, head-to-tail	3.44E-06	0.5	49.53%
140	TDL_R13	S10_57427894	-	-	5.26E-06	0.4967	49.12%
141	TDL_R13	S10_57453669	-	-	8.17E-06	0.4474	48.70%
142	TDL_R13	S10_57400347	Sb10g027610	" EF-hand Ca2+-binding protein CCD1"	1.30E-05	0.4737	48.27%
143	TDL_R13	S10_57773700	-	-	1.40E-05	0.4737	48.20%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
144	TDL_R13	S10_57963498	-	-	1.47E-05	0.4803	48.15%
145	TDL_R13	S10_57103838	Sb10g027350	p450; Cytochrome P450	2.21E-05	0.4934	47.77%
146	TDL_R13	S10_57145296	Sb10g027370	PBP1_GABAb_receptor	2.36E-05	0.4901	47.71%
147	TDL_R13	S10_56730378	Sb10g027090	serine/arginine repetitive matrix protein 2-like	2.49E-05	0.4836	47.66%
148	TDL_R13	S10_58069749	-	-	2.50E-05	0.4704	47.65%
149	TDL_R13	S10_57548453	-	-	2.87E-05	0.4901	47.53%
150	TDL_R13	S10_57403166	-	-	3.05E-05	0.3059	47.47%
151	TDL_R13	S10_57449076	-	-	3.09E-05	0.4868	47.46%
152	TDL_R13	S10_57936436	-	-	4.63E-05	0.4671	47.08%
153	TDL_R13	S10_57490614	Sb10g027680	" Armadillo/beta-catenin repeat protein-like"	4.76E-05	0.4803	47.06%
154	TDL_R13	S10_57760921	Sb10g027920	COG0724; RNA-binding proteins	5.04E-05	0.4934	47.00%
155	TDL_R13	S10_57482523	-	-	6.84E-05	0.4539	46.72%
156	TDL_R13	S10_58035226	Sb10g028140	AA-amino acid hydrolase	7.11E-05	0.4638	46.69%
157	TDL_R13	S10_56930141	-	-	9.23E-05	0.4868	46.45%
158	TDL_R13	S10_56773166	Sb10g027100	NAC domain protein NAC1	9.38E-05	0.4934	46.44%
159	TDL_R13	S10_59571447	-	-	1.01E-04	0.4309	46.37%
160	TDL_R13	S10_57623967	Sb10g027790	" Auxin response factor 18"	1.35E-04	0.4638	46.11%
161	TDL_R13	S10_57936524	-	-	1.40E-04	0.4474	46.07%
162	TDL_R13	S10_56922022	-	-	1.91E-04	0.477	45.80%
163	TDL_R13	S10_57831107	Sb10g027980	Cysteine protease Mir1	1.97E-04	0.4901	45.77%
164	TDL_R13	S10_57251912	Sb10g027450	" 40S ribosomal protein S14-1"	2.10E-04	0.4934	45.71%
165	TDL_R13	S10_57423429	-	-	2.21E-04	0.4737	45.67%
166	TDL_R13	S10_57549720	-	-	2.31E-04	0.477	45.63%
167	TDL_R13	S10_56728443	-	-	2.37E-04	0.4704	45.61%
168	TDL_R13	S10_56932767	-	-	2.42E-04	0.5	45.59%
169	TDL_R13	S10_57024276	Sb10g027275	pyrrolidone-carboxylate peptidase; K01304 pyroglutamyl-peptidase	3.18E-04	0.4967	45.34%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
170	TDL_R13	S10_58039503	-	-	3.41E-04	0.477	45.28%
171	TDL_R13	S10_58039505	-	-	3.41E-04	0.477	45.28%
172	TDL_R13	S10_58039451	-	-	3.41E-04	0.477	45.28%
173	TDL_R13	S10_58039504	-	-	3.41E-04	0.477	45.28%
174	TDL_R13	S10_58039507	-	-	3.41E-04	0.477	45.28%
175	TDL_R13	S10_58039508	-	-	3.41E-04	0.477	45.28%
176	TDL_R13	S10_57331278	-	-	3.54E-04	0.5	45.25%
177	TDL_R13	S10_57303312	-	-	3.83E-04	0.3618	45.18%
178	TDL_R13	S10_60213638	Sb10g030550	GDSL-lipase-like	4.45E-04	0.4145	45.05%
179	TDL_R13	S10_58079690	Sb10g028200	Catalytic domain of Protein Kinases	4.75E-04	0.4474	44.99%
180	TDL_R13	S10_58022908	Sb10g028130	" Putative thaumatin-protein"	4.83E-04	0.4408	44.98%
181	TDL_R13	S10_57547037	-	-	4.95E-04	0.4671	44.95%
182	TDL_R13	S10_58299415	-	-	5.23E-04	0.4474	44.91%
183	TDL_R13	S10_57022696	-	-	5.51E-04	0.4474	44.86%
184	TDL_R13	S10_58022907	Sb10g028130	" Putative thaumatin-protein"	5.90E-04	0.4441	44.80%
185	TDL_R13	S10_57252006	Sb10g027450	" 40S ribosomal protein S14-1"	6.37E-04	0.4803	44.73%
186	TDL_R13	S10_57252004	Sb10g027450	" 40S ribosomal protein S14-1"	6.37E-04	0.4803	44.73%
187	TDL_R13	S10_57252007	Sb10g027450	" 40S ribosomal protein S14-1"	6.37E-04	0.4803	44.73%
188	TDL_R13	S10_57808411	Sb10g027950	AT5MAT; O-malonyltransferase/ transferase	7.04E-04	0.4868	44.65%
189	TDL_R13	S10_57808412	Sb10g027950	AT5MAT; O-malonyltransferase/ transferase	7.04E-04	0.4868	44.65%
190	TDL_R13	S10_57785077	-	-	7.10E-04	0.4243	44.64%
191	TDL_R13	S10_57830499	-	-	7.62E-04	0.4704	44.58%
192	TDL_R13	S10_57830472	-	-	7.62E-04	0.4704	44.58%
193	TDL_R13	S10_57830492	-	-	7.62E-04	0.4704	44.58%
194	TDL_R13	S10_57453732	-	-	8.03E-04	0.4704	44.53%
195	TDL_R13	S10_56728353	-	-	8.39E-04	0.4803	44.49%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
196	TDL_R13	S10_57522978	Sb10g027730	F-box domain	9.14E-04	0.4836	44.42%
197	TDL_R13	S10_56199661	Sb10g026750	Zinc finger POZ domain protein	9.14E-04	0.4375	44.42%
198	TDL_R13	S10_56199672	Sb10g026750	Zinc finger POZ domain protein	9.14E-04	0.4375	44.42%
199	TDL_R13	S10_57006286	-	-	9.52E-04	0.4803	44.39%
200	TDL_R13	S10_57547066	-	-	9.69E-04	0.4605	44.37%
201	TDL_R13	S10_57032564	Sb10g027280	similar to Putative transcription factor GAMyb	9.78E-04	0.4934	44.36%
202	Tdl across	S10_57432493	Sb10g027640	O methyl transferase	3.67E-10	0.4704	54.53%
203	Tdl across	S10_57427894	-	-	2.82E-09	0.4967	52.33%
204	Tdl across	S10_56958200	Sb10g027240	Trans_IPPS_HT; Trans-Isoprenyl Diphosphate Synthases, head-to-tail	3.88E-09	0.5	51.99%
205	Tdl across	S10_57484592	-	-	4.75E-09	0.5	51.78%
206	Tdl across	S10_57484595	-	-	4.75E-09	0.5	51.78%
207	Tdl across	S10_57484597	-	-	4.75E-09	0.5	51.78%
208	Tdl across	S10_57484598	-	-	4.75E-09	0.5	51.78%
209	Tdl across	S10_57453669	-	-	5.13E-09	0.4474	51.70%
210	Tdl across	S10_57548453	-	-	6.69E-09	0.4901	51.42%
211	Tdl across	S10_57253966	Sb10g027460	OsENODL1_like; Early nodulin-like protein	7.00E-09	0.4967	51.37%
212	Tdl across	S10_57253967	Sb10g027460	OsENODL1_like; Early nodulin-like protein	7.00E-09	0.4967	51.37%
213	Tdl across	S10_57773700	-	-	9.14E-09	0.4737	51.09%
214	Tdl across	S10_57145296	Sb10g027370	PBP1_GABAb_receptor	1.23E-08	0.4901	50.78%
215	Tdl across	S10_57963498	-	-	1.27E-08	0.4803	50.75%
216	Tdl across	S10_57400347	Sb10g027610	" EF-hand Ca2+-binding protein CCD1"	1.59E-08	0.4737	50.52%
217	Tdl across	S10_58069749	-	-	3.23E-08	0.4704	49.78%
218	Tdl across	S10_57490614	Sb10g027680	" Armadillo/beta-catenin repeat protein-like"	6.86E-08	0.4803	49.02%
219	Tdl across	S10_58035226	Sb10g028140	AA-amino acid hydrolase	8.18E-08	0.4638	48.84%
220	Tdl across	S10_56730378	Sb10g027090	serine/arginine repetitive matrix protein 2-like	1.10E-07	0.4836	48.54%
221	Tdl across	S10_57936436	-	-	1.21E-07	0.4671	48.44%
222	Tdl across	S10_57403166	-	-	1.41E-07	0.3059	48.29%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
223	Tdl across	S10_57623967	Sb10g027790	" Auxin response factor 18"	1.47E-07	0.4638	48.25%
224	Tdl across	S10_56930141	-	-	1.81E-07	0.4868	48.04%
225	Tdl across	S10_57449076	-	-	2.10E-07	0.4868	47.89%
226	Tdl across	S10_58079690	Sb10g028200	Catalytic domain of Protein Kinases	3.83E-07	0.4474	47.30%
227	Tdl across	S10_57453732	-	-	3.95E-07	0.4704	47.26%
228	Tdl across	S10_57103838	Sb10g027350	p450; Cytochrome P450	4.34E-07	0.4934	47.17%
229	Tdl across	S10_57022696	-	-	5.28E-07	0.4474	46.98%
230	Tdl across	S10_56932767	-	-	5.42E-07	0.5	46.95%
231	Tdl across	S10_57423429	-	-	6.38E-07	0.4737	46.79%
232	Tdl across	S10_57331278	-	-	7.08E-07	0.5	46.69%
233	Tdl across	S10_57936524	-	-	9.54E-07	0.4474	46.40%
234	Tdl across	S10_57251912	Sb10g027450	" 40S ribosomal protein S14-1"	9.76E-07	0.4934	46.38%
235	Tdl across	S10_56773166	Sb10g027100	NAC domain protein NAC1	9.83E-07	0.4934	46.37%
236	Tdl across	S10_58022908	Sb10g028130	" Putative thaumatin-protein"	1.15E-06	0.4408	46.22%
237	Tdl across	S10_58022907	Sb10g028130	" Putative thaumatin-protein"	1.67E-06	0.4441	45.85%
238	Tdl across	S10_56922022	-	-	1.75E-06	0.477	45.81%
239	Tdl across	S10_57785077	-	-	2.04E-06	0.4243	45.66%
240	Tdl across	Xtxp141	-	-	2.06E-06	0.4704	45.65%
241	Tdl across	S10_59571447	-	-	2.17E-06	0.4309	45.60%
242	Tdl across	S10_57303312	-	-	2.67E-06	0.3618	45.41%
243	Tdl across	S10_56945933	-	-	2.88E-06	0.4934	45.33%
244	Tdl across	S10_59020325	Sb10g029190	" Squamosa promoter-binding-like protein 12"	2.92E-06	0.4375	45.32%
245	Tdl across	S10_58299415	-	-	2.99E-06	0.4474	45.30%
246	Tdl across	S10_57482523	-	-	3.05E-06	0.4539	45.28%
247	Tdl across	S10_56869829	-	-	3.14E-06	0.5	45.25%
248	Tdl across	S10_56869835	-	-	3.14E-06	0.5	45.25%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
249	Tdl across	S10_58532130	Sb10g028670	Protein of unknown function (DUF1336)	3.82E-06	0.4211	45.06%
250	Tdl across	S10_57808411	Sb10g027950	AT5MAT; O-malonyltransferase/ transferase	4.69E-06	0.4868	44.87%
251	Tdl across	S10_57808412	Sb10g027950	AT5MAT; O-malonyltransferase/ transferase	4.69E-06	0.4868	44.87%
252	Tdl across	S10_58954853	-	-	5.84E-06	0.4211	44.66%
253	Tdl across	S10_58356653	-	-	5.86E-06	0.4507	44.66%
254	Tdl across	S10_57403220	-	-	5.97E-06	0.4342	44.64%
255	Tdl across	S10_56728443	-	-	6.04E-06	0.4704	44.63%
256	Tdl across	S10_57760921	Sb10g027920	COG0724; RNA-binding proteins	6.41E-06	0.4934	44.57%
257	Tdl across	S10_58048950	Sb10g028160	N-terminal domain (domain I) of transcription elongation factor S-II (TFIIS)	6.79E-06	0.4638	44.52%
258	Tdl across	S10_56728353	-	-	6.87E-06	0.4803	44.51%
259	Tdl across	S10_57831107	Sb10g027980	Cysteine protease Mir1	7.01E-06	0.4901	44.49%
260	Tdl across	S10_57549720	-	-	7.45E-06	0.477	44.43%
261	Tdl across	S10_59082132	Sb10g029270	" Delta-aminolevulinic acid dehydratase"	8.12E-06	0.4178	44.35%
262	Tdl across	S10_59082143	Sb10g029270	" Delta-aminolevulinic acid dehydratase"	8.12E-06	0.4178	44.35%
263	Tdl across	S10_57830472	-	-	8.34E-06	0.4704	44.32%
264	Tdl across	S10_57830492	-	-	8.34E-06	0.4704	44.32%
265	Tdl across	S10_57830499	-	-	8.34E-06	0.4704	44.32%
266	Tdl across	S10_57522978	Sb10g027730	F-box domain	9.87E-06	0.4836	44.17%
267	Tdl across	S10_58954854	-	-	1.18E-05	0.4178	44.00%
268	Tdl across	S10_58954867	-	-	1.18E-05	0.4178	44.00%
269	Tdl across	S10_56943507	-	-	1.30E-05	0.4934	43.91%
270	Tdl across	S10_58310186	Sb10g028500	" Peroxidase 16 protein"	1.31E-05	0.4441	43.90%
271	Tdl across	S10_56937708	-	-	1.38E-05	0.4704	43.86%
272	Tdl across	S10_58652432	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	1.41E-05	0.4243	43.83%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
273	Tdl across	S10_56626709	-	-	1.46E-05	0.4605	43.80%
274	Tdl across	S10_57024276	Sb10g027275	pyrrolidone-carboxylate peptidase; K01304 pyroglutamyl-peptidase	1.99E-05	0.4967	43.51%
275	Tdl across	S10_56655783	Sb10g027070	Putative uncharacterized protein	2.12E-05	0.4572	43.46%
276	Tdl across	S10_58050683	Sb10g028160	N-terminal domain (domain I) of transcription elongation factor S-II (TFIIS)	2.25E-05	0.477	43.40%
277	Tdl across	S10_56078885	-	-	2.40E-05	0.4309	43.34%
278	Tdl across	S10_58365099	-	-	2.52E-05	0.4408	43.29%
279	Tdl across	S10_58345765	-	-	2.60E-05	0.4441	43.27%
280	Tdl across	S10_58039451	-	-	2.68E-05	0.477	43.24%
281	Tdl across	S10_58039503	-	-	2.68E-05	0.477	43.24%
282	Tdl across	S10_58039504	-	-	2.68E-05	0.477	43.24%
283	Tdl across	S10_58039505	-	-	2.68E-05	0.477	43.24%
284	Tdl across	S10_58039507	-	-	2.68E-05	0.477	43.24%
285	Tdl across	S10_58039508	-	-	2.68E-05	0.477	43.24%
286	Tdl across	S10_56199661	Sb10g026750	Zinc finger POZ domain protein	2.75E-05	0.4375	43.21%
287	Tdl across	S10_56199672	Sb10g026750	Zinc finger POZ domain protein	2.75E-05	0.4375	43.21%
288	Tdl across	S10_57006286	-	-	2.78E-05	0.4803	43.21%
289	Tdl across	S10_59434640	Sb10g029690	" GCIP-interacting family protein-like"	3.31E-05	0.4046	43.04%
290	Tdl across	S10_57252004	Sb10g027450	" 40S ribosomal protein S14-1"	3.34E-05	0.4803	43.03%
291	Tdl across	S10_57252006	Sb10g027450	" 40S ribosomal protein S14-1"	3.34E-05	0.4803	43.03%
292	Tdl across	S10_57252007	Sb10g027450	" 40S ribosomal protein S14-1"	3.34E-05	0.4803	43.03%
293	Tdl across	S10_58460662	-	-	4.74E-05	0.4211	42.71%
294	Tdl across	S10_56393810	-	-	6.11E-05	0.4605	42.48%
295	Tdl across	S10_56393811	-	-	6.11E-05	0.4605	42.48%
296	Tdl across	S10_59030462	-	-	7.04E-05	0.4309	42.36%
297	Tdl across	S10_57036097	Sb10g027290	Galactosyl_T; Galactosyltransferase	7.08E-05	0.4803	42.35%
298	Tdl across	S10_59770217	-	-	7.25E-05	0.4474	42.33%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
299	Tdl across	S10_57024290	Sb10g027275	pyrrolidone-carboxylate peptidase; K01304 pyroglutamyl-peptidase	7.53E-05	0.4901	42.29%
300	Tdl across	S10_56596575	-	-	7.56E-05	0.4507	42.29%
301	Tdl across	Xiabt466	SSR	-	7.74E-05	0.4342	42.27%
302	Tdl across	S10_57547037	-	-	7.79E-05	0.4671	42.26%
303	Tdl across	S10_58022779	Sb10g028130	" Putative thaumatin-protein"	7.88E-05	0.4638	42.25%
304	Tdl across	S10_58663475	-	-	8.11E-05	0.4375	42.23%
305	Tdl across	S10_56678635	-	-	8.60E-05	0.477	42.17%
306	Tdl across	S10_56678638	-	-	8.60E-05	0.477	42.17%
307	Tdl across	S10_59202679	-	-	8.66E-05	0.4276	42.17%
308	Tdl across	S10_57088032	Sb10g027340	O-methyltransferase ZRP4	9.01E-05	0.4934	42.13%
309	Tdl across	S10_58048884	-	-	9.49E-05	0.4507	42.09%
310	TDU_K13	S10_57432493	Sb10g027640	Omethyl transferase	1.38E-04	0.4704	28.71%
311	TDU_K13	S10_57427894	-	-	2.80E-04	0.4967	27.88%
312	TDU_K13	S10_57548453	-	-	1.03E-03	0.4901	26.36%
313	TDU_K13	S10_59483624	Sb10g029750	" Putative uncharacterized protein OJ1119_H02.21	1.18E-03	0.3914	26.21%
314	TDU_K13	S10_57403166	-	-	1.21E-03	0.3059	26.18%
315	TDU_K13	S10_59434640	Sb10g029690	" GCIP-interacting family protein-like"	1.24E-03	0.4046	26.16%
316	TDU_K13	S10_57963498	-	-	1.29E-03	0.4803	26.12%
317	TDU_K13	S10_59571447	-	-	1.42E-03	0.4309	26.01%
318	TDU_K13	S10_57453732	-	-	1.61E-03	0.4704	25.86%
319	TDU_K13	S10_57831107	Sb10g027980	Cysteine protease Mir1	1.66E-03	0.4901	25.83%
320	TDU_K13	S10_56958200	Sb10g027240	Trans_IPPS_HT; Trans-Isoprenyl Diphosphate Synthases, head-to-tail	1.71E-03	0.5	25.80%
321	TDU_K13	S10_57251912	Sb10g027450	" 40S ribosomal protein S14-1"	2.16E-03	0.4934	25.53%
322	TDU_K13	S10_58299415	-	-	2.29E-03	0.4474	25.47%
323	TDU_K13	S10_57484592	-	-	2.35E-03	0.5	25.44%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
324	TDU_K13	S10_57484595	-	-	2.35E-03	0.5	25.44%
325	TDU_K13	S10_57484597	-	-	2.35E-03	0.5	25.44%
326	TDU_K13	S10_57484598	-	-	2.35E-03	0.5	25.44%
327	TDU_K13	S10_53826992	Sb10g024670	unknown protein	3.22E-03	0.4276	25.09%
328	TDU_K13	S10_57453669	-	-	3.24E-03	0.4474	25.08%
329	TDU_K13	S10_57400347	Sb10g027610	" EF-hand Ca2+-binding protein CCD1"	3.38E-03	0.4737	25.04%
330	TDU_K13	S10_57449076	-	-	3.54E-03	0.4868	24.99%
331	TDU_K13	S10_57036097	Sb10g027290	Galactosyl_T; Galactosyltransferase	3.56E-03	0.4803	24.98%
332	TDU_K13	S10_58356653	-	-	3.92E-03	0.4507	24.87%
333	TDU_K13	S10_54646100	Sb10g025360	SAM (Sterile Alpha Motif)	3.93E-03	0.3816	24.87%
334	TDU_K13	S10_57490614	Sb10g027680	" Armadillo/beta-catenin repeat protein-like"	4.15E-03	0.4803	24.81%
335	TDU_K13	S10_57623967	Sb10g027790	" Auxin response factor 18"	4.23E-03	0.4638	24.79%
336	TDU_K13	S10_57423429	-	-	4.91E-03	0.4737	24.63%
337	TDU_K13	S10_51517153	Sb10g023140	Galactose oxidase/kelch repeat superfamily protein	5.09E-03	0.3224	24.59%
338	TDU_K13	S10_58652432	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	5.23E-03	0.4243	24.56%
339	TDU_K13	S10_57145296	Sb10g027370	PBP1_GABAb_receptor	5.38E-03	0.4901	24.53%
340	TDU_K13	S10_56930141	-	-	5.67E-03	0.4868	24.47%
341	TDU_K13	S10_58261872	Sb10g028430	Catylatic activity in RNA Degradation pathway-Chromosome chr12 scaffold_18, whole genome	5.82E-03	0.4901	24.44%
342	TDU_K13	S10_58839711	Sb10g029010	DUF617; Protein of unknown function	6.26E-03	0.4243	24.36%
343	TDU_K13	S10_58640688	Sb10g028790	4Oxalocrotonate_Tautomerase	6.34E-03	0.4408	24.35%
344	TDU_K13	S10_57253966	Sb10g027460	OsENODL1_like; Early nodulin-like protein	6.48E-03	0.4967	24.33%
345	TDU_K13	S10_57253967	Sb10g027460	OsENODL1_like; Early nodulin-like protein	6.48E-03	0.4967	24.33%
346	TDU_K13	S10_51517147	Sb10g023140	Galactose oxidase/kelch repeat superfamily protein	6.67E-03	0.3257	24.29%
347	TDU_K13	S10_57036100	Sb10g027290	Galactosyl_T; Galactosyltransferase	6.96E-03	0.4836	24.25%
348	TDU_K13	S10_57036103	Sb10g027290	Galactosyl_T; Galactosyltransferase	6.96E-03	0.4836	24.25%
349	TDU_K13	S10_57036106	Sb10g027290	Galactosyl_T; Galactosyltransferase	6.96E-03	0.4836	24.25%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
350	TDU_K13	S10_57036107	Sb10g027290	Galactosyl_T; Galactosyltransferase	6.96E-03	0.4836	24.25%
351	TDU_K13	S10_59525199	-	-	7.03E-03	0.4408	24.24%
352	TDU_K13	S10_56393810	-	-	7.64E-03	0.4605	24.15%
353	TDU_K13	S10_56393811	-	-	7.64E-03	0.4605	24.15%
354	TDU_K13	Xtxp141	-	-	7.71E-03	0.4704	24.14%
355	TDU_K13	S10_50855577	-	-	7.82E-03	0.4507	24.12%
356	TDU_K13	S10_52042465	Sb10g023430	CESA1 (CELLULOSE SYNTHASE 1); cellulose synthase/ transferase	8.13E-03	0.375	24.08%
357	TDU_K13	S10_59566699	Sb10g029820	AdoMet_Mtases(involves the direct methylation of oleic acid esterified as a component of phospholipids/it directly interacts with DNA,RNA also)	8.46E-03	0.4276	24.04%
358	TDU_K13	S10_57103838	Sb10g027350	p450; Cytochrome P450	8.77E-03	0.4934	24.00%
359	TDU_K13	S10_54646082	Sb10g025360	SAM (Sterile Alpha Motif)	9.07E-03	0.375	23.96%
360	TDU_K13	S10_57773700	-	-	9.66E-03	0.4737	23.90%
361	TDU_K13	S10_57830472	-	-	9.72E-03	0.4704	23.89%
362	TDU_K13	S10_57830492	-	-	9.72E-03	0.4704	23.89%
363	TDU_K13	S10_57830499	-	-	9.72E-03	0.4704	23.89%
364	TDU_K13	S10_57760921	Sb10g027920	COG0724; RNA-binding proteins	9.79E-03	0.4934	23.88%
365	TDU_R13	S10_57427894	-	-	2.59E-06	0.4967	50.61%
366	TDU_R13	S10_57963498	-	-	2.60E-06	0.4803	50.61%
367	TDU_R13	S10_57253966	Sb10g027460	OsENODL1_like; Early nodulin-like protein	2.90E-06	0.4967	50.51%
368	TDU_R13	S10_57253967	Sb10g027460	OsENODL1_like; Early nodulin-like protein	2.90E-06	0.4967	50.51%
369	TDU_R13	S10_58299415	-	-	3.84E-06	0.4474	50.24%
370	TDU_R13	S10_57145296	Sb10g027370	PBP1_GABAb_receptor	4.01E-06	0.4901	50.20%
371	TDU_R13	S10_57400347	Sb10g027610	" EF-hand Ca2+-binding protein CCD1"	5.13E-06	0.4737	49.96%
372	TDU_R13	S10_57251912	Sb10g027450	" 40S ribosomal protein S14-1"	6.61E-06	0.4934	49.73%
373	TDU_R13	S10_57831107	Sb10g027980	Cysteine protease Mir1	7.98E-06	0.4901	49.55%
374	TDU_R13	S10_58069749	-	-	9.76E-06	0.4704	49.36%
375	TDU_R13	S10_57773700	-	-	9.94E-06	0.4737	49.34%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
376	TDU_R13	S10_57936524	-	-	1.01E-05	0.4474	49.33%
377	TDU_R13	S10_57453669	-	-	1.17E-05	0.4474	49.19%
378	TDU_R13	S10_57484592	-	-	1.26E-05	0.5	49.13%
379	TDU_R13	S10_57484595	-	-	1.26E-05	0.5	49.13%
380	TDU_R13	S10_57484597	-	-	1.26E-05	0.5	49.13%
381	TDU_R13	S10_57484598	-	-	1.26E-05	0.5	49.13%
382	TDU_R13	S10_58079690	Sb10g028200	Catalytic domain of Protein Kinases	1.63E-05	0.4474	48.89%
383	TDU_R13	S10_57432493	Sb10g027640	Omethyl transferase/WRKY	2.78E-05	0.4704	48.40%
384	TDU_R13	S10_57103838	Sb10g027350	p450; Cytochrome P450	3.55E-05	0.4934	48.17%
385	TDU_R13	S10_57548453	-	-	3.72E-05	0.4901	48.13%
386	TDU_R13	S10_56958200	Sb10g027240	Trans_IPPS_HT; Trans-Isoprenyl Diphosphate Synthases, head-to-tail	6.59E-05	0.5	47.61%
387	TDU_R13	S10_57453732	-	-	7.22E-05	0.4704	47.53%
388	TDU_R13	S10_58035226	Sb10g028140	AA-amino acid hydrolase	7.75E-05	0.4638	47.47%
389	TDU_R13	S10_58048884	-	-	8.14E-05	0.4507	47.42%
390	TDU_R13	S10_57024276	Sb10g027275	pyrrolidone-carboxylate peptidase; K01304 pyroglutamyl-peptidase	8.73E-05	0.4967	47.36%
391	TDU_R13	S10_58022908	Sb10g028130	" Putative thaumatin-protein"	1.11E-04	0.4408	47.14%
392	TDU_R13	S10_58261872	Sb10g028430	catylatic activity in RNA Degradation pathway-Chromosome chr12 scaffold_18, whole genome	1.30E-04	0.4901	47.01%
393	TDU_R13	S10_57403166	-	-	1.34E-04	0.3059	46.98%
394	TDU_R13	S10_57490614	Sb10g027680	" Armadillo/beta-catenin repeat protein-like"	1.34E-04	0.4803	46.98%
395	TDU_R13	S10_58022907	Sb10g028130	" Putative thaumatin-protein"	1.68E-04	0.4441	46.78%
396	TDU_R13	S10_58310186	Sb10g028500	" Peroxidase 16 protein"	1.76E-04	0.4441	46.74%
397	TDU_R13	Xtxp141	-	-	1.95E-04	0.4704	46.65%
398	TDU_R13	S10_57623967	Sb10g027790	" Auxin response factor 18"	2.08E-04	0.4638	46.59%
399	TDU_R13	S10_57036097	Sb10g027290	Galactosyl_T; Galactosyltransferase	2.31E-04	0.4803	46.50%
400	TDU_R13	S10_57936436	-	-	2.72E-04	0.4671	46.36%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
401	TDU_R13	S10_57022696	-	-	2.91E-04	0.4474	46.30%
402	TDU_R13	S10_57449076	-	-	3.09E-04	0.4868	46.25%
403	TDU_R13	S10_57036100	Sb10g027290	Galactosyl_T; Galactosyltransferase	3.44E-04	0.4836	46.15%
404	TDU_R13	S10_57036103	Sb10g027290	Galactosyl_T; Galactosyltransferase	3.44E-04	0.4836	46.15%
405	TDU_R13	S10_57036106	Sb10g027290	Galactosyl_T; Galactosyltransferase	3.44E-04	0.4836	46.15%
406	TDU_R13	S10_57036107	Sb10g027290	Galactosyl_T; Galactosyltransferase	3.44E-04	0.4836	46.15%
407	TDU_R13	S10_58050683	Sb10g028160	N-terminal domain (domain I) of transcription elongation factor S-II (TFIIS)	4.32E-04	0.477	45.95%
408	TDU_R13	xiabt466	-	-	5.87E-04	0.4342	45.69%
409	TDU_R13	S10_58163876	Sb10g028300	" Zinc finger protein-like"	6.45E-04	0.4474	45.61%
410	TDU_R13	S10_57808411	Sb10g027950	AT5MAT; O-malonyltransferase/ transferase	6.61E-04	0.4868	45.59%
411	TDU_R13	S10_57808412	Sb10g027950	AT5MAT; O-malonyltransferase/ transferase	6.61E-04	0.4868	45.59%
412	TDU_R13	S10_57024290	Sb10g027275	pyrrolidone-carboxylate peptidase; K01304 pyroglutamyl-peptidase	6.94E-04	0.4901	45.55%
413	TDU_R13	S10_57252004	Sb10g027450	" 40S ribosomal protein S14-1"	6.98E-04	0.4803	45.54%
414	TDU_R13	S10_57252006	Sb10g027450	" 40S ribosomal protein S14-1"	6.98E-04	0.4803	45.54%
415	TDU_R13	S10_57252007	Sb10g027450	" 40S ribosomal protein S14-1"	6.98E-04	0.4803	45.54%
416	TDU_R13	S10_57785077	-	-	9.79E-04	0.4243	45.25%
417	TDU_R13	S10_57760921	Sb10g027920	COG0724; RNA-binding proteins	1.05E-03	0.4934	45.19%
418	TDU_R13	S10_57331278	-	-	1.20E-03	0.5	45.08%
419	TDU_R13	S10_57549720	-	-	1.26E-03	0.477	45.04%
420	TDU_R13	S10_58048950	Sb10g028160	N-terminal domain (domain I) of transcription elongation factor S-II (TFIIS)	1.53E-03	0.4638	44.87%
421	TDU_R13	S10_57522978	Sb10g027730	F-box domain	1.88E-03	0.4836	44.71%
422	TDU_R13	S10_57303312	-	-	1.96E-03	0.3618	44.67%
423	TDU_R13	S10_57032564	Sb10g027280	similar to Putative transcription factor GAMyb	2.41E-03	0.4934	44.50%
424	TDU_R13	S10_58155733	-	-	2.41E-03	0.4704	44.50%
425	TDU_R13	S10_57088032	Sb10g027340	O-methyltransferase ZRP4	2.50E-03	0.4934	44.47%
426	TDU_R13	S10_57423429	-	-	2.77E-03	0.4737	44.38%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
427	TDU_R13	S10_57331385	-	-	3.70E-03	0.4671	44.15%
428	TDU_R13	S10_57547037	-	-	4.56E-03	0.4671	43.97%
429	TDU_R13	S10_57036758	Sb10g027290	Galactosyl_T; Galactosyltransferase	4.69E-03	0.4737	43.95%
430	TDU_R13	S10_57036791	Sb10g027290	Galactosyl_T; Galactosyltransferase	4.69E-03	0.4737	43.95%
431	TDU_R13	S10_57830472	-	-	5.77E-03	0.4704	43.78%
432	TDU_R13	S10_57830492	-	-	5.77E-03	0.4704	43.78%
433	TDU_R13	S10_57830499	-	-	5.77E-03	0.4704	43.78%
434	TDU_R13	S10_57482523	-	-	5.86E-03	0.4539	43.77%
435	TDU_R13	S10_57036796	Sb10g027290	Galactosyl_T; Galactosyltransferase	7.73E-03	0.4671	43.55%
436	TDU_R13	S10_57403220	-	-	8.87E-03	0.4342	43.44%
437	TDU_R13	S10_57403113	-	-	9.14E-03	0.4836	43.42%
438	TDU_R13	S10_57341007	Sb10g027550	"weakly Zinc finger (C2H2 type) protein-like	9.50E-03	0.4803	43.39%
439	TDU_R13	S10_57547066	-	-	9.73E-03	0.4605	43.37%
440	Tdu across	S10_57427894	-	-	2.66E-07	0.4967	42.89%
441	Tdu across	S10_57432493	Sb10g027640	Omethyl transferase	6.91E-07	0.4704	41.86%
442	Tdu across	S10_57963498	-	-	1.06E-06	0.4803	41.40%
443	Tdu across	S10_57548453	-	-	1.51E-06	0.4901	41.03%
444	Tdu across	S10_58299415	-	-	2.08E-06	0.4474	40.70%
445	Tdu across	S10_57400347	Sb10g027610	" EF-hand Ca2+-binding protein CCD1"	2.09E-06	0.4737	40.69%
446	Tdu across	S10_57145296	Sb10g027370	PBP1_GABAb_receptor	2.10E-06	0.4901	40.69%
447	Tdu across	S10_57253966	Sb10g027460	OsENODL1_like; Early nodulin-like protein	2.39E-06	0.4967	40.55%
448	Tdu across	S10_57253967	Sb10g027460	OsENODL1_like; Early nodulin-like protein	2.39E-06	0.4967	40.55%
449	Tdu across	S10_57251912	Sb10g027450	" 40S ribosomal protein S14-1"	2.73E-06	0.4934	40.41%
450	Tdu across	S10_56958200	Sb10g027240	Trans_IPPS_HT; Trans-Isoprenyl Diphosphate Synthases, head-to-tail	3.27E-06	0.5	40.22%
451	Tdu across	S10_57453732	-	-	5.94E-06	0.4704	39.60%
452	Tdu across	S10_57403166	-	-	7.14E-06	0.3059	39.41%
453	Tdu across	S10_57831107	Sb10g027980	Cysteine protease Mir1	7.58E-06	0.4901	39.35%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
454	Tdu across	S10_57773700	-	-	8.45E-06	0.4737	39.24%
455	Tdu across	S10_57453669	-	-	9.59E-06	0.4474	39.11%
456	Tdu across	S10_57484592	-	-	1.16E-05	0.5	38.92%
457	Tdu across	S10_57484595	-	-	1.16E-05	0.5	38.92%
458	Tdu across	S10_57484597	-	-	1.16E-05	0.5	38.92%
459	Tdu across	S10_57484598	-	-	1.16E-05	0.5	38.92%
460	Tdu across	S10_57490614	Sb10g027680	" Armadillo/beta-catenin repeat protein-like"	1.24E-05	0.4803	38.85%
461	Tdu across	S10_57103838	Sb10g027350	p450; Cytochrome P450	1.33E-05	0.4934	38.78%
462	Tdu across	S10_57036097	Sb10g027290	Galactosyl_T; Galactosyltransferase	1.47E-05	0.4803	38.67%
463	Tdu across	S10_57449076	-	-	2.40E-05	0.4868	38.18%
464	Tdu across	S10_57623967	Sb10g027790	" Auxin response factor 18"	2.43E-05	0.4638	38.17%
465	Tdu across	S10_58079690	Sb10g028200	Catalytic domain of Protein Kinases	2.59E-05	0.4474	38.10%
466	Tdu across	S10_57936524	-	-	2.66E-05	0.4474	38.07%
467	Tdu across	S10_59571447	-	-	3.00E-05	0.4309	37.95%
468	Tdu across	S10_56930141	-	-	3.16E-05	0.4868	37.90%
469	Tdu across	S10_57036100	Sb10g027290	Galactosyl_T; Galactosyltransferase	3.33E-05	0.4836	37.85%
470	Tdu across	S10_57036103	Sb10g027290	Galactosyl_T; Galactosyltransferase	3.33E-05	0.4836	37.85%
471	Tdu across	S10_57036106	Sb10g027290	Galactosyl_T; Galactosyltransferase	3.33E-05	0.4836	37.85%
472	Tdu across	S10_57036107	Sb10g027290	Galactosyl_T; Galactosyltransferase	3.33E-05	0.4836	37.85%
473	Tdu across	Xtxp141	-	-	3.82E-05	0.4704	37.71%
474	Tdu across	S10_56932767	-	-	3.91E-05	0.5	37.69%
475	Tdu across	S10_58261872	Sb10g028430	catylatic activity in RNA Degradation pathway-Chromosome chr12 scaffold_18, whole genome	4.02E-05	0.4901	37.66%
476	Tdu across	S10_58356653	-	-	4.37E-05	0.4507	37.58%
477	Tdu across	S10_58035226	Sb10g028140	AA-amino acid hydrolase	4.54E-05	0.4638	37.54%
478	Tdu across	S10_58345765	-	-	5.59E-05	0.4441	37.33%
479	Tdu across	S10_58069749	-	-	6.68E-05	0.4704	37.16%

Table 23: (Contd..)

S NO:	Trait	SNP/SSR	Gene Id	Functional annotation	P.value	maf	%R^2
480	Tdu across	S10_58652432	Sb10g028810	UDP-glucoronosyl/UDP-glucosyl transferase family protein	7.88E-05	0.4243	36.99%
481	Tdu across	S10_57024276	Sb10g027275	pyrrolidone-carboxylate peptidase; K01304 pyroglutamyl-peptidase	8.75E-05	0.4967	36.89%
482	Tdu across	S10_59434640	Sb10g029690	" GCIP-interacting family protein-like"	8.77E-05	0.4046	36.89%
483	Tdu across	S10_58022907	Sb10g028130	" Putative thaumatin-protein"	8.80E-05	0.4441	36.88%
484	Tdu across	S10_58022908	Sb10g028130	" Putative thaumatin-protein"	9.03E-05	0.4408	36.86%

Table 24: Stay-green candidate genes in the target region of sorghum chromosome SBI-10L

S No:	Trait	SNP	Gene ID	Functional Annotation	Rice Homologs
1	%GL7	S10_58311699	Sb10g028500	similar to Peroxidase 16 protein	LOC_Os06g48030.1
2	%GL7	S10_58357039	Sb10g028550	similar to Putative uncharacterized protein	LOC_Os06g48160.1
3	%GL7	S10_58683017	Sb10g028870	similar to Putative meiotic serine proteinase	LOC_Os06g48650.1
4	%GL7	S10_59833299	Sb10g030080	weakly similar to Putative uncharacterized protein	LOC_Os06g50070.1
5	%GL7	S10_59148610	Sb10g029305	similar to Os02g0137100 protein	LOC_Os06g49180.1
6	%GL7	S10_58839857	Sb10g029010	similar to Putative uncharacterized protein	LOC_Os05g20030.1
7	%GL7	S10_58652548	Sb10g028810	similar to Os03g0824600 protein; UDP glycosyl transferase	LOC_Os03g60960.1
8	%GL7	S10_59316155	Sb10g029530	similar to Pentatricopeptide (PPR) repeat-containing protein-like	LOC_Os09g24640.1
9	%GL7	S10_59024190	Sb10g029190	similar to Squamosa promoter-binding-like protein 12	LOC_Os06g49010.1
10	%GL7	S10_59069052	Sb10g029245	similar to translation initiation factor IF-2	-
11	%GL7	S10_59833250	Sb10g030080	weakly similar to Putative uncharacterized protein	LOC_Os06g50070.1
12	%GL7	S10_58839711	Sb10g029010	similar to Putative uncharacterized protein	LOC_Os05g20030.1
13	%GL7	S10_59020155	Sb10g029180	similar to Putative uncharacterized protein	LOC_Os06g48980.1
14	%GL7	S10_59020363	Sb10g029190	similar to Squamosa promoter-binding-like protein 12	LOC_Os06g49010.1
15	%GL7	S10_59062420	Sb10g029230	similar to hAT dimerisation domain-containing protein-like	LOC_Os06g49050.1
16	%GL7	S10_59554262	Sb10g029810	similar to MADS box transcription factor	LOC_Os06g49840.1

Table 24: (Contd..)

S No:	Trait	SNP	Gene ID	Functional Annotation	Rice Homologs
17	%GL7	S10_58634237	Sb10g028780	similar to Mitogen-activated protein kinase 3	LOC_Os06g48590.1
18	%GL7	S10_59419567	Sb10g029670	similar to Putative uncharacterized protein	LOC_Os06g49700.1
19	%GL7	S10_59958913	Sb10g030270	similar to Putative receptor protein kinase	LOC_Os06g50340.1
20	%GL7	S10_59970887	-	-	-
21	%GL7	S10_60024056	Sb10g030330	similar to Aspartic proteinase nepenthesin II-like	LOC_Os06g50390.1
22	%GL14	S10_52940776	Sb10g024110	*Helix-loop-helix DNA-binding*	-
23	%GL14	S10_54877607	Sb10g025540	*Putative prolylcarboxypeptidase isoform 1*	-
24	%GL14	S10_53576112	Sb10g024500	NADP binding domain	-
25	%GL14	S10_54081973	Sb10g024920	weakly similar to Putative uncharacterized protein	LOC_Os06g43060.1
26	%GL14	S10_54532995	Sb10g025283	*NBS-LRR disease resistance protein*	-
27	%GL14	S10_54585199	Sb10g025310	similar to Ankyrin repeat-containing protein-like	LOC_Os06g43680.1
28	%GL14	S10_54535306	Sb10g025283	*NBS-LRR disease resistance protein*	-
29	%GL14	S10_54185546	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1
30	%GL14	S10_54185539	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1
31	%GL14	S10_60024056	Sb10g030330	similar to Aspartic proteinase nepenthesin II-like	LOC_Os06g50390.1
32	%GL14	S10_60333532	Sb10g030700	Predicted protein (autophagy related transport to vacuole)	LOC_Os05g30640.1
33	%GL14	S10_60413173	Sb10g030770	*No apical meristem (NAM) protein*	-
34	%GL14	S10_60423900	Sb10g030776	" Starch branching enzyme I precursor"	-
35	%GL14	S10_60194381	Sb10g030520	similar to Senescence-associated protein	LOC_Os06g50930.1
36	%GL14	S10_60194379	Sb10g030520	similar to Senescence-associated protein	LOC_Os06g50930.1
37	%GL14	S10_60308140	Sb10g030660	*Exo70 exocyst complex subunit (autophagycytosis/programmed cell death mechanism)*	-
38	%GL14	S10_60297335	Sb10g030640	*Exo70 exocyst complex subunit (autophagycytosis/programmed cell death mechanism)*	LOC_Os05g30660.1

Table 24: (Contd..)

S No:	Trait	SNP	Gene ID	Functional Annotation	Rice Homologs
39	%GL14	S10_60287963	Sb10g030620	*Exo70 exocyst complex subunit (autophagycytosis/programmed cell death mechanism)*	LOC_Os09g17810.1
40	%GL14	S10_60349808	Sb10g030720	similar to Cell division protease ftsH homolog, chloroplast precursor	LOC_Os06g51029.1
41	%GL14	S10_59946860	Sb10g030260	similar to Putative senescence-associated protein	LOC_Os06g50330.1
42	%GL14	S10_59946877	Sb10g030260	similar to Putative senescence-associated protein	LOC_Os06g50330.1
43	%GL14	S10_60701880	Sb10g031030	similar to Putative AGO1 homologous protein	LOC_Os06g51310.1
44	%GL21	S10_58634237	Sb10g028780	similar to Mitogen-activated protein kinase 3	LOC_Os06g48590.1
45	%GL21	S10_59419567	Sb10g029670	similar to Putative uncharacterized protein	LOC_Os06g49700.1
46	%GL21	S10_59342804	Sb10g029570	similar to Putative uncharacterized protein P0655A07.24	LOC_Os06g49650.1
47	%GL21	S10_59564696	Sb10g029820	similar to Putative uncharacterized protein	LOC_Os06g49860.1
48	%GL21	S10_59215385	Sb10g029392	MATE efflux family protein	-
49	%GL21	S10_59342804	Sb10g029570	similar to Putative uncharacterized protein P0655A07.24	LOC_Os06g49650.1
50	%GL21	S10_59564696	Sb10g029820	similar to Putative uncharacterized protein	LOC_Os06g49860.1
51	%GL21	S10_59564696	Sb10g029820	similar to Putative uncharacterized protein	LOC_Os06g49860.1
52	%GL21	S10_59564696	Sb10g029820	similar to Putative uncharacterized protein	LOC_Os06g49860.1
53	%GL21	S10_59564696	Sb10g029820	similar to Putative uncharacterized protein	LOC_Os06g49860.1
54	%GL21	S10_59419567	Sb10g029670	similar to Putative uncharacterized protein	LOC_Os06g49700.1
55	%GL21	S10_59476045	Sb10g029740	similar to Putative uncharacterized protein	LOC_Os06g49770.1
56	%GL21	S10_59413371	Sb10g029660	anthocyanin1	-
57	%GL21	S10_59835807	Sb10g030090	similar to Os06g0714800 protein	LOC_Os06g50080.1
58	%GL28	S10_53160198	Sb10g024190	similar to Putative leucine zipper	LOC_Os06g41100.1
59	%GL28	S10_53834366	Sb10g024680	similar to Chromosome chr14 scaffold_9, whole genome shotgun sequence	LOC_Os06g42560.1
60	%GL28	S10_52676228	Sb10g023920	similar to Pentatricopeptide (PPR) repeat-containing protein-like	LOC_Os06g40860.1
61	%GL28	S10_53058516	Sb10g024170	Signal transduction receptor-regulatr domain	-

Table 24: (Contd..)

S No:	Trait	SNP	Gene ID	Functional Annotation	Rice Homologs
62	%GL28	S10_54269620	Sb10g025053	similar to Glossy15/Apetal2	-
63	%GL28	S10_49366136	Sb10g022090	similar to Chromosome chr9 scaffold_7, whole genome shotgun sequence	LOC_Os06g36360.1
64	%GL28	S10_54532995	Sb10g025283	*NBS-LRR disease resistance protein*	-
65	%GL28	S10_54585199	Sb10g025310	similar to Ankyrin repeat-containing protein-like	LOC_Os06g43680.1
66	%GL28	S10_60282257	Sb10g030610	similar to Putative uncharacterized protein P0548E04.19	LOC_Os06g51010.1
67	%GL28	S10_59946988	Sb10g030260	similar to Putative senescence-associated protein	LOC_Os06g50330.1
68	%GL28	S10_60024056	Sb10g030330	similar to Aspartic proteinase nepenthesin II-like	LOC_Os06g50390.1
69	%GL28	S10_60333532	Sb10g030700	Predicted protein (autophagy related transport to vacuole)	LOC_Os05g30640.1
70	%GL28	S10_60413173	Sb10g030770	*No apical meristem (NAM) protein*	-
71	%GL28	S10_60423900	Sb10g030776	" Starch branching enzyme I precursor"	-
72	%GL28	S10_60194381	Sb10g030520	similar to Senescence-associated protein	LOC_Os06g50930.1
73	%GL28	S10_60194381	Sb10g030520	similar to Senescence-associated protein	LOC_Os06g50930.1
74	%GL28	S10_60308140	Sb10g030660	Predicted protein;cullin protein	-
75	%GL35	S10_54877607	Sb10g025540	Putative prolylcarboxypeptidase isoform 1	-
76	%GL35	S10_60900987	Sb10g031250	similar to Os06g0731700 protein	LOC_Os06g51500.1
77	%GL35	S10_60900987	Sb10g031250	similar to Os06g0731700 protein	LOC_Os06g51500.1
78	%GL35	S10_60344348	Sb10g030710	Predicted protein	LOC_Os08g13570.1
79	%GL35	S10_59889374	Sb10g030175	*rod shape-determining protein MreC*	-
80	%GL35	S10_59946988	Sb10g030260	*Putative senescence-associated protein*	LOC_Os06g50330.1
81	%GL35	S10_60287963	Sb10g030620	Predicted protein	LOC_Os09g17810.1
82	%GL35	S10_60349808	Sb10g030720	similar to Cell division protease ftsH homolog, chloroplast precursor	LOC_Os06g51029.1
83	%GL35	S10_59946860	Sb10g030260	similar to Putative senescence-associated protein	LOC_Os06g50330.1
84	%GL35	S10_59946877	Sb10g030260	similar to Putative senescence-associated protein	LOC_Os06g50330.1
85	%GL35	S10_60701880	Sb10g031030	similar to Putative AGO1 homologous protein	LOC_Os06g51310.1
86	%GL35	S10_59930523	Sb10g030230	similar to Putative uncharacterized protein	LOC_Os06g50240.1

Table 24: (Contd..)

S No:	Trait	SNP	Gene ID	Functional Annotation	Rice Homologs
87	%GL42	S10_52781712	Sb10g023955	similar to Seven transmembrane protein Mlo7	LOC_Os02g10350.1
88	%GL42	S10_53058516	Sb10g024170	Signal transduction receptor-regulatr domain	-
89	%GL42	S10_54269620	Sb10g025053	similar to *glossy15*	-
90	%GL42	S10_49366136	Sb10g022090	similar to Chromosome chr9 scaffold_7, whole genome shotgun sequence	LOC_Os06g36360.1
91	%GL42	S10_53714284	Sb10g024610	similar to Os01g0609200 protein	LOC_Os01g42380.1
92	%GL42	S10_54185069	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1
93	%GL42	S10_53576112	Sb10g024500	NADP binding domain	-
94	%GL42	S10_55599913	Sb10g026200	similar to SBP-domain protein 4	LOC_Os06g44860.1
95	%GL42	S10_57400347	Sb10g027610	similar to EF-hand Ca2+-binding protein CCD1	LOC_Os06g46950.1
96	%GL42	S10_57552719	Sb10g027760	F-box/LRR	-
97	%GL42	S10_57248800	Sb10g027440	similar to Putative uncharacterized protein	LOC_Os06g46700.1
98	%GL42	S10_57432493	Sb10g027640	weakly similar to O-methyltransferase ZRP4	-
99	%GL42	S10_57522978	Sb10g027730	Predicted protein	LOC_Os02g06520.1
100	%GL42	S10_58640688	Sb10g028790	similar to Putative uncharacterized protein	LOC_Os06g48600.1
101	%GL42	S10_58022779	Sb10g028130	similar to Putative thaumatin-protein	LOC_Os06g47600.1
102	%GL42	S10_58311699	Sb10g028500	similar to Peroxidase 16 protein	LOC_Os06g48030.1
103	%GL42	S10_58357039	Sb10g028550	similar to Putative uncharacterized protein	LOC_Os06g48160.1
104	%GL42	S10_60282257	Sb10g030610	similar to Putative uncharacterized protein P0548E04.19	LOC_Os06g51010.1
105	%GL42	S10_59946988	Sb10g030260	similar to Putative senescence-associated protein	LOC_Os06g50330.1
106	%GL42	S10_60024056	Sb10g030330	similar to Aspartic proteinase nepenthesin II-like	LOC_Os06g50390.1
107	%GL42	S10_60333532	Sb10g030700	Predicted protein (autophagy related transport to vacuole)	LOC_Os05g30640.1
108	%GL42	S10_60413173	Sb10g030770	*No apical meristem (NAM) protein*	-
109	%GL42	S10_60423900	Sb10g030776	" Starch branching enzyme I precursor"	-
110	%GL42	S10_60194381	Sb10g030520	similar to Senescence-associated protein	LOC_Os06g50930.1

Table 24: (Contd..)

S No:	Trait	SNP	Gene ID	Functional Annotation	Rice Homologs
111	%GL42	S10_60194379	Sb10g030520	similar to Senescence-associated protein	LOC_Os06g50930.1
112	%GL42	S10_60308140	Sb10g030660	Predicted protein;cullin protein	-
113	%GL49	S10_52700141	Sb10g023930	similar to Type III chlorophyll a/b-binding protein	LOC_Os02g10390.1
114	%GL49	S10_52940776	Sb10g024110	*Helix-loop-helix DNA-binding*	-
115	%GL49	S10_54877607	Sb10g025540	*Putative prolylcarboxypeptidase isoform 1*	-
116	%GL49	S10_53576112	Sb10g024500	NADP binding domain	-
117	%GL49	S10_54081973	Sb10g024920	weakly similar to Putative uncharacterized protein	LOC_Os06g43060.1
118	%GL49	S10_54532995	Sb10g025283	*NBS-LRR disease resistance protein*	-
119	%GL49	S10_54585199	Sb10g025310	similar to Ankyrin repeat-containing protein-like	LOC_Os06g43680.1
120	%GL49	S10_54535306	Sb10g025283	*NBS-LRR disease resistance protein*	-
121	%GL49	S10_54185546	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1
122	%GL49	S10_54185539	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1
123	%GL49	S10_60413173	Sb10g030770	*No apical meristem (NAM) protein*	LOC_Os06g51070.1
124	%GL49	S10_60423900	Sb10g030776	*" Starch branching enzyme I precursor"*	-

Table 25: Candidate genes for agronomic and yield related traits in the mapped QTL regions of SBI-10

S. NO.	Trait	SNP	Gene ID	Functional annotaation	Rice homolog	Pos in CM
1	FT	S10_51071502	Sb10g022800	similar to Putative nitrate transporter NTL1	LOC_Os06g38294.1	25.88
2	FT	S10_51228412	Sb10g022900	weakly similar to Chromosome undetermined scaffold_151, whole genome shotgun sequence	LOC_Os06g38980.1	26.918
3	FT	S10_52042465	Sb10g023430	similar to Putative uncharacterized protein	LOC_Os06g39970.1	27.524
4	FT	S10_52676228	Sb10g023920	similar to Pentatricopeptide (PPR) repeat-containing protein-like	LOC_Os06g40860.1	27.785
5	FT	S10_51224387	Sb10g022890	weakly similar to Chromosome chr1 scaffold_46, whole genome shotgun sequence	LOC_Os06g38970.1	28.41
6	FT	S10_52677221	Sb10g023920	similar to Pentatricopeptide (PPR) repeat-containing protein-like	LOC_Os06g40860.1	29.365
7	FT	S10_52676281	Sb10g023920	similar to Pentatricopeptide (PPR) repeat-containing protein-like	LOC_Os06g40860.1	29.715
8	FT	S10_51919897	Sb10g023350	similar to Elongation factor 1-alpha	LOC_Os03g08050.1	30.959
9	FT	S10_52781712	Sb10g023955	similar to Seven transmembrane protein Mlo7	LOC_Os02g10350.1	32.291
10	FT	S10_53682073	Sb10g024575	DUF597		34.972
11	FT	S10_53160198	Sb10g024190	similar to Putative leucine zipper	LOC_Os06g41100.1	35.678
12	FT	S10_53834366	Sb10g024680	similar to Chromosome chr14 scaffold_9, whole genome shotgun sequence	LOC_Os06g42560.1	35.758
13	FT	S10_52675727	Sb10g023920	similar to Pentatricopeptide (PPR) repeat-containing protein-like	LOC_Os06g40860.1	35.997
14	FT	S10_53058516	Sb10g024170	Signal transduction receptor-regulatr domain		36.145
15	FT	S10_54269620	Sb10g025053	similar to *glossy15/AP2*		36.401
16	FT	S10_49366136	Sb10g022090	similar to Chromosome chr9 scaffold_7, whole genome shotgun sequence	LOC_Os06g36360.1	37.73
17	FT	S10_53714284	Sb10g024610	similar to Os01g0609200 protein	LOC_Os01g42380.1	38.079
18	FT	S10_54185069	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1	38.342
19	FT	S10_50232566	Sb10g022450	Role in Cytochrome P_450 (photosynthesis)		39.38
20	FT	S10_50232568	Sb10g022450	Role in cytochrome P_450 (photosynthesis)		39.38

Table 25: (Contd..)

S. NO.	Trait	SNP	Gene ID	Functional annotaation	Rice homolog	Pos in CM
21	FT	S10_53544398	Sb10g024460	Predicted protein	LOC_Os09g39020.1	39.394
22	FT	S10_52700141	Sb10g023930	similar to Type III chlorophyll a/b-binding protein	LOC_Os02g10390.1	39.782
23	FT	S10_52940776	Sb10g024110	Catalytic activity		40.377
24	FT	S10_54877607	Sb10g025540	Putative prolylcarboxypeptidase isoform 1		40.677
25	FT	S10_53576112	Sb10g024500	NADP binding domain		40.76
26	FT	S10_54081973	Sb10g024920	weakly similar to Putative uncharacterized protein	LOC_Os06g43060.1	41.371
27	FT	S10_54532995	Sb10g025283	NBS-LRR disease resistance protein		43.958
28	FT	S10_54585199	Sb10g025310	similar to Ankyrin repeat-containing protein-like	LOC_Os06g43680.1	44.416
29	FT	S10_54535306	Sb10g025283	NBS-LRR disease resistance protein		44.945
30	FT	S10_54185546	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1	45.857
31	FT	S10_54185539	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1	45.985
32	Pl Ht	S10_57088032	Sb10g027340	weakly similar to O-methyltransferase ZRP4	LOC_Os12g25450.1	96.055
33	Pl Ht	S10_57400347	Sb10g027610	similar to EF-hand Ca2+-binding protein CCD1	LOC_Os06g46950.1	97.265
34	Pl Ht	S10_57552719	Sb10g027760	F-box/LRR	0	98.396
35	Pl Ht	S10_57248800	Sb10g027440	similar to Putative uncharacterized protein	LOC_Os06g46700.1	99.044
36	Pl Ht	S10_57432493	Sb10g027640	weakly similar to O-methyltransferase ZRP4	0	99.608
37	Pl Ht	S10_57522978	Sb10g027730	Predicted protein	LOC_Os02g06520.1	101.244
38	Pl Ht	S10_58640688	Sb10g028790	similar to Putative uncharacterized protein	LOC_Os06g48600.1	102.44
39	Pl Ht	S10_58022779	Sb10g028130	similar to Putative thaumatin-protein	LOC_Os06g47600.1	103.623
40	Pl Ht	S10_58311699	Sb10g028500	similar to Peroxidase 16 protein	LOC_Os06g48030.1	104.802
41	Pl Ht	S10_58357039	Sb10g028550	similar to Putative uncharacterized protein	LOC_Os06g48160.1	108.032
42	Pl Ht	S10_58683017	Sb10g028870	similar to Putative meiotic serine proteinase	LOC_Os06g48650.1	108.615
43	Pl Ht	S10_59833299	Sb10g030080	weakly similar to Putative uncharacterized protein	LOC_Os06g50070.1	109.008
44	Pl Ht	S10_59148610	Sb10g029305	similar to Os02g0137100 protein	LOC_Os06g49180.1	109.224
45	Pl Ht	S10_58839857	Sb10g029010	similar to Putative uncharacterized protein	LOC_Os05g20030.1	109.466

Table 25: (Contd..)

S. NO.	Trait	SNP	Gene ID	Functional annotaation	Rice homolog	Pos in CM
46	Pl Ht	S10_58652548	Sb10g028810	similar to Os03g0824600 protein	LOC_Os03g60960.1	109.901
47	Pl Ht	S10_59316155	Sb10g029530	similar to Pentatricopeptide (PPR) repeat-containing protein-like	LOC_Os09g24640.1	110.125
48	Pl Ht	S10_59024190	Sb10g029190	similar to Squamosa promoter-binding-like protein 12	LOC_Os06g49010.1	110.337
49	Pl Ht	S10_59069052	Sb10g029245	similar to Putative uncharacterized protein/translation initiation factor IF-2	-	110.528
50	HGM	S10_59342804	Sb10g029570	similar to Putative uncharacterized protein P0655A07.24	LOC_Os06g49650.1	114.593
51	HGM	S10_59564696	Sb10g029820	similar to Putative uncharacterized protein	LOC_Os06g49860.1	114.809
52	HGM	S10_59215385	Sb10g029392	MATE efflux family protein	-	115.101
53	HGM	S10_59342820	Sb10g029570	similar to Putative uncharacterized protein P0655A07.24	LOC_Os06g49650.1	115.321
54	HGM	S10_59566700	Sb10g029820	similar to Putative uncharacterized protein	LOC_Os06g49860.1	115.589
55	HGM	S10_59566699	Sb10g029820	similar to Putative uncharacterized protein	LOC_Os06g49860.1	115.632
56	HGM	S10_59565625	Sb10g029820	similar to Putative uncharacterized protein	LOC_Os06g49860.1	116.207
57	HGM	S10_59565627	Sb10g029820	similar to Putative uncharacterized protein	LOC_Os06g49860.1	116.207
58	HGM	S10_59418734	Sb10g029670	similar to Putative uncharacterized protein	LOC_Os06g49700.1	116.702
59	HGM	S10_59476045	Sb10g029740	similar to Putative uncharacterized protein	LOC_Os06g49770.1	117.456
60	HGM	S10_59413371	Sb10g029660	anthocyanin1	-	117.994
61	HGM	S10_59835807	Sb10g030090	similar to Os06g0714800 protein	LOC_Os06g50080.1	118.575
62	HGM	S10_59775260	Sb10g030040	*" Calcium/calmodulin-dependent protein kinase;LYR motif containing protein*	-	118.728
63	HGM	S10_59866581	Sb10g030150	*" Calcium-dependent protein kinase CPK1 adapter protein 2*	-	119.287
64	HGM	S10_59826585	Sb10g030060	similar to Chromosome chr16 scaffold_86, whole genome shotgun sequence	LOC_Os06g50050.1	119.868
65	HGM	S10_59525199	Sb10g029810	29kb down-stream to MADS box transcriptional factor		116.941
66	GNP/plot & GNPP	S10_57522978	Sb10g027730	Predicted protein	LOC_Os02g06520.1	101.244
67	GNP/plot & GNPP	S10_58640688	Sb10g028790	similar to Putative uncharacterized protein	LOC_Os06g48600.1	102.44

Table 25: (Contd..)

S. NO.	Trait	SNP	Gene ID	Functional annotaation	Rice homolog	Pos in CM
68	GNP/plot & GNPP	S10_58022779	Sb10g028130	similar to Putative thaumatin-protein	LOC_Os06g47600.1	103.623
69	GNP/plot & GNPP	S10_58634237	Sb10g028780	similar to Mitogen-activated protein kinase 3	LOC_Os06g48590.1	113.523
70	GNP/plot & GNPP	S10_59419567	Sb10g029670	similar to Putative uncharacterized protein	LOC_Os06g49700.1	113.698
71	GNP/plot & GNPP	S10_60282257	Sb10g030610	similar to Putative uncharacterized protein P0548E04.19	LOC_Os06g51010.1	122.169
72	GNP/plot & GNPP	S10_59946988	Sb10g030260	similar to Putative senescence-associated protein	LOC_Os06g50330.1	122.798
73	GNP/plot & GNPP	S10_60024056	Sb10g030330	similar to Aspartic proteinase nepenthesin II-like	LOC_Os06g50390.1	123.601
74	GNP/plot & GNPP	S10_60287963	Sb10g030620	Predicted protein	LOC_Os09g17810.1	129.512
75	GNP/plot & GNPP	S10_60349808	Sb10g030720	similar to Cell division protease ftsH homolog, chloroplast precursor	LOC_Os06g51029.1	129.798
76	GNP/plot & GNPP	S10_60245187	Sb10g030580	similar to Putative uncharacterized protein	LOC_Os06g50980.1	133.343
77	PnDW/Plot & GDW/plot	S10_58357039	Sb10g028550	similar to Putative uncharacterized protein	LOC_Os06g48160.1	108.032
78	PnDW/Plot & GDW/plot	S10_58683017	Sb10g028870	similar to Putative meiotic serine proteinase	LOC_Os06g48650.1	108.615
79	PnDW/Plot & GDW/plot	S10_59833299	Sb10g030080	weakly similar to Putative uncharacterized protein	LOC_Os06g50070.1	109.008
80	PnDW/Plot & GDW/plot	S10_59148610	Sb10g029305	similar to Os02g0137100 protein	LOC_Os06g49180.1	109.224
81	PnDW/Plot & GDW/plot	S10_58839857	Sb10g029010	similar to Putative uncharacterized protein	LOC_Os05g20030.1	109.466
82	PnDW/Plot & GDW/plot	S10_58652548	Sb10g028810	similar to Os03g0824600 protein/Glycosyl transferase protein	LOC_Os03g60960.1	109.901

Table 25: (Contd..)

S. NO.	Trait	SNP	Gene ID	Functional annotaation	Rice homolog	Pos in CM
83	PHI	S10_60308140	Sb10g030660	Exo70 exocyst complex subunit	-	125.703
84	PHI	S10_59419567	Sb10g029670	Transcription termination factor Rho; Provisional	-	113.698
85	PHI	S10_60324251	-	INTERGENIC	-	122.46
86	PHI	S10_50140543	-	INTERGENIC	-	35.288
87	PHI	S10_56252649	Sb10g026810	RF4; DNA polymerase sigma	-	73.641
88	PHI	S10_51263932	-	INTERGENIC	-	18.488

Table 26: Shoot fly resistance candidate genes in the target region of sorghum chromosome SBI-10L

S No.	Trait	SNP	Gene ID	Functional Annotation	Rice homolog	Pos in cM
1	Gls	S10_54223864	Sb10g025040	C2 CaLB binds to membrane lipids and mediate signal transduction	-	54.416
2	Gls	S10_53058516	Sb10g024170	Signal transduction receptor-regulatr domain	-	36.145
3	Gls	S10_54269620	Sb10g025053	similar to *glossy15*/AP2	-	36.401
4	Gls	S10_53099908	Sb10g024180	MYB transcriptional factor alters WIN1/SHN1 which encodes AP2/EREBP	-	-
5	Gls	S10_52940776	Sb10g024110	Basic helix loop helix family protein	-	40.377
6	Gls	S10_54877607	Sb10g025540	Putative prolylcarboxypeptidase isoform 1	-	40.677
7	Gls	S10_53576112	Sb10g024500	NADP binding domain	-	40.76
8	Gls	S10_54081973	Sb10g024920	weakly similar to Putative uncharacterized protein	LOC_Os06g43060.1	41.371
9	Gls	S10_54532995	Sb10g025283	NBS-LRR disease resistance protein	-	43.958
10	Gls	S10_54585199	Sb10g025310	similar to Ankyrin repeat-containing protein-like	LOC_Os06g43680.1	44.416
11	Gls	S10_54535306	Sb10g025283	NBS-LRR disease resistance protein	-	44.945
12	Gls	S10_54185546	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1	45.857
13	Gls	S10_54185539	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1	45.985

Table 26: (Contd..)

S No.	Trait	SNP	Gene ID	Functional Annotation	Rice homolog	Pos in cM
14	Gls	S10_54185186	Sb10g025010	similar to Putative uncharacterized protein	LOC_Os01g33090.1	46.461
15	TDU/TDL	S10_54966382	Sb10g025600	WRKY transcription factor		47.424
16	TDU/TDL	S10_56186177	Sb10g026730	Speckle POZ protein		-
17	TDU/TDL	S10_57031008	Sb10g027280	MYB transcryption factor		-
18	TDU/TDL	S10_57341007	Sb10g027550	weakly similar to Zinc finger (C2H2 type) protein-like	LOC_Os06g46910.1	91.295
19	TDU/TDL	S10_57145296	Sb10g027370	similar to Os06g0680500 protein	LOC_Os06g46670.2	93.122
20	TDU/TDL	S10_57552456	Sb10g027760	F-box/LRR	-	94.409
21	TDU/TDL	S10_57088032	Sb10g027340	weakly similar to O-methyltransferase ZRP4	LOC_Os12g25450.1	96.055
22	TDU/TDL	S10_57400347	Sb10g027610	similar to EF-hand Ca2+-binding protein CCD1	LOC_Os06g46950.1	97.265
23	TDU/TDL	S10_57552719	Sb10g027760	F-box/LRR	-	98.396
24	TDU/TDL	S10_57248800	Sb10g027440	similar to Putative uncharacterized protein	LOC_Os06g46700.1	99.044
25	TDU/TDL	S10_57432493	Sb10g027640	weakly similar to O-methyltransferase ZRP4	-	99.608
26	TDU/TDL	S10_57522978	Sb10g027730	Predicted protein: F-box domain	LOC_Os02g06520.1	101.244
27	TDU/TDL	S10_58640688	Sb10g028790	similar to Putative uncharacterized protein	LOC_Os06g48600.1	102.44
28	TDU/TDL	S10_58022779	Sb10g028130	similar to Putative thaumatin-protein	LOC_Os06g47600.1	103.623
29	TDU/TDL	S10_58311699	Sb10g028500	similar to Peroxidase 16 protein	LOC_Os06g48030.1	104.802
30	TDU/TDL	S10_58357039	Sb10g028550	similar to Putative uncharacterized protein	LOC_Os06g48160.1	108.032
31	TDU/TDL	S10_58683017	Sb10g028870	similar to Putative meiotic serine proteinase	LOC_Os06g48650.1	108.615
32	TDU/TDL	S10_59833299	Sb10g030080	weakly similar to Putative uncharacterized protein	LOC_Os06g50070.1	109.008
33	TDU/TDL	S10_59148610	Sb10g029305	similar to Os02g0137100 protein	LOC_Os06g49180.1	109.224
34	TDU/TDL	S10_58839857	Sb10g029010	similar to Putative uncharacterized protein	LOC_Os05g20030.1	109.466
35	TDU/TDL	S10_58652548	Sb10g028810	similar to Os03g0824600 protein/Glycosyl transferase protein	LOC_Os03g60960.1	109.901
36	TDU/TDL	S10_57490614	Sb10g027680	Armidillo repeat protein	-	-

Table 27: Selected double recombinants performing better than parent or nearby parental donors for further generation advancing (pyramiding)

	Agronomic_ Summer13							Agronomic_Summer14							Agronomic_Across season						
S No.	F2: GH _name	FT_12	PlHt_12	PnDW/plot_12	GDW/plot_12	HGM_12	GNP/plot_12	GNPP_12	FT_13	PlHt_13	PnDW/plot_13	GDW/plot_13	HGM_13	GNP/plot_13	GNPP_13	FT	meanPlHt	GDW/plot	HGM	PnDW/plot	GNP/plot	GNPP
1	U120020	59.82	88.44	552.53	355.46	1.51	21514.95	827.50	77.17	108.56	861.66	607.50	2.37	21662.33	833.17	68.51	92.65	490.05	1.95	706.39	21717.79	835.30
2	U120201	66.76	123.19	634.12	440.04	2.31	20853.83	802.07	85.03	178.38	1005.55	639.14	3.00	20052.44	771.25	76.13	151.30	531.81	2.67	815.57	20894.50	803.63
3	U120356	63.45	114.32	475.81	310.36	2.31	18777.70	722.22	83.32	155.68	975.70	670.20	3.40	21610.73	831.18	73.81	135.03	506.85	2.84	746.09	20241.13	778.50
4	U120609	62.35	109.05	596.10	410.98	2.09	20991.15	807.35	79.58	169.97	1069.35	777.47	3.22	25743.95	990.15	71.19	138.80	587.45	2.67	836.83	22652.56	871.25
5	**U120807**	**61.74**	**99.33**	**584.64**	**392.61**	**1.68**	**21828.95**	**839.57**	**78.95**	**138.85**	**1030.67**	**673.83**	**2.94**	**23273.36**	**895.13**	**70.47**	**118.08**	**536.44**	**2.32**	**813.61**	**22370.48**	**860.40**
6	U120860	61.74	130.22	533.63	367.26	1.81	20774.32	799.01	81.20	187.05	946.41	680.02	2.84	23782.34	914.71	71.67	159.61	529.24	2.33	746.54	21942.85	843.96
7	**U121011**	**61.74**	**101.51**	**637.85**	**443.47**	**1.75**	**22799.60**	**876.91**	**82.82**	**148.17**	**923.65**	**659.26**	**2.62**	**24203.82**	**930.92**	**72.49**	**123.87**	**542.51**	**2.18**	**767.90**	**23141.02**	**890.04**
8	U121021	64.88	117.36	624.40	427.15	1.99	21435.07	824.43	77.15	149.97	819.02	571.53	2.57	19404.10	746.31	70.90	134.13	492.53	2.30	705.65	20997.98	807.61
9	U121062	70.07	134.91	619.06	444.88	2.13	21314.83	819.80	84.55	177.83	844.32	600.03	2.89	19453.12	748.20	77.03	157.95	507.34	2.52	710.45	20961.34	806.21
10	**U121113**	**59.50**	**122.35**	**651.02**	**427.66**	**2.15**	**20956.19**	**806.01**	**78.16**	**172.21**	**973.00**	**664.49**	**2.91**	**22674.27**	**872.09**	**69.06**	**147.86**	**531.44**	**2.53**	**791.85**	**21714.47**	**835.17**
11	U121114	60.49	104.56	647.69	462.69	2.01	22085.10	849.43	83.87	145.23	929.67	613.12	3.31	19782.24	760.86	71.55	124.13	523.61	2.66	774.26	21494.51	826.71
12	**U121124**	**70.74**	**84.97**	**529.97**	**343.01**	**1.74**	**20534.17**	**789.78**	**83.27**	**112.74**	**956.89**	**643.30**	**2.42**	**24577.23**	**945.28**	**76.63**	**96.42**	**504.78**	**2.07**	**753.52**	**22040.04**	**847.69**
13	U121147	65.53	138.65	651.86	461.72	1.81	22893.16	880.51	80.45	196.41	837.24	598.07	3.70	19006.27	731.01	72.94	169.54	519.03	2.71	726.33	21688.44	834.17
14	U121163	64.23	84.77	416.17	274.42	1.95	18765.92	721.77	82.79	105.19	1078.75	736.37	3.33	25509.50	981.13	73.61	92.90	535.64	2.62	791.51	21388.82	822.65
15	U121168	61.10	104.09	535.37	349.00	1.69	20767.77	798.76	77.51	175.37	903.73	599.70	2.76	20719.36	796.90	69.46	138.71	483.95	2.23	725.09	21047.97	809.54
16	**U121564**	**67.09**	**108.45**	**511.44**	**354.04**	**2.06**	**19979.55**	**768.44**	**83.34**	**166.45**	**911.93**	**658.80**	**2.99**	**22151.68**	**851.99**	**75.15**	**137.18**	**516.99**	**2.52**	**720.94**	**21032.26**	**808.93**
17	U121644	62.05	119.09	640.54	438.18	1.83	22155.67	852.14	76.46	184.61	993.06	708.01	2.86	24539.82	943.84	69.43	152.33	559.39	2.36	805.46	22887.45	880.29
18	**U121658**	**64.22**	**127.83**	**574.01**	**389.04**	**2.18**	**20248.06**	**778.77**	**76.54**	**185.87**	**964.18**	**654.10**	**2.89**	**22437.08**	**862.96**	**70.86**	**157.63**	**523.21**	**2.53**	**773.94**	**21252.37**	**817.40**
19	**U121700**	**65.53**	**128.51**	**732.15**	**456.65**	**2.16**	**21433.33**	**824.36**	**83.09**	**188.57**	**1079.77**	**675.45**	**3.61**	**20702.58**	**796.25**	**74.74**	**159.19**	**551.31**	**2.88**	**887.26**	**21401.02**	**823.12**
20	U121717	65.49	98.63	585.64	384.09	2.11	20304.12	780.93	83.29	143.74	1004.66	671.66	2.94	23247.13	894.12	74.50	120.02	527.05	2.49	793.97	21529.13	828.04

Table 27: (Contd..)

		%GL _*rabi*/Summer 2013							%GLA _*rabi*/Summer 2014							%GL _Across season						
S No.	**F_2: GH _name**	**GL13_W1**	**GL13_W2**	**GL13_W3**	**GL13_W4**	**GL13_W5**	**GL13_W6**	**GL13_W7**	**GL14_W1**	**GL14_W2**	**GL14_W3**	**GL14_W4**	**GL14_W5**	**GL14_W6**	**GL14_W7**	**GL(1week)**	**GL(2 week)**	**GL(3week)**	**GL(4week)**	**GL(5week)**	**GL(6 week)**	**GL(7 week)**
1	U120020	97.72	95.91	93.57	70.44	52.68	34.28	18.49	98.89	95.30	79.40	70.08	58.36	24.42	24.34	98.53	96.68	87.20	71.07	56.24	30.64	21.71
2	U120201	92.43	85.91	62.46	46.06	33.43	25.63	17.27	99.01	85.99	76.93	70.10	54.94	49.67	43.03	95.74	85.79	70.51	58.71	44.42	36.71	29.30
3	U120356	92.40	85.32	69.22	51.45	42.41	32.02	22.52	99.07	85.00	73.41	66.40	46.54	31.54	26.14	95.82	85.11	71.33	59.00	43.69	32.44	24.66
4	U120609	97.72	95.83	88.18	66.44	50.21	34.21	23.58	98.87	91.63	79.67	65.92	56.09	45.47	36.86	98.49	94.63	85.09	66.88	53.71	39.69	30.38
5	**U120807**	**97.74**	**96.06**	**86.72**	**64.79**	**43.57**	**28.70**	**16.15**	**98.94**	**90.85**	**76.38**	**68.90**	**57.93**	**39.85**	**35.18**	**98.50**	**93.96**	**81.85**	**67.58**	**51.45**	**34.29**	**25.24**
6	U120860	97.73	95.93	86.84	64.91	48.74	31.07	17.53	99.03	87.87	73.64	66.34	50.04	40.17	32.76	98.49	91.91	79.85	65.50	48.93	35.55	24.76
7	**U121011**	**97.76**	**96.43**	**82.59**	**63.33**	**44.47**	**31.67**	**20.41**	**98.87**	**78.46**	**68.22**	**61.89**	**44.12**	**39.85**	**34.68**	**98.38**	**87.15**	**74.48**	**62.18**	**43.84**	**36.04**	**27.50**
8	U121021	97.73	95.66	76.11	57.20	45.57	30.22	23.05	99.10	96.30	83.68	74.26	66.05	46.82	35.22	98.62	96.68	80.71	66.22	56.61	37.92	28.85
9	U121062	83.05	73.27	62.40	49.96	39.51	29.60	18.33	99.10	82.39	69.22	61.44	49.14	45.60	38.27	91.14	77.62	65.32	55.50	43.95	37.30	27.63
10	**U121113**	**97.77**	**96.49**	**86.70**	**69.74**	**51.65**	**40.10**	**27.32**	**98.98**	**90.44**	**76.08**	**68.85**	**56.13**	**45.55**	**40.89**	**98.41**	**93.95**	**81.73**	**69.81**	**54.17**	**43.30**	**33.99**
11	U121114	97.78	96.59	89.34	69.70	51.98	37.10	21.26	99.14	87.02	76.98	69.29	52.75	46.93	36.55	98.46	91.91	83.10	70.02	52.64	42.13	28.78
12	**U121124**	**87.07**	**74.71**	**62.39**	**44.55**	**35.72**	**27.35**	**16.30**	**98.98**	**85.43**	**77.22**	**70.07**	**51.38**	**37.20**	**33.52**	**93.08**	**79.67**	**69.90**	**57.46**	**43.60**	**32.49**	**24.27**
13	U121147	97.74	96.13	66.42	53.98	39.62	27.22	20.58	98.91	83.24	71.95	61.68	53.47	39.82	35.97	98.48	89.87	69.19	57.86	46.69	33.63	28.10
14	U121163	97.76	96.20	74.56	55.34	40.75	27.22	19.25	96.56	87.33	76.60	67.30	53.39	40.00	20.45	97.15	91.91	75.83	61.47	47.25	33.67	20.55
15	U121168	97.78	96.64	88.03	67.49	48.49	31.74	22.36	99.12	91.21	79.08	71.12	62.75	51.24	41.24	98.45	93.95	83.77	69.71	55.76	40.93	31.15
16	**U121564**	**89.71**	**84.94**	**65.16**	**48.68**	**34.69**	**25.37**	**18.64**	**99.02**	**79.98**	**69.55**	**62.61**	**48.90**	**34.51**	**11.80**	**94.49**	**82.39**	**67.18**	**55.31**	**41.40**	**30.09**	**16.04**
17	U121644	97.76	96.45	81.25	59.35	45.06	30.11	19.59	98.87	90.96	75.59	66.77	58.28	38.57	41.03	98.38	93.95	78.45	63.41	52.11	34.31	28.86
18	**U121658**	**97.75**	**96.19**	**79.96**	**60.58**	**40.76**	**31.87**	**22.36**	**99.03**	**93.13**	**76.47**	**69.01**	**61.17**	**45.59**	**39.45**	**98.44**	**94.63**	**77.84**	**64.76**	**51.06**	**38.49**	**30.47**
19	**U121700**	**91.10**	**80.49**	**65.14**	**49.97**	**38.19**	**27.80**	**20.61**	**98.98**	**90.68**	**78.68**	**72.24**	**59.61**	**48.40**	**28.39**	**95.01**	**85.11**	**71.97**	**61.55**	**49.11**	**37.33**	**24.67**
20	U121717	97.74	95.99	82.67	65.89	52.35	37.56	27.74	99.01	84.57	73.77	67.48	45.91	31.60	7.83	98.52	90.55	78.45	67.28	48.81	35.50	22.23

Table 27: (Contd..)

				SFR_*kharif* 2013			SFR_*rabi* 2013			SFR Across season		
S No.	**Geno**	**F3:F4 Field name(seed packet name)**	**F2: GH _name**	**Glossy_K13**	**TRICHOME UP_K13**	**TRICHOME LOW_K13**	**Glossy_K13**	**TRICHOME UP_K13**	**TRICHOME LOW_K13**	**Trichome low**	**Glossy score**	**Trichome up**
1	3	U122011	U120020	1.84	78.24	16.99	2.46	101.03	47.37	32.90	2.01	91.00
2	10	U122064	U120201	2.07	97.92	20.24	2.44	75.09	42.72	32.24	2.14	89.07
3	14	U122086	U120356	2.31	74.54	29.01	2.70	79.13	41.84	36.36	2.43	78.48
4	26	U122138	U120609	2.77	78.25	22.14	3.07	108.94	59.40	42.59	2.90	94.67
5	**31**	**U122162**	**U120807**	**2.78**	**118.41**	**38.99**	**2.43**	**110.86**	**47.00**	**42.99**	**2.56**	**117.32**
6	36	U122173	U120860	2.07	106.72	30.85	1.87	81.83	55.18	45.16	1.87	98.00
7	**52**	**U122210**	**U121011**	**1.82**	**68.63**	**29.47**	**2.14**	**68.91**	**26.16**	**27.82**	**1.88**	**68.12**
8	53	U122211	U121021	2.31	67.83	30.48	2.72	63.42	43.20	37.71	2.43	66.35
9	60	U122223	U121062	2.78	91.40	22.84	2.76	83.13	38.01	30.87	2.73	90.69
10	**70**	**U122236**	**U121113**	**3.01**	**72.97**	**17.90**	**2.19**	**79.24**	**33.62**	**25.54**	**2.59**	**77.65**
11	71	U122237	U121114	2.54	99.31	17.85	3.07	75.56	65.94	43.65	2.73	88.79
12	**73**	**U122241**	**U121124**	**1.83**	**94.72**	**29.97**	**2.74**	**92.27**	**54.87**	**43.34**	**2.16**	**95.10**
13	77	U122247	U121147	2.78	78.88	19.73	2.72	56.86	43.86	31.94	2.72	66.82
14	78	U122250	U121163	2.54	99.61	24.50	3.04	109.26	39.99	32.99	2.74	105.66
15	81	U122253	U121168	1.84	86.46	26.01	2.47	67.32	34.20	30.47	2.00	79.15
16	**107**	**U122349**	**U121564**	**2.55**	**63.66**	**13.85**	**2.72**	**93.69**	**51.05**	**32.82**	**2.56**	**77.75**
17	116	U122365	U121644	2.77	65.29	36.93	3.06	63.70	29.28	33.67	2.89	62.56
18	**119**	**U122369**	**U121658**	**2.30**	**73.00**	**39.90**	**2.43**	**61.36**	**40.34**	**41.06**	**2.30**	**69.63**
19	**124**	**U122376**	**U121700**	**2.79**	**57.78**	**12.60**	**2.44**	**100.48**	**25.06**	**18.13**	**2.57**	**77.26**
20	126	U122382	U121717	2.55	107.54	50.67	2.46	97.74	62.27	57.66	2.43	105.41

Table 28: Shoot fly meta QTL analysis on SBI-10

Reference	Trait of SFR	Marker interval	genetic map dist	Closest marker	Physical map positions (Mb)	Size of QTL in physical positions	Pedegree	QTL mapping methods	Population
Sajjanar 2002	Gls*	*Xgap001-Xtxp141*	34cM	*Xgap001*	54.50-58.24	3.74Mb	Btx623/IS18551	CIM/Cartographer	259 RIL
Sajjanar 2002	TD*	*Xgap001-Xtxp141*	34cM	*Xtxp141*	54.50-58.24	3.74Mb	Btx623/IS18551	–	259 RIL
Deshpandae 2005	TD	*Xgap001-Xcup67*	22cM	–	54.50-12.27	42Mb	296B/IS18551	–	213 RIL
Mehtre 2006	TD	*Xgap001-Xcup67*	22cM	–	54.50-12.27	42Mb	296B/IS18551	–	213 RIL
Satish *et al.* ,2009	Gls*	*Xgap001-Xnhsbm1043*	10cM	–	54.50-56.88	2.38Mb	296B/IS18551	–	168 RIL
Satish *et al.* ,2009	TD	*Xgap001-Xnhsbm1043*	10cM	–	54.50-56.88	2.38Mb	296B/IS18551	–	168 RIL
Satish *et al.*, 2009	TD*	*Xnhsbm1013-Xnhsbm1048*	11cM	–	55.04-57.39	2.35Mb	296B/IS18551	–	168 RIL
Aruna *et al*., 2011	Gls***	*Xtxp320 -Xcup16*	17cM	–	55.38-57.76	2.38Mb	296B/IS18551	–	168 RIL
Aruna *et al*., 2011	TD*	*Xtxp320 -Xcup16*	17cM	–	55.38-57.76	2.38Mb	27B/IS2122	–	210 RIL
Aruna *et al*., 2011	TD	*Xgap001-Xtxp320*	4cM	–	54.50-55.38	880kb	27B/IS2122	–	210 RIL
Satish *et al*., 2012	Gls*	*Xgap001-Xnhsbm1011*	5cM	*Xgap001*	54.50-54.90	400kb	296B/IS18551	–	168 RIL
Satish *et al*., 2012	Gls**	*SvpepcA- XnhsbmSFC4*	5.9cM	–	47.13-46.50	630Mb	296B/IS18551	–	168 RIL
Satish *et al*., 2012	Gls***	*XnhsbmSFC34-Xnhsbm1039*	8cM	–	57.83-58.24	410kb	296B/IS18551	–	168 RIL
Satish *et al*., 2012	TD	*Xgap001-Xnhsbm1011*	5cM	*Xgap001*	54.50-54.90	400kb	296B/IS18551	–	168 RIL
Satish *et al*., 2012	TD*	*XnhsbmSFC34-Xnhsbm1039*	8cM	–	57.83-58.24	410kb	296B/IS18551	–	168 RIL
kiranmayee *et al.*, 2016	Gls*	*Xgap001-Xnhsbm1044*	10cM	*Xgap001*	54.50-56.96	2.46Mb	RSG04008-6/J2614-11	CIM/QTLCartographer	1894 F2
kiranmayee *et al.*,2016	TD*	*Xnhsbm1044-Xtxp141*	8cM	–	57.00-58.24	740kb	RSG04008-6/J2614-11	CIM/QTLCartographer	1894 F2
kiranmayee *et al.*,2016	Gls*	*XIsp10263-Xgap001*	14cM	*Xgap001*	49.67-54.50	4.83Mb	RSG04008-6/J2614-11	PlaB QTL	369 F2:3
kiranmayee *et al.*,2016	TD*	*Xnhsbm1044-Xtxp141*	8cM	–	57.00-58.24	740kb	RSG04008-6/J2614-11	PlaB QTL	369 F2:3
Usha Thesis 2016	Gls	54185546-54532800	S10_54269620	S10_54269620	54.18-54.55	384kb	RSG04008-6/J2614-11	CIM/QTLCartographer	152 F4
Usha Thesis 2016	TDL	S10_57331385-S10_57552719	S10_57432493	S10_57432493	57.34-57.56	221kb	RSG04008-6/J2614-11	CIM/QTLCartographer	152 F4
Usha Thesis 2016	TDU	S10_57331385-S10_57552719	S10_57432493	S10_57432493	57.34-57.56	221kb	RSG04008-6/J2614-11	CIM/QTLCartographer	152 F4

Table 29: Candidate genes in mapped intervals of seedling leaf blade glossiness and trichome density QTLs on sorghum chromosome SBI-10L

Marker interval:Trait	Sorghum gene ID	Description	Functional role	Reference
Xgap001-Xnhsbm1044 (2Mb; *Gls*)	Sb10g025040	C2 calcium lipid-binding domain	C2 CaLB binds to membrane lipids and mediate signal transduction	De Silva et al. (2011)
	Sb10g025110	Cytochrome P450	Oxidoreductase activity in wax/cutin biosynthesis	Li-Beisson et al. (2009)
Gls fine mapped region (384kb)	Sb10g025053	glossy15/AP2/EF/ EREBP transcryptional factor	Controls juvenile epidermal leaf trait and epicuticular wax synthesis and cutin deposition in maize	Foerster et al. (2015)
	Sb10g024950	MYB transcription factor and DNA binding domain	Over expression of MYB transcriptional factor alters WIN1/SHN1 encodes AP2/EREBP family that encodes glossy	Cominelli. et al. (2008)
Xisep0630-Xtxp141(800kb;*Td*)	Sb10g025600	WRKY40 transcription factor	Transparent Testa Glabra2 (TTG2) encodes WRKY transcription factor and control trichome out growth	Ishida et al. (2007)
	Sb10g026780	Speckle-type POZ protein	Expressed in Arabidopsis trichomes	Jakoby et al. (2008)
	Sb10g027280	MYB transcription factor	WD40-HLH-MYB complex regulates trichome development in Arabidopsis	Liang et al. (2014)
	Sb10g027550	C2H2 Zinc finger protein	C2H2 zinc finger protein regulates trichome cell initiation in arabidopsis	Zhou et al. (2013)
	Sb10g027610	EF-hand Ca2+-binding protein CCD1	Interacts with a microtubule motor protein and regulates trichome morphogenesis	Reddy et al. (2004)
Td fine mapped region (221kb)	Sb10g027670	Cyclin deppendant kinase (CDKB2;1)	Involved in endoreduplication cycle of trichome cell development process	Schnittger et al. (2003)
	Sb10g027680	Armadillo repeat protein	Sequence-specific DNA binding functional transcriptional regulator for plant development activity	Patra et al. (2013)
	Sb10g027730	F-box domain	Acts as transcriptional factors in developmental and degradation process	Coates et al 2007)

FIGURES

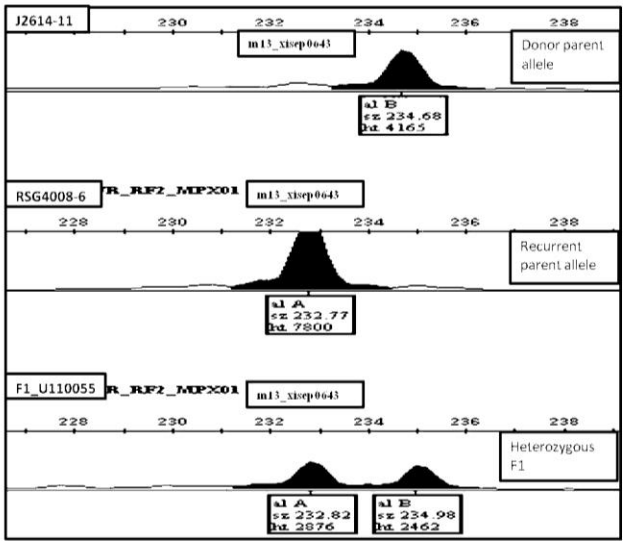

Fig .3F1 hybrid identification (Gene mapper profile)

Recombinant selection based on 7 SSR markers genotyping data

HHBBBBH	AAHAAAA	HHHBBBB	HHHBBHH	AAHABBB	AAAB-H-	HHBBBBB	AABA-AH	HHAA-AA
AAABBBB	HHHAAA-	HHHBBBB	HHHAAHH	HHHBAAA	AAHBAAB	HHHBBHH	AAAA-AH	AAAAAAH
HHHBBHH	BBAAAAA	BH-AAAA	HHHAAAH	HHBABBB	AAA-HBB	HHAAAAA	HHHBBBB	BBBBABH
AAHBBBB	BBBBBBH	AA----H	AHHAAAA	HHHHBBB	BBBAHAB	AAAAAAH	HAAAAAA	AHBABBB
AAAAAAA	HHHAAAA	AABBBBB	BBBBHBB	HHHHBBB	BBBAAAA	HHAAAAA	BBBB-BB	AAAHBHH
AAAAAAH	AAAHHBB	AAABBBB	AAABBBH	BBBBAAA	HHABAAA	HHAAAAA	AAAA-AH	AHHBABB
AAAAAAH	AAAAAAH	HHHBBBB	HHHBBBB	AAAHAAA	AAA-AAB	AAAB-BB	AAAAAAH	HAAAAAH
AAAAAAH	AAABBBB	HHBBBB-	HHA-AAA	BBBBBAH	AAAB-AA	AAAH-BB	AAAAAAH	BBBBABH
HAAAAAA	AAAAABB	HHHHAAA	HHHBBBB	AAAHAAA	HHBABHB	AAAAAAH	BBBBBBB	HAAAAAH
AAABBBB	AAAAAAH	HHHBBHH	AAAHBBB	HHHBBBH	AAHABBB	AABB-BB	BBBAAAA	AAAABHA
HBBBBBB	HHHBBBB	HHBBBBB	AAHHBBB	AAAHAAA	AAAAAAA	HHHA-BB	AAAABHH	AAAAAAB
AHHBBBB	HHBBBBB	AAAAAAH	HHABHHH	AAAB-HB	AAAHHBB	AAAAABB	HHBBABB	AHHBAB-
HHBBBBH	HHHBBBH	AAABBBB	HHBB-BB	HH-A-AB	BBBAAHH	AAAHHBB	AAAHBHH	HHBBABB
AAAAAAA	HHHBBBH	HHBBBBB	AAAB-BB	AAHBAAA	HHHABBB	AAAAAAH	HHH-ABB	AAAAAAH
HBBBBBH	AAAHAAA	HHHBBBB	HHBBBBB	-HHABHB	AAA-AAH	HHHBBBB	H-BHABB	HHHHBBB
HHHAAAA	AAAHAAH	AAAAAAH	BBBBAAA	AAABAAA	AAA-BBB	AAABBBB	AAAHBH-	BBBABAA
AAAAAAH	HHBBHBH	HAAAAAA	HHAAAAA	AAAAAAA	HHABAAA	AAAABAA	AHAAAAA	AAAABAA
HHHBBHH	AAHBBHH	AAAHBBB	AAHBHHB	HHHBBBA	AAAHAAH	HHHB-BH	ABHBABB	AAABABB
HH--AAA	HHBBBBB	HBBBBBB	HHHBBBB	HAABAAA	AAHABBB	HHBBBBB	BAHBABB	AAABABB
HH--AAA	HHHBBBB	AHHBBBB	AAAAAAB	AAAAAAH	AAABAAA	AAAAAAH	AAAABHB	AAAABAA
HH-BBBB	BHBBBBB	HHBHBBH	AAABBBB	HHHBAAH	AHHABBB	AABAAAH	AH-AAAH	HHBAAHH
BBBBBBB	HHBBBHH	AAHAAAH	AAABBBB	AAABAAA	AHBABHH	AAABBBB	AAAHAAH	AABBBBB
AAAA-AH	HHHHAAH	HHHHBBB	HHBBBBB	HHHBBBB	HHAHAAA	AAAAAAB	AAAHAAA	HHHBBBB
HHHBBBH	HHBBBBB	AHHAAAA	HHHBBHH	AAAHAAA	HHHHHHH	HHHBBBB	AHAAAAA	AAAAAAB
AAAAAHA	AAHBBBB	HHHAAAA	AHHHBBB	HHABAAA	BBABAAA	AAHBBBB	HAAABAA	AAAAAAB
HH-AAAA	BBBBBBB	HHHAAAA	HHHA-BH	HHHBBBB	AAABAAA	AAAAAAH	BBBAAAA	AAHBABB
BB--AAA	BBBBBBH	HHBBBBH	AAAHAAH	AAAAAAH	AAAHAAH	AAABBHH	ABAAAAA	-HBHAB-
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AHAAAAA	HHHBBBH	HHHAAAA	HHAHAAH	AAABAAB	BBBABBH	AAAAAAH	BBHABAA	-HHBAB-
AAAAAAH	AAAHAAA	AAAAAAH	AAAHAAH	HBBABBA	AHBABHB	AAAA-AH	AAAAHBB	-AAABA-
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HH-AAAA	HHHBBBB	HBBBHBB	HHHAAAA	HBBB-AB	BBB--AA	HHBB-BB	HHAAAAH	HHBBHBB
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HHHHBBB	AAABBBB	HHHAAAA	HHAHAAA	AAABBBH	HHAA-AA	HHAAAAA	AAAABAA	
		HHHHAAH	HHHABBH	HBBABBH	AAAAAAB	HAAABHH	AHHBABB	

a) RSG4008-6 b) J2614-11

Fig .5 a) RSG04008-6 parent showing non–glossy leaves b)J2614-11 parent showing glossy leaves c) F_2 population sown in pots

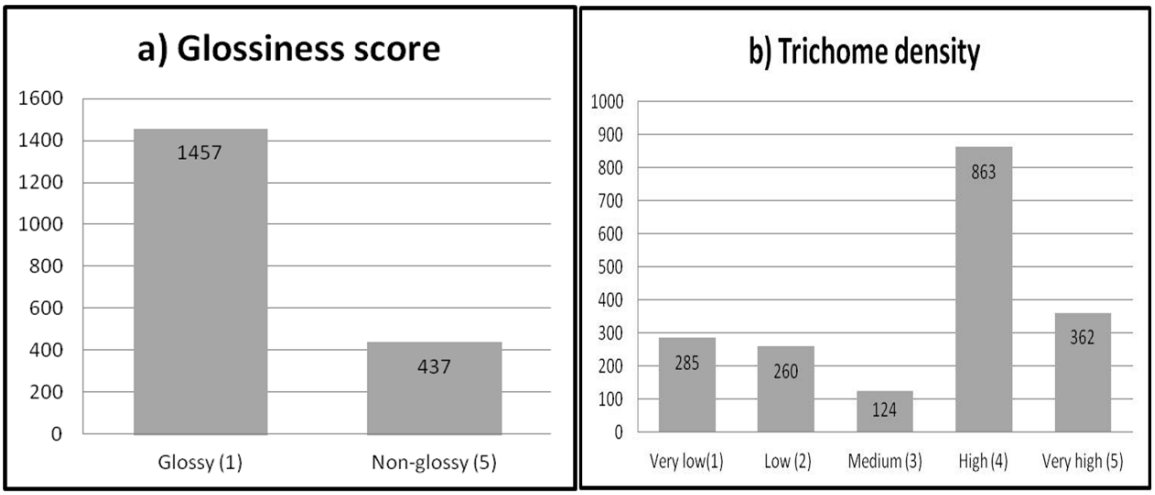


Fig .6 Trait segregation among 1,894 F2 individuals for glossy score and trichome density score

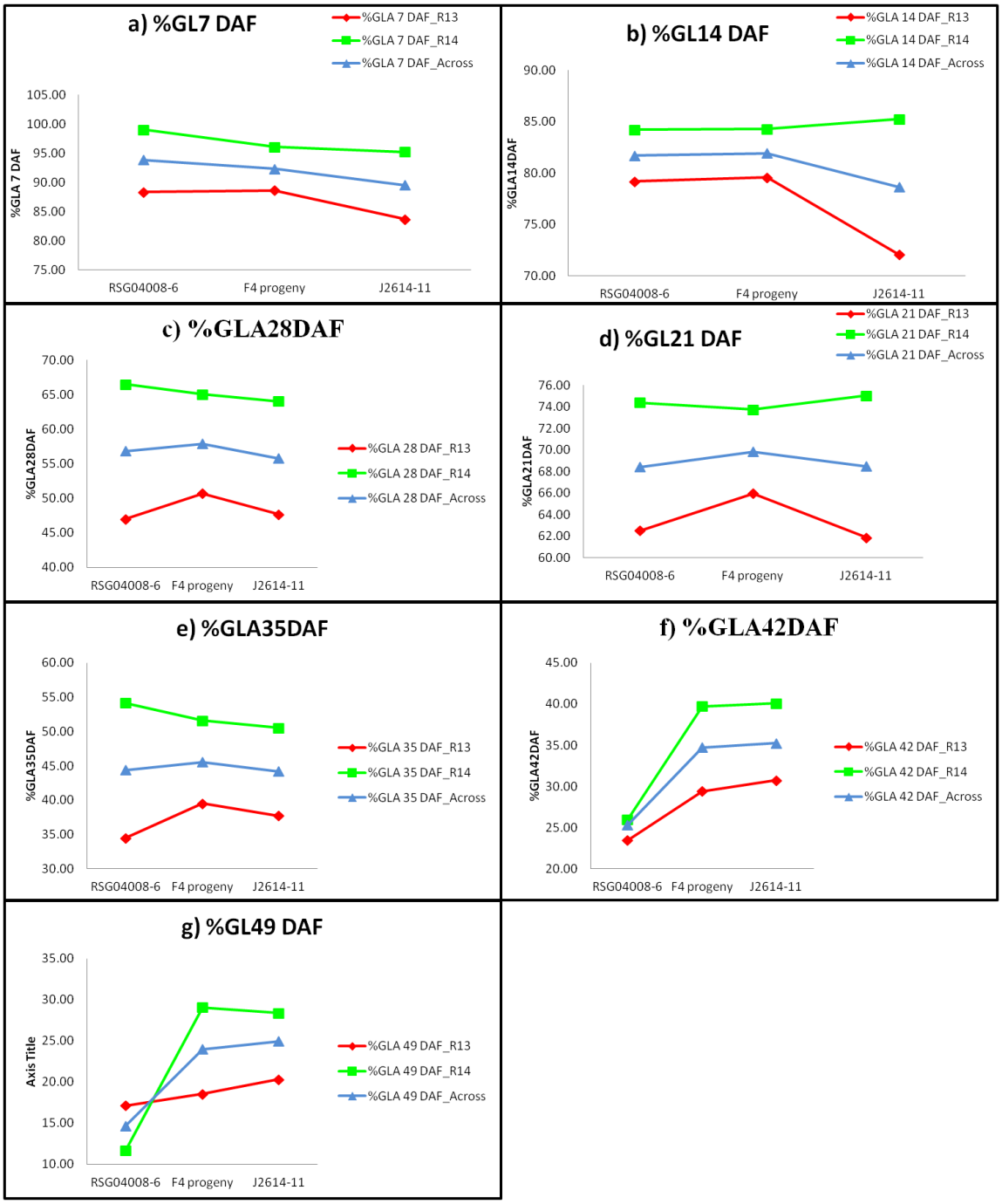


Fig .7 Stay-green mean performance during 2013 and 2014 Post-rainy (rabi) a) %GL7 DAF b) %GL14 DAF c) %GL21 DAF d) %GL28 DAF e) %GL35 DAF f) %GL42 DAF g) %GL9 DAF

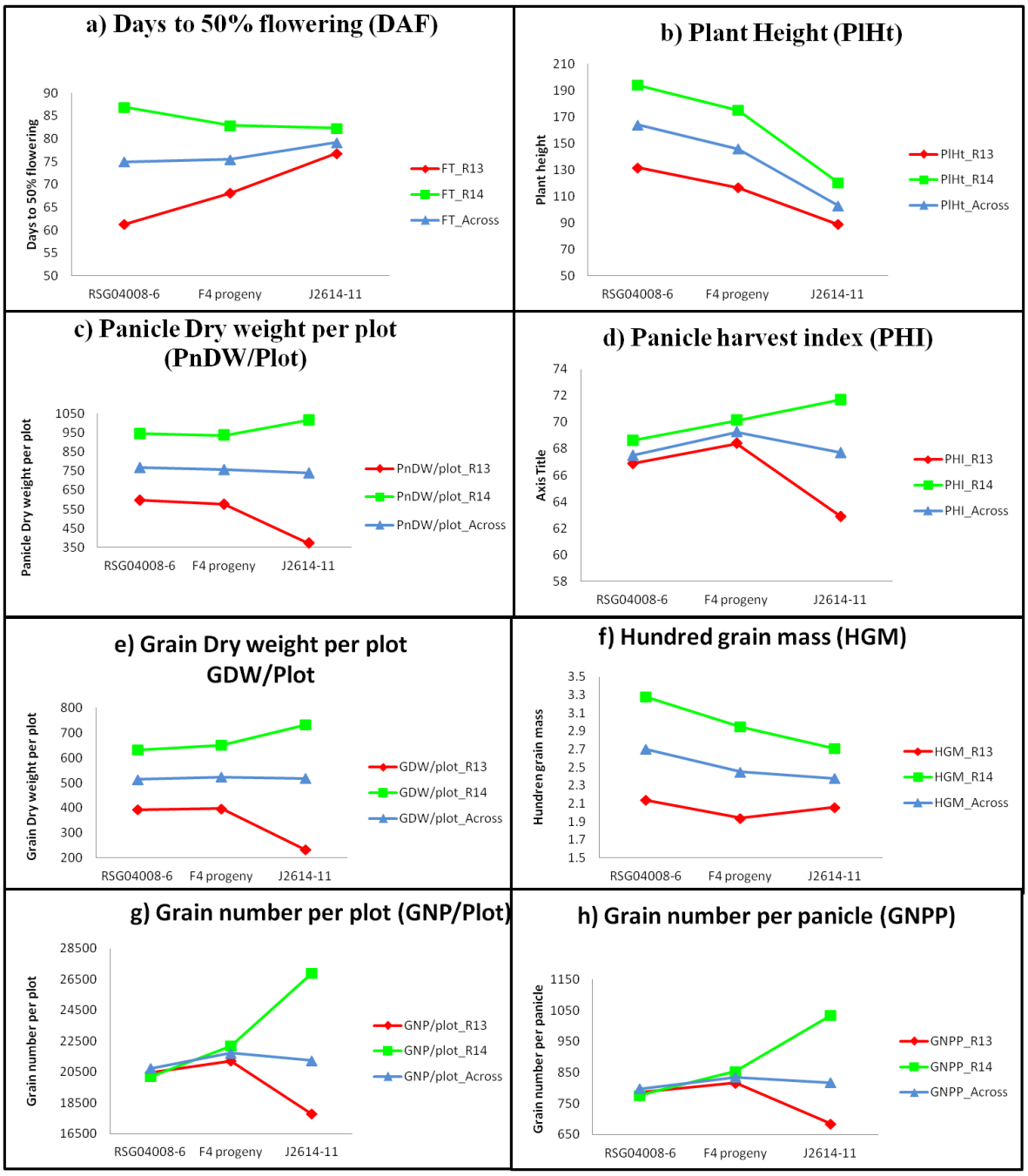


Fig .8 Agronomic and yield related traits mean performance during 2013 and 2014 post-rainy (rabi) season a) Days to 50% flowering (DAF) b) Plant height (PlHt) e) Grain dry weight per plot (GDW/Plot) f) Hundred grain mass (HGM) g) Grain no. per plot (GNP/plot) h) Grain no. per panicle (GNPP)

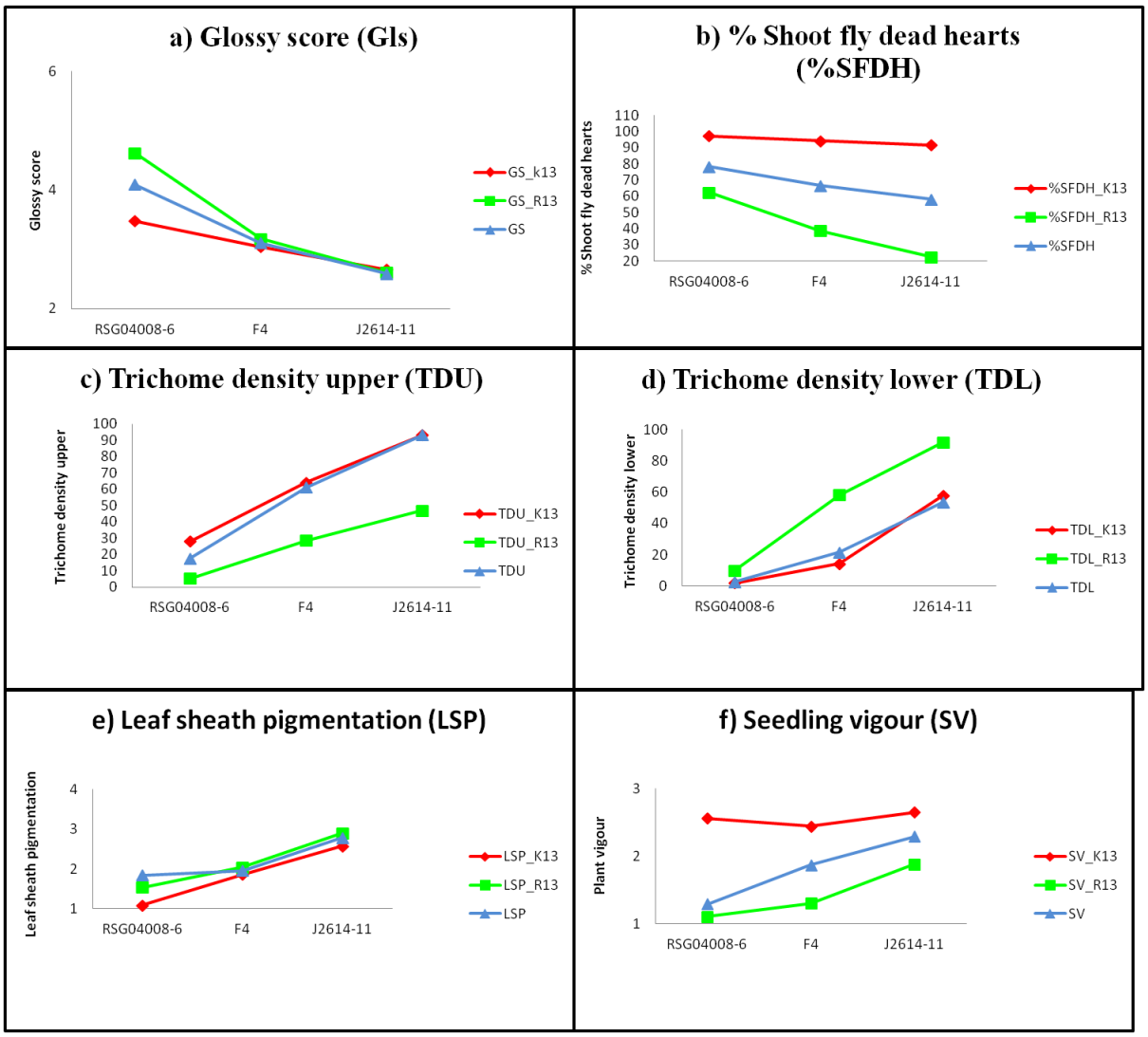


Fig .9 Shoot fly morphological traits mean performance in parents and progeny during rainy (kharif) and Post-rainy (rabi) 2013 season a) Glossy score b) %Shoot fly dead heart (%SFDH) c) Trichome density upper (TDU) d) Trichome density lower (TDL) e) Leaf sheath Pigmentation (LSP) f) Seedling vigour (SV)

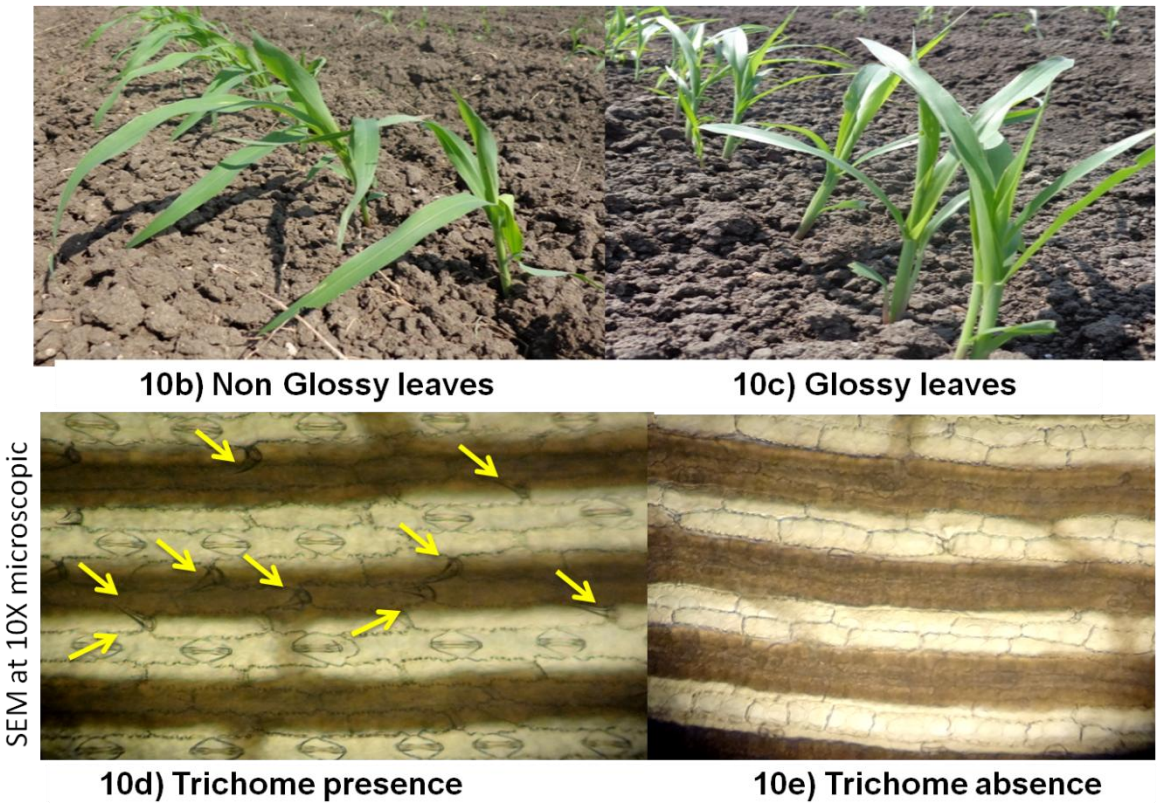


Fig .10 F_4 population showing non glossy, glossy leaves and Trichomes presence and absence profiles

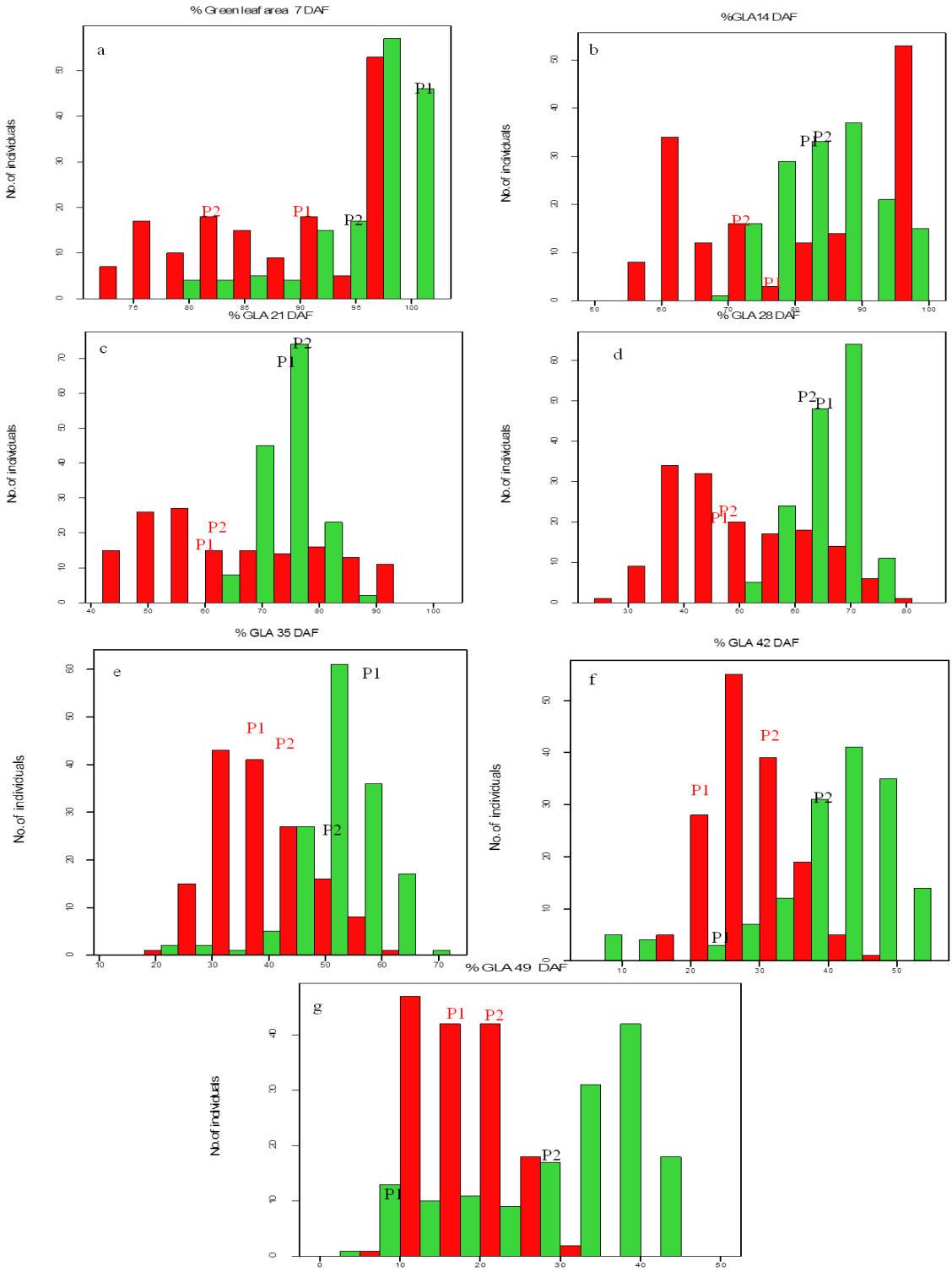


Fig .11 Frequency distribution graphs of F_4 progeny for Stay-green traits during Post-rainy 2013 and 2014 seasons a) %GL7DAF b) %GL14DAF c) %GL21DAF d) GL28DAF e) %GL35 DAF f) %GL42 DAF g) %GL49DAF

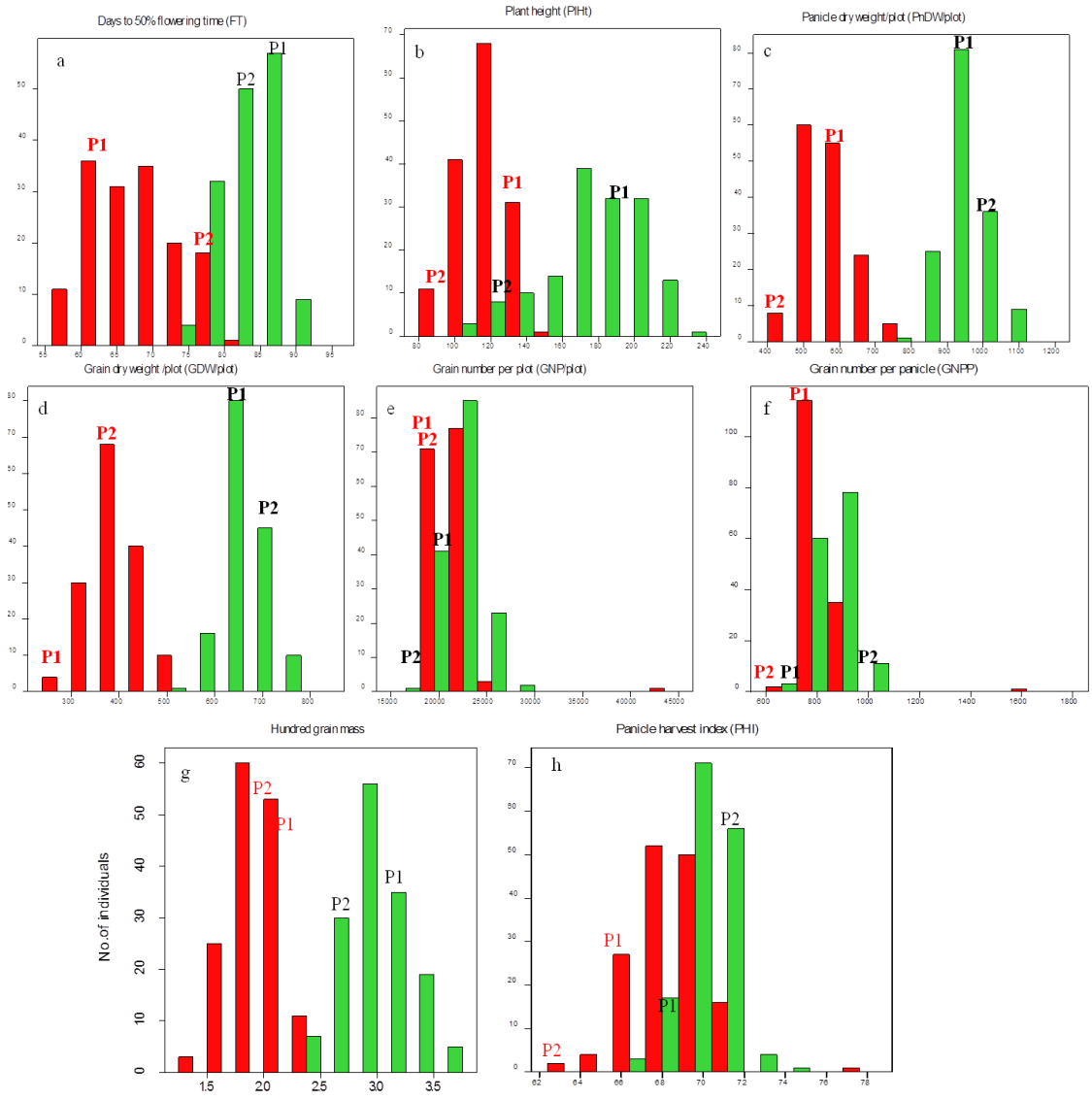


Fig .12 Frequency distribution graphs of F_4 progeny for agronomic and yield traits during Post-rainy 2013 and 2014 seasons a) FT b) PlHt c) PnDW/Plot d) GDW/Plot e) GNP/Plot f) GNPP g) Hundred grain mass (HGM) h)Panicle harvest index (PHI) Frequency distribution of 152, F4 recombinant progeny derived from cross RSG04008-6 x J2614-11 for agronomic traits in two screening environments *rabi* 2012-2013 (summer 2013) and *rabi* 2013-2014 (summer 2014) at ICRISAT , Patancheru. X-axis groups of concerned traits, Y-axis no. of individual frequencies of each group

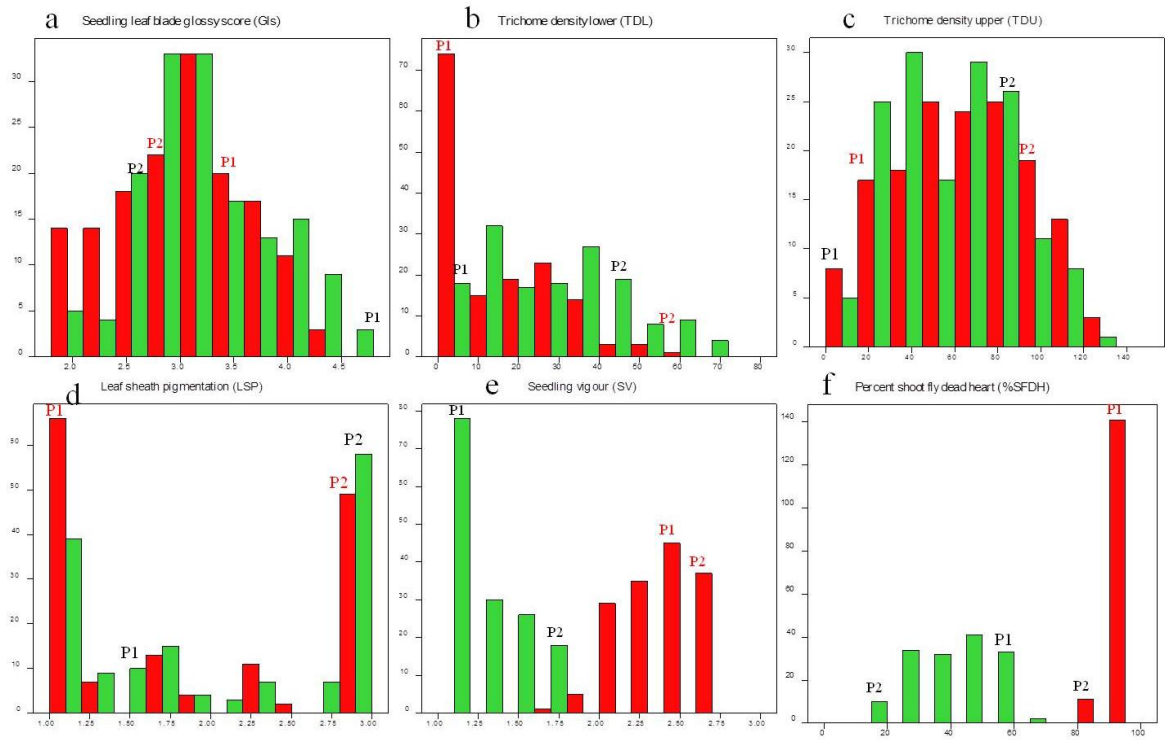


Fig .13 Frequency distribution graphs of F_4 progeny for shoot fly morphological traits during post-rainy 2013 and 2014 seasons a) Glossy score b) Trichome density lower (TDL) c) Trichome density upper (TDL) d) Leaf sheath pigmentation (LSP) e) Seedling vigour (SV) f) Percent shoot fly dead heart (%SFDH)

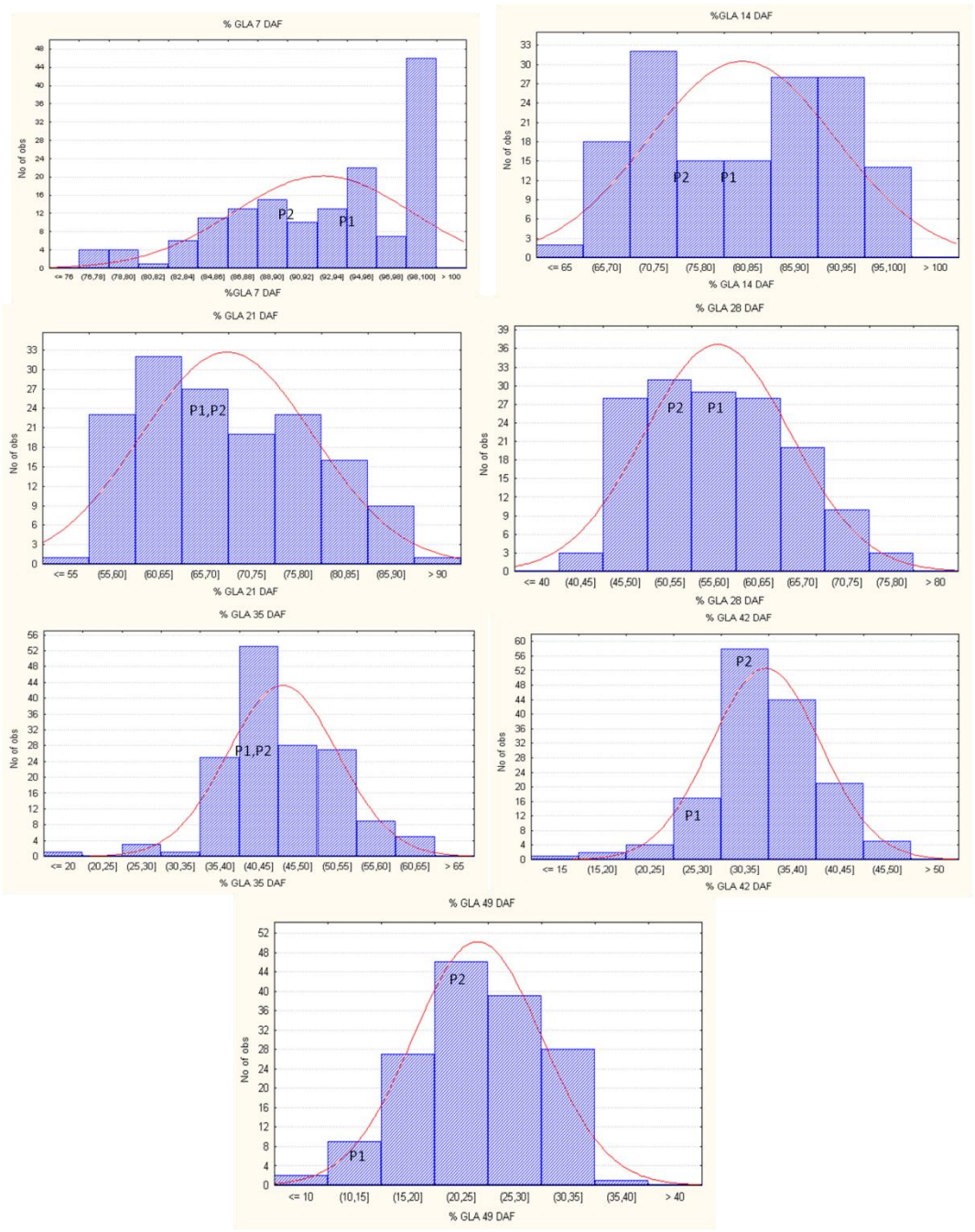


Fig .14 Frequency distribution graphs of F_4 progeny for Stay-green traits for across season a) %GL7DAF b) %GL14DAF c) %GL21DAF d) GL28DAF e) %GL35 DAF f) %GL42 DAF g) %GL49DAF

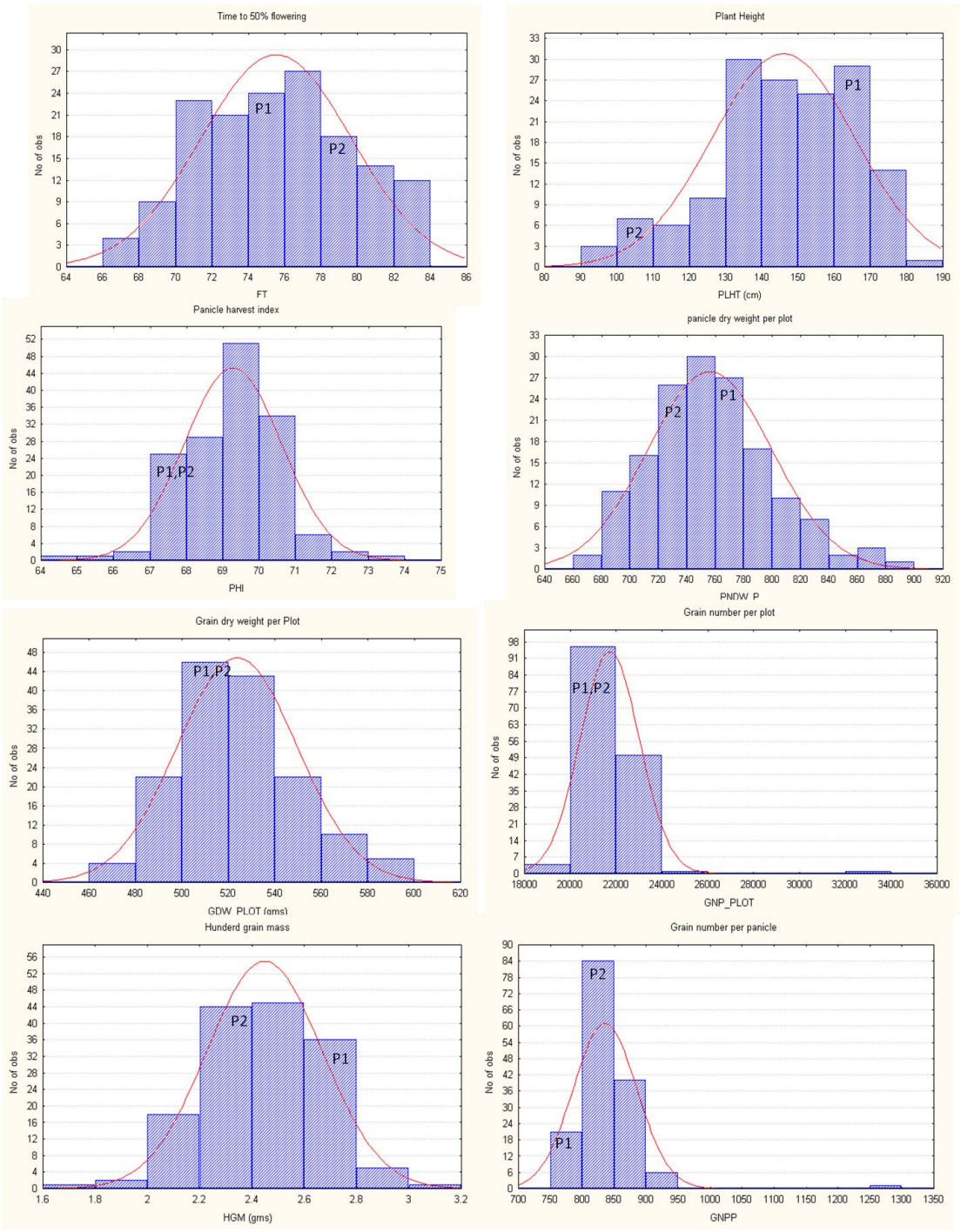


Fig .15 Frequency distribution graphs of F_4 progeny for agronomic and yield traits for across season a) FT b) PlHt c) PHI d) PnDW/Plot e) GDW/Plot f) GNP/Plot g) HGM h) GNPP

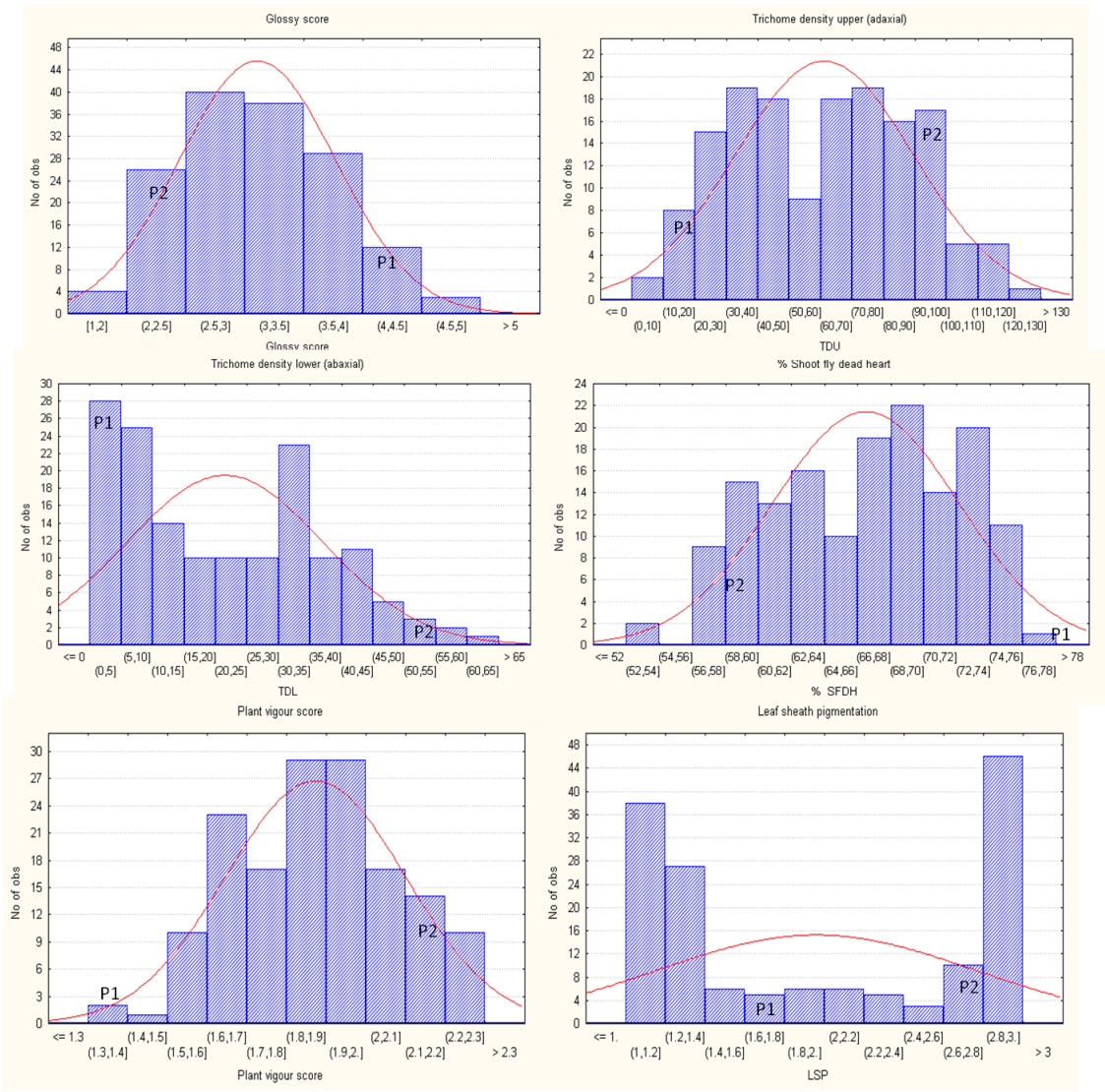


Fig .16 Frequency distribution graphs of F_4 progeny for shoot fly morphological traits for across season a) Glossy score b) Trichome density upper (TDU) c) Trichome density lower (TDL) d) % Shoot fly dead heart (%SFGDH) e) Plant vigour f) Leaf sheath pigmentation (LSP)

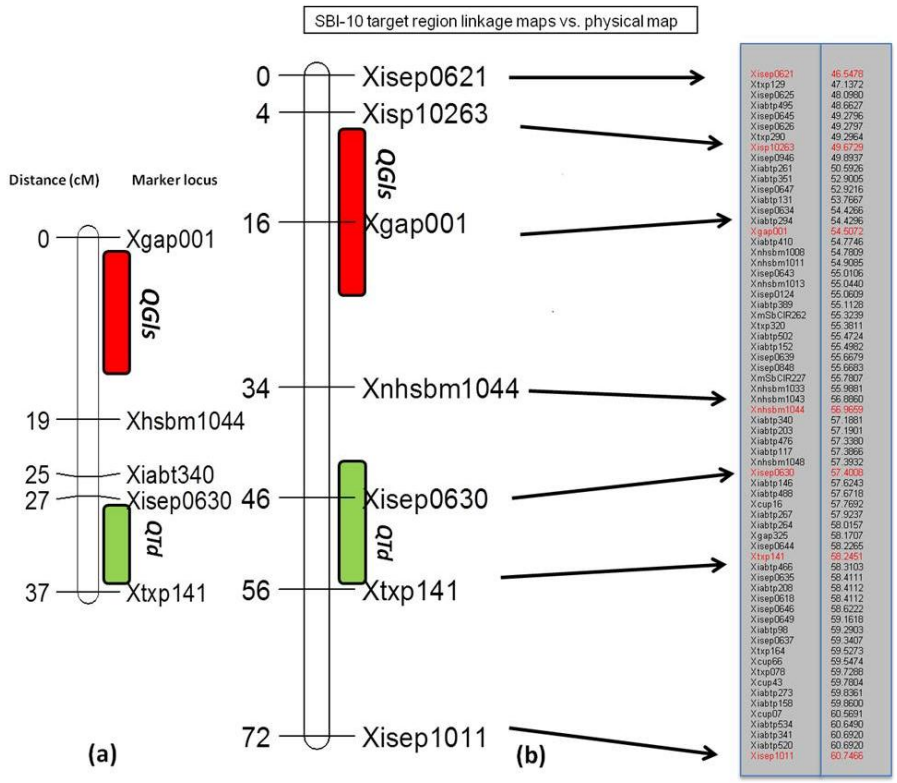


Fig .17 Genetic linkage map constructed based on SSR markers on SBI-10L

a) 5 SSR markers on 1894 F_2 population b) with 7 SSRs on 369 selective informative recombinant individuals and linkage map *vs* physical map

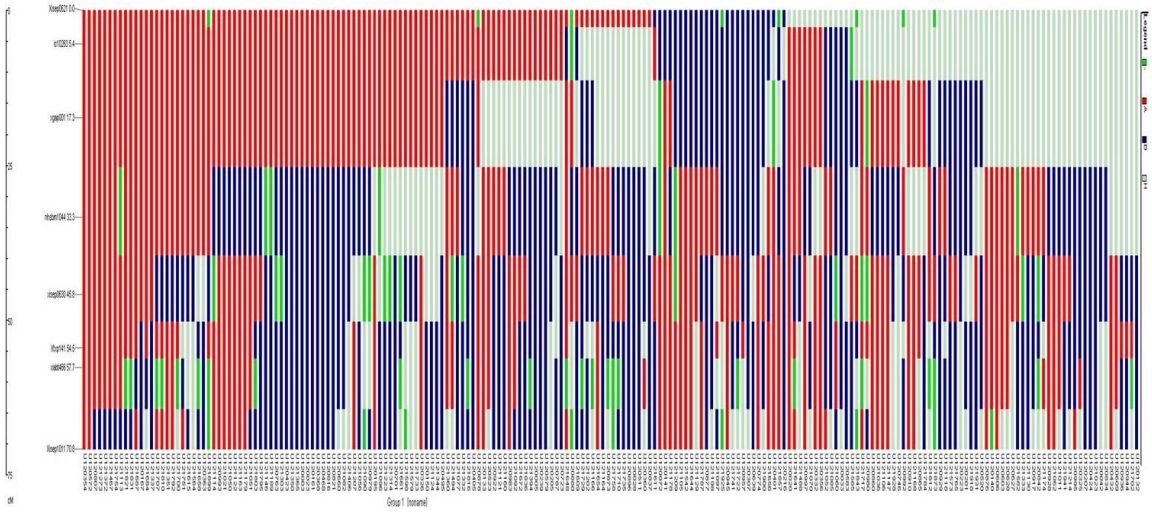


Fig .18 Graphical Genotype representation (GGT) of 182 selected recombinants

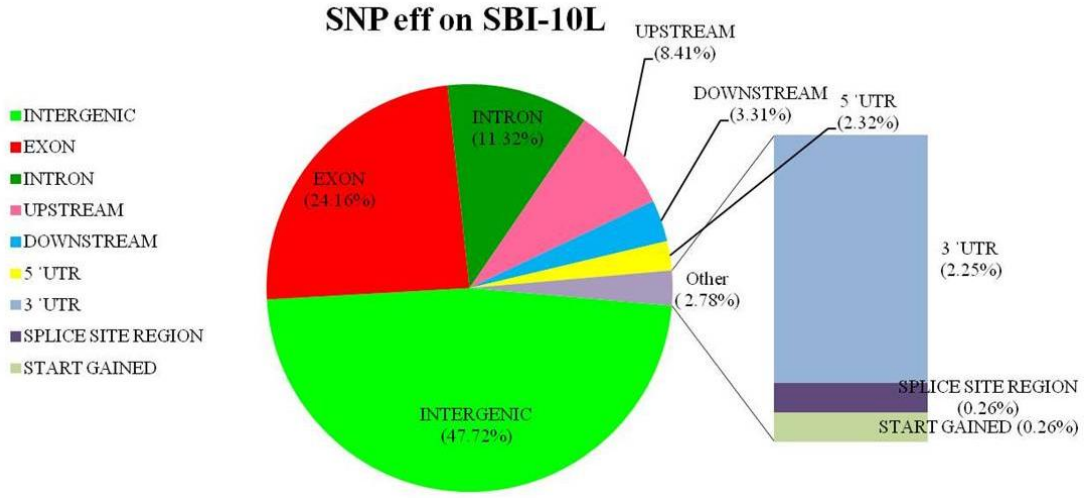


Fig .19 SNP effect of the identified SNPs in SBI-10L

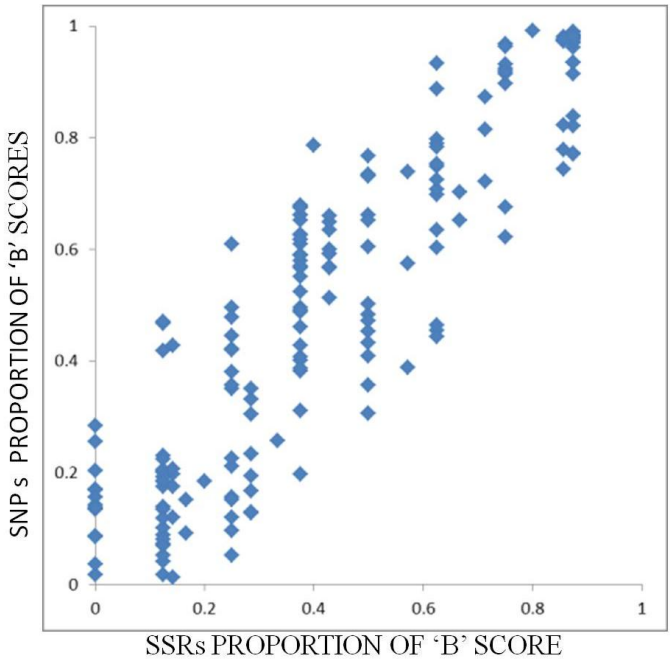


Fig .20 Proportion of 'B' alleles of SNPs plotted against proportion 'B' alleles of SSRs

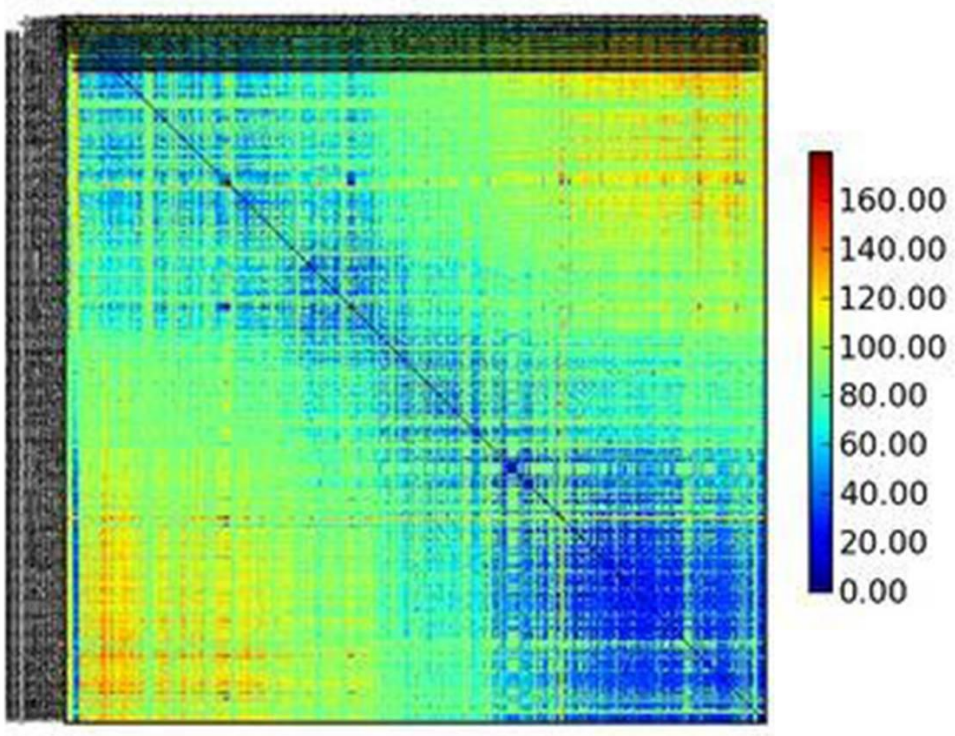


Fig .21 Distance matrix 1 calculated from THREaD mapper for 392 SNPs and 7SSR markers

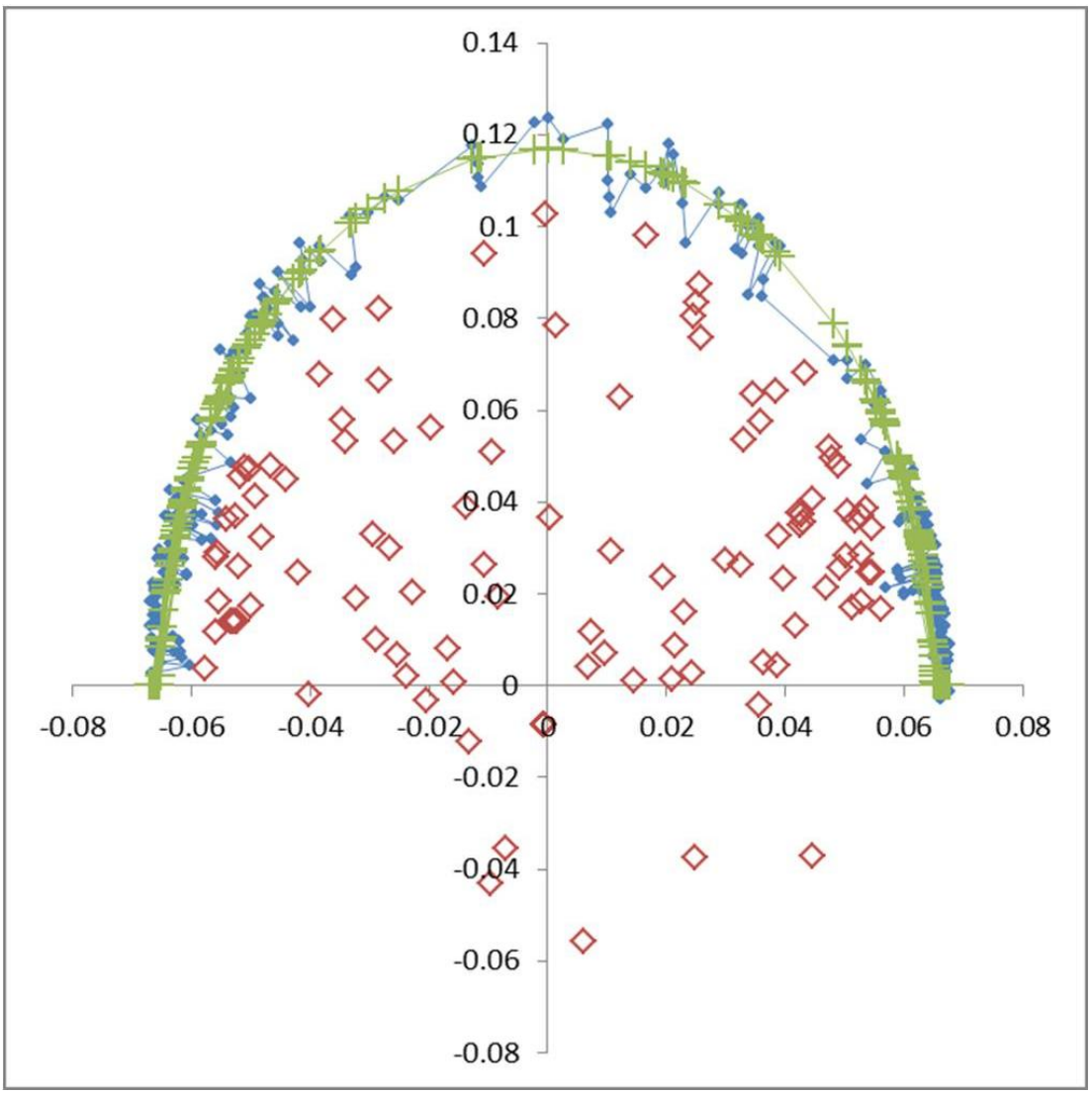


Fig .22 Horseshoe effect of markers in PCA plot

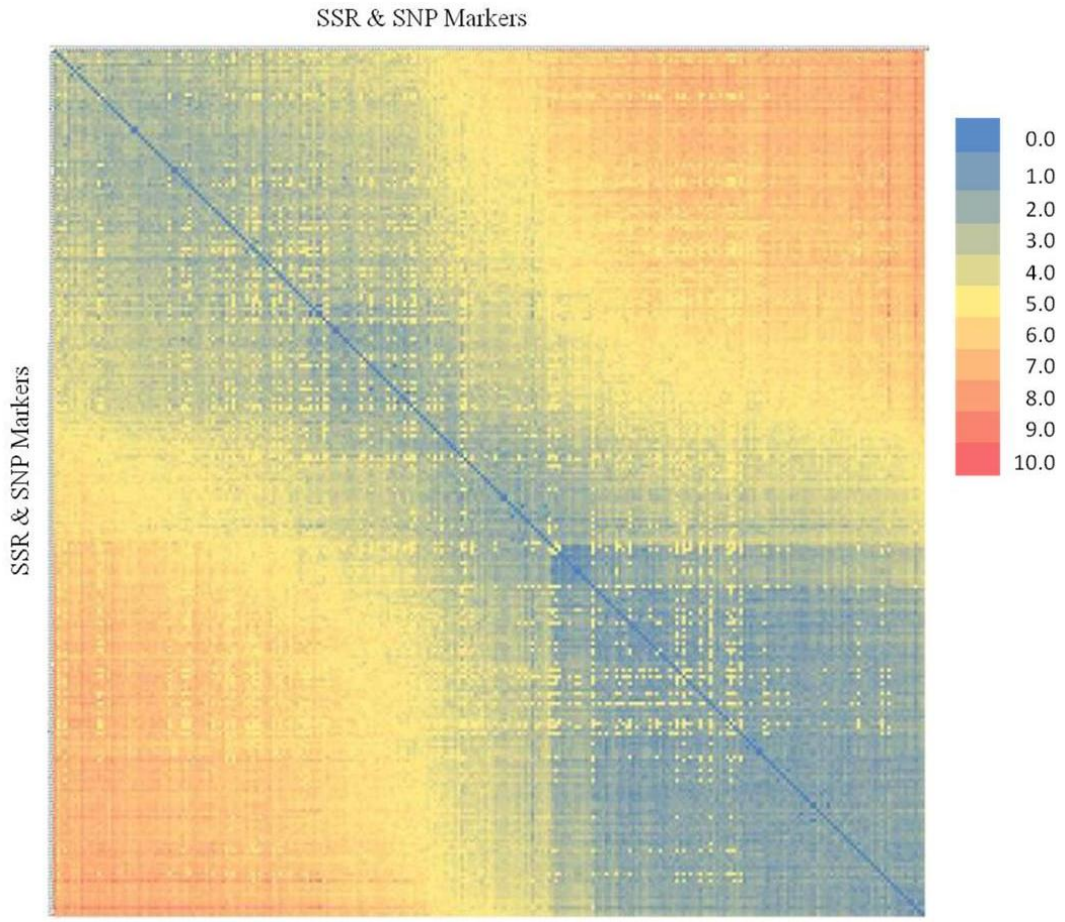


Fig .23 Distance matrix plot for 265 markers in Horseshoe line

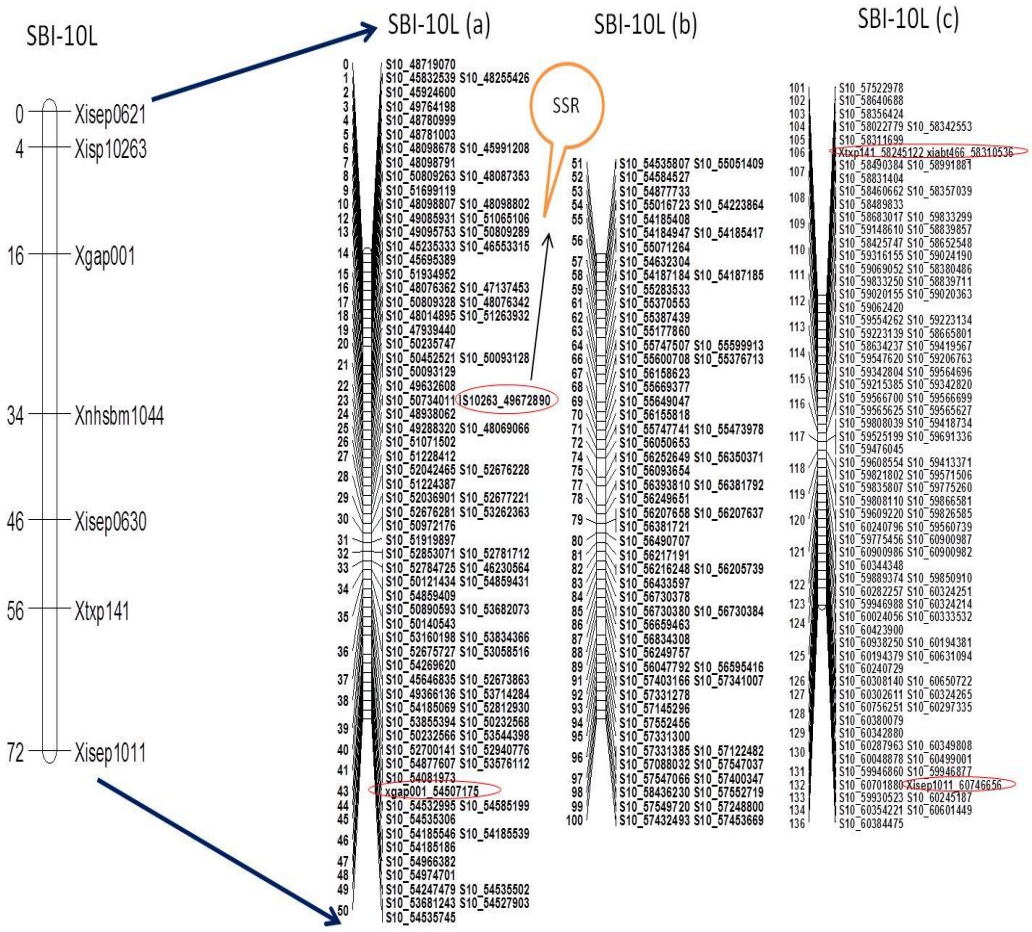


Fig .24 High density linkage map of SNPs integrated in SSR map

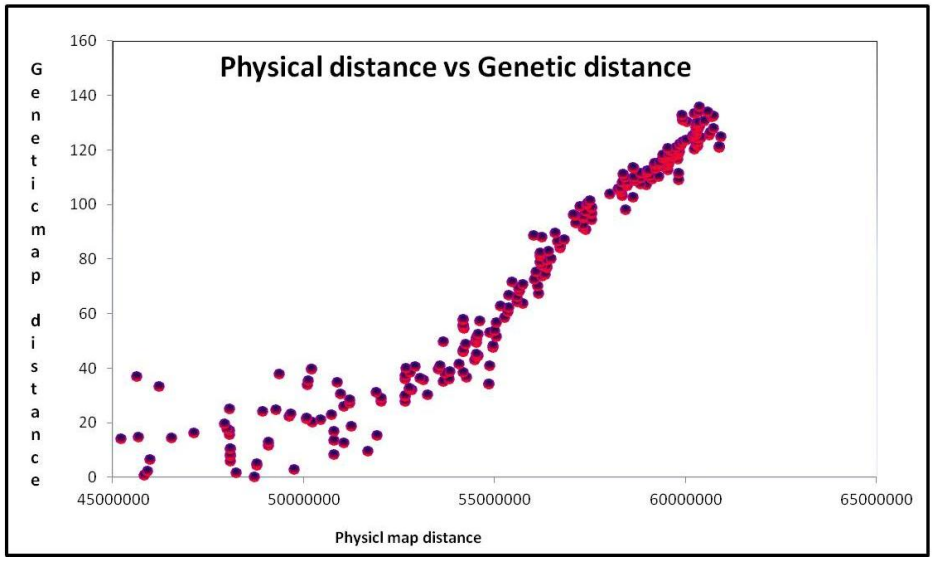


Fig .25 Genetic map distances plotted against physical map distances on SBI-10L

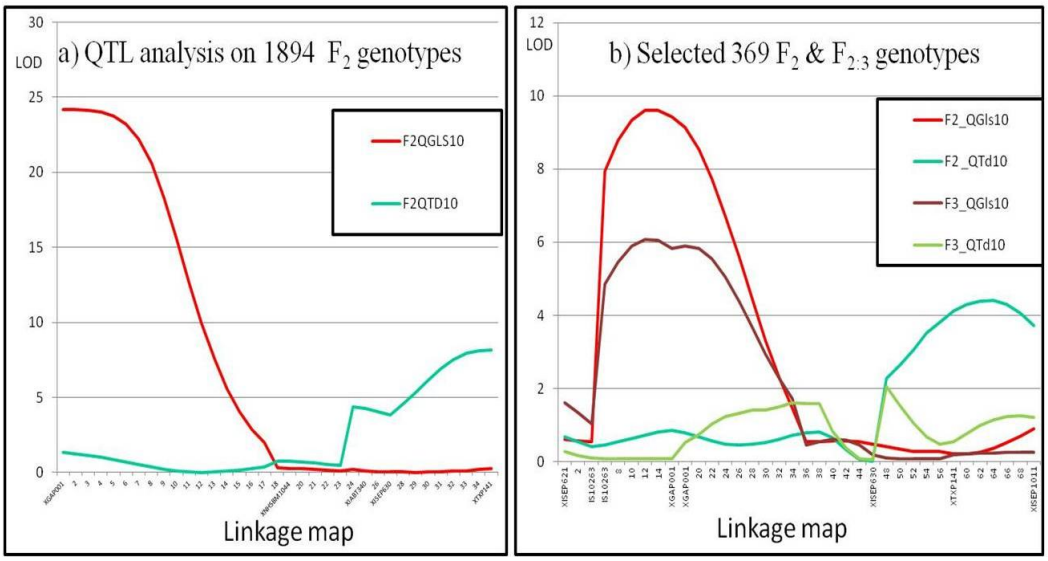


Fig .26 a) Map for glossiness score and trichome density score QTLs on SBI-10L among 1,894 F_2 individuals evaluated in *rabi* season of 2010-2011, b) QTL confirmation among 369 selected informative recombinant F_2 individuals evaluated in *rabi* season of 2010-2011 and their derived $F_{2:3}$ progenies evaluated in a late *kharif* season 2012 sowing.

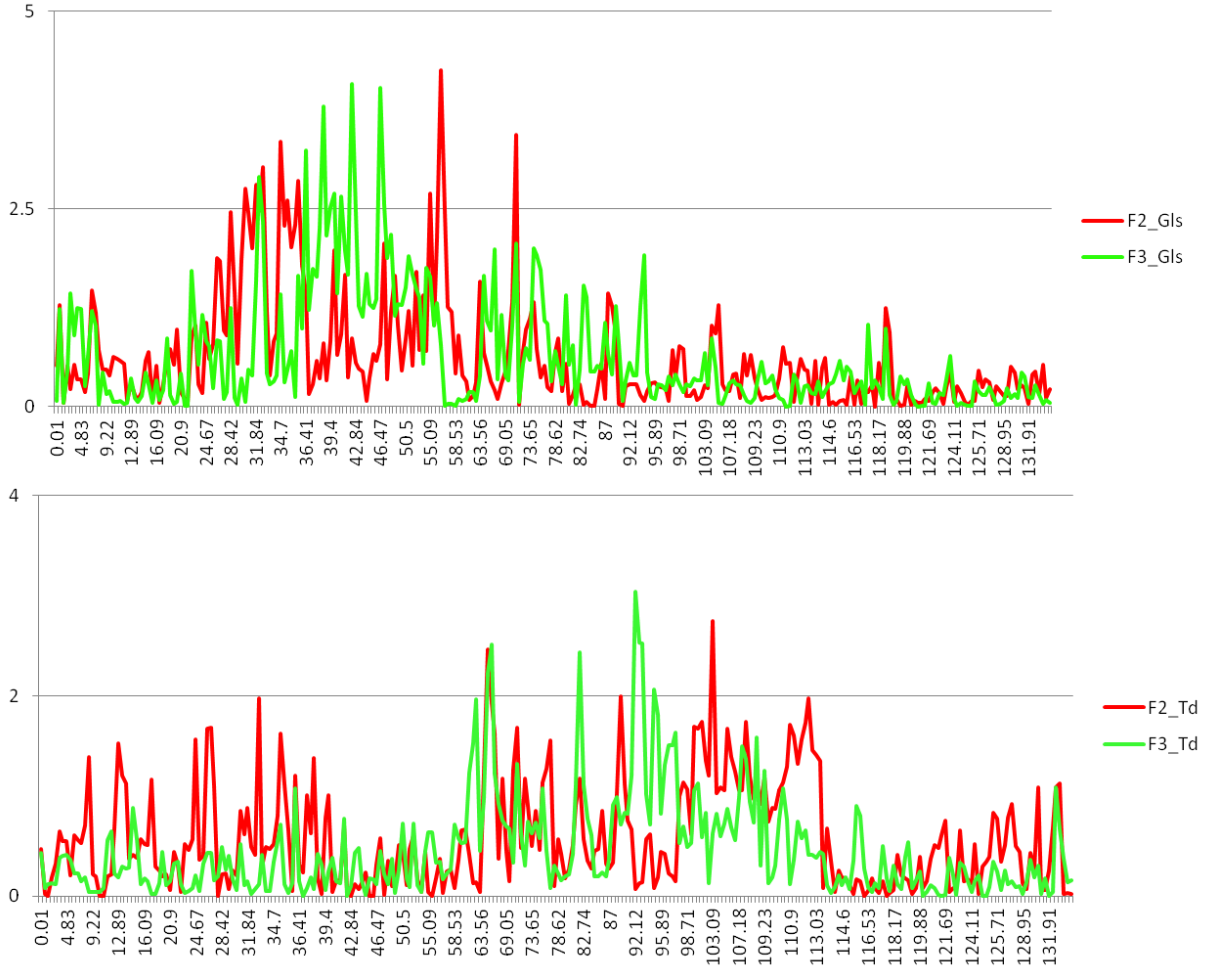


Fig .27: F_2, $F_{2:3}$ QTL mapping for a) seedling leaf blade glossiness b) trichome density on high density map

Fig .28 F_4 QTL mapping of stay-green, shoot fly morphological , agronomic and yield related traits for individual seasons

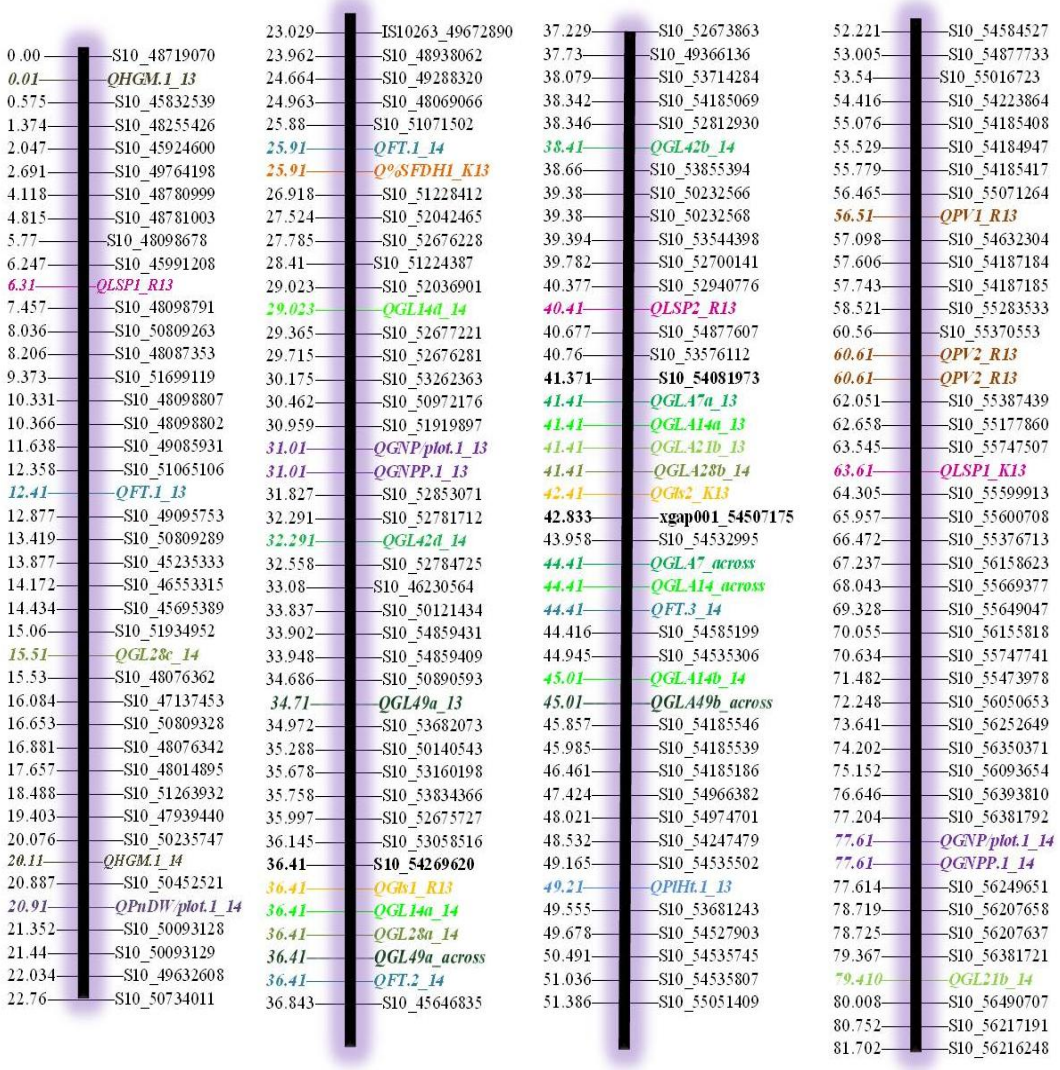


Fig .28 contd.. F4 QTL mapping of stay-green, shoot fly morphological , agronomic and yield related traits for individual seasons

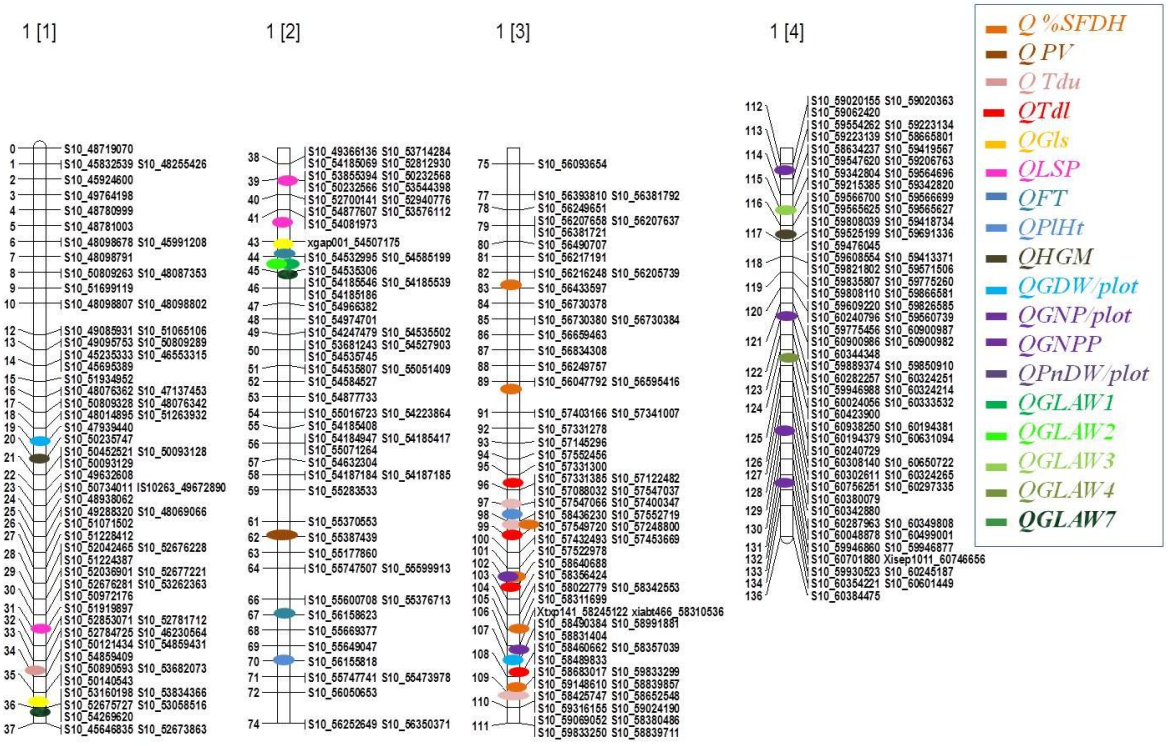


Fig .29 F4 QTL mapping of stay-green, shoot fly morphological, agronomic and yield related traits for across season

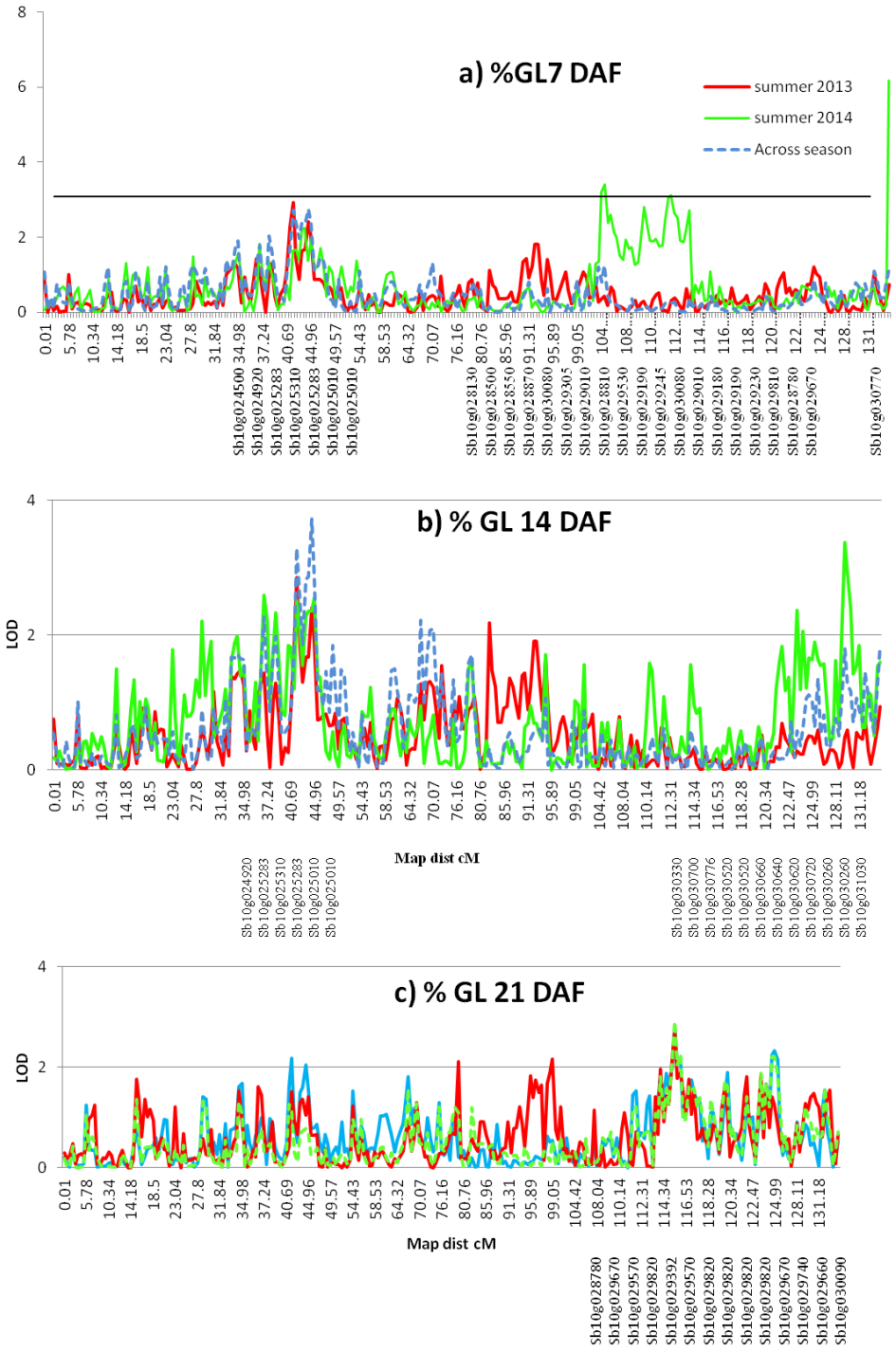


Fig .30 QTL LOD graphs for stay-green traits with candidate genes underlying

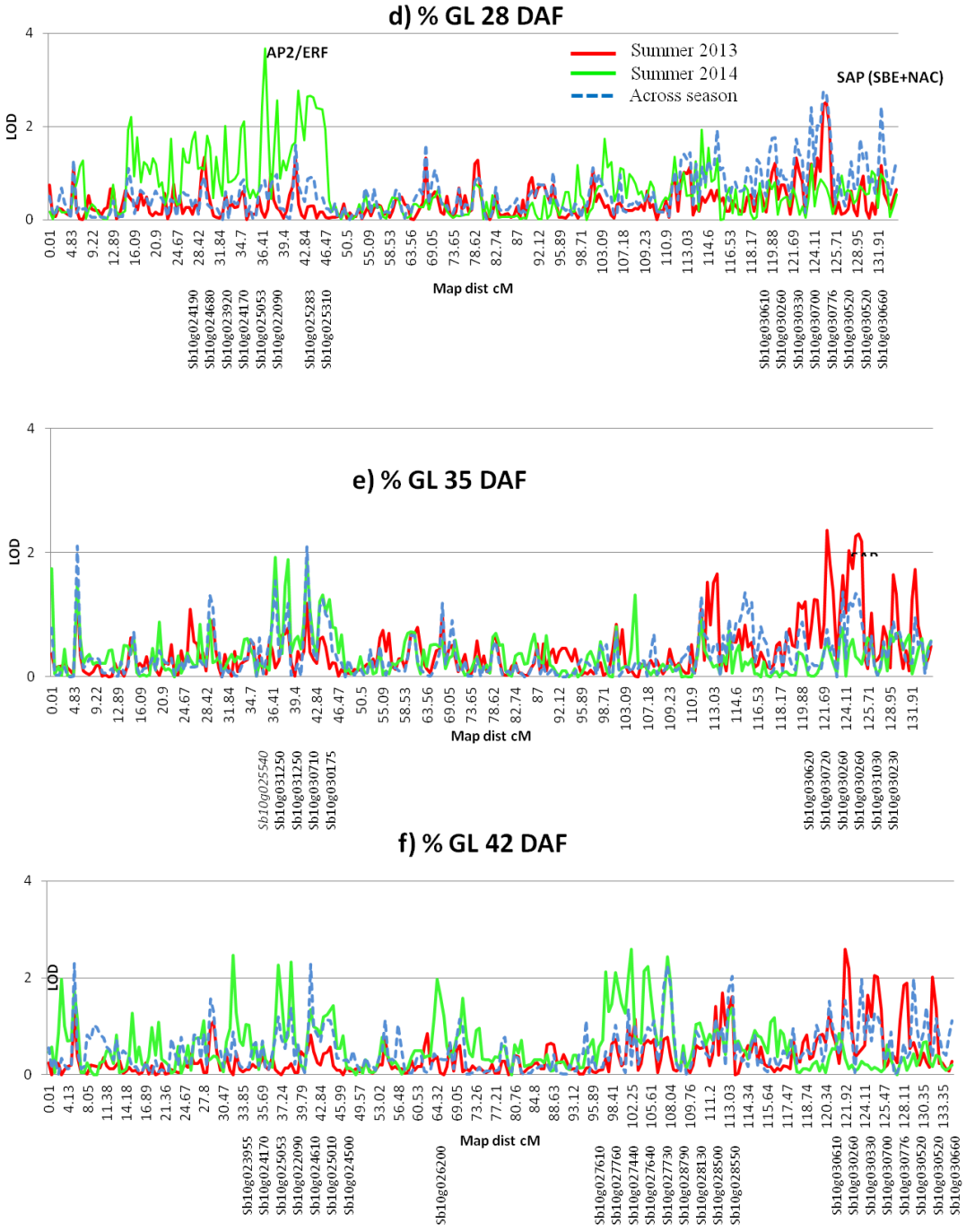


Fig .30 Contd.. QTL LOD graphs for stay-green traits with candidate genes

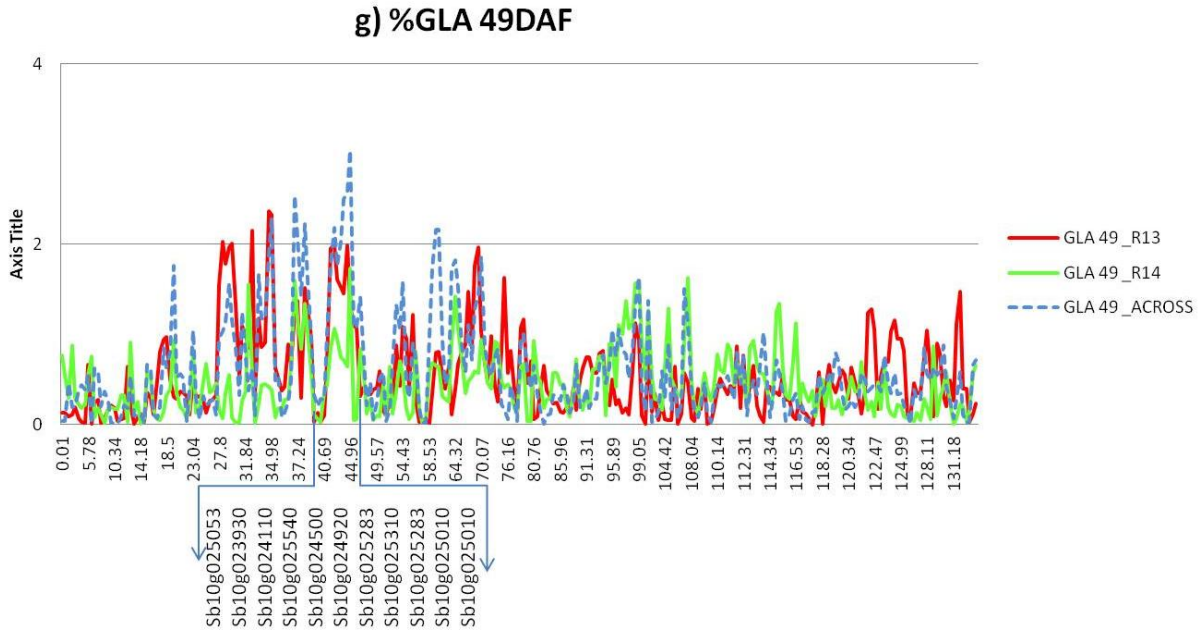


Fig .30 Contd.. QTL LOD graphs for stay-green traits with candidate genes

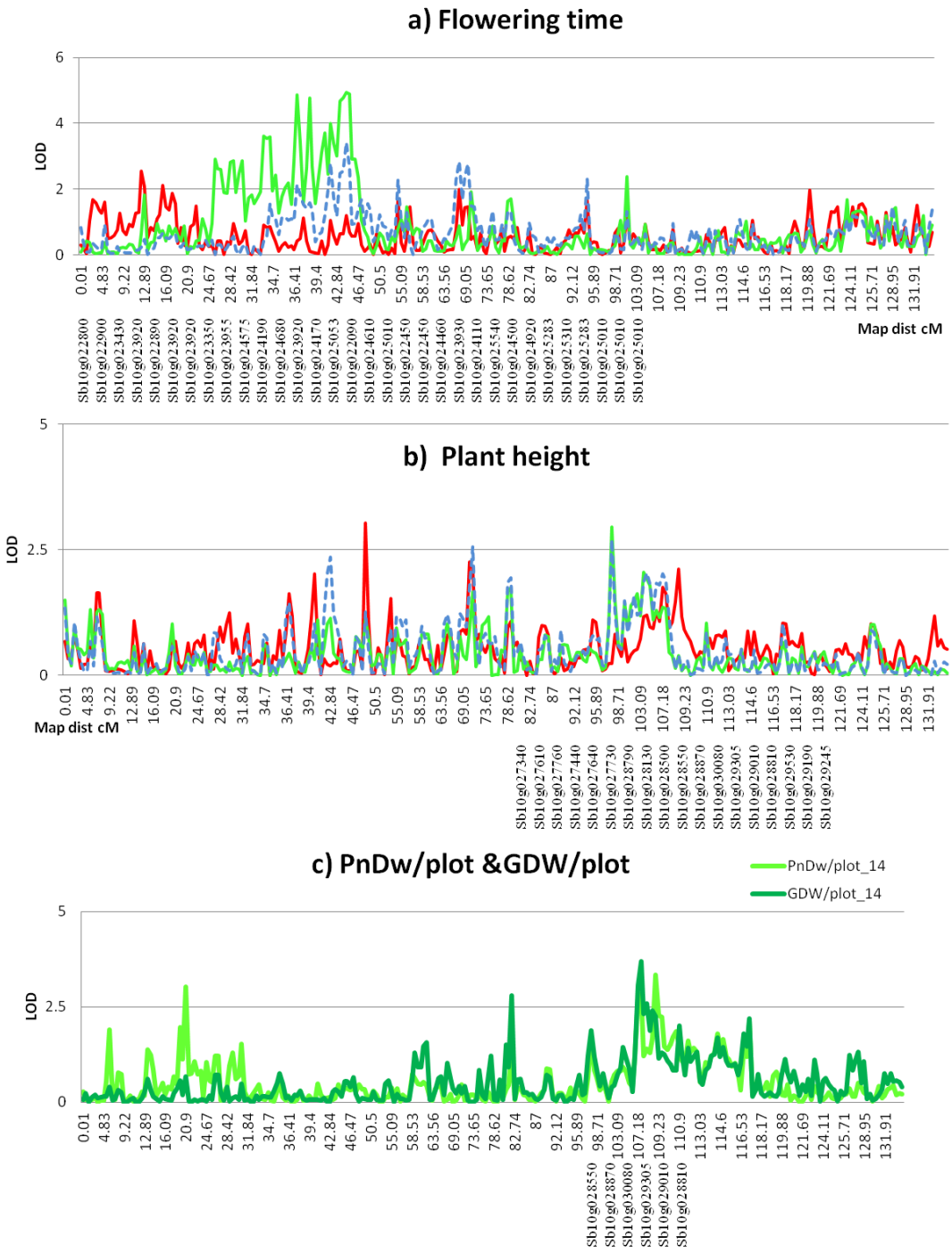


Fig .31 QTL LOD graphs for agronomic traits with candidate genes underlying

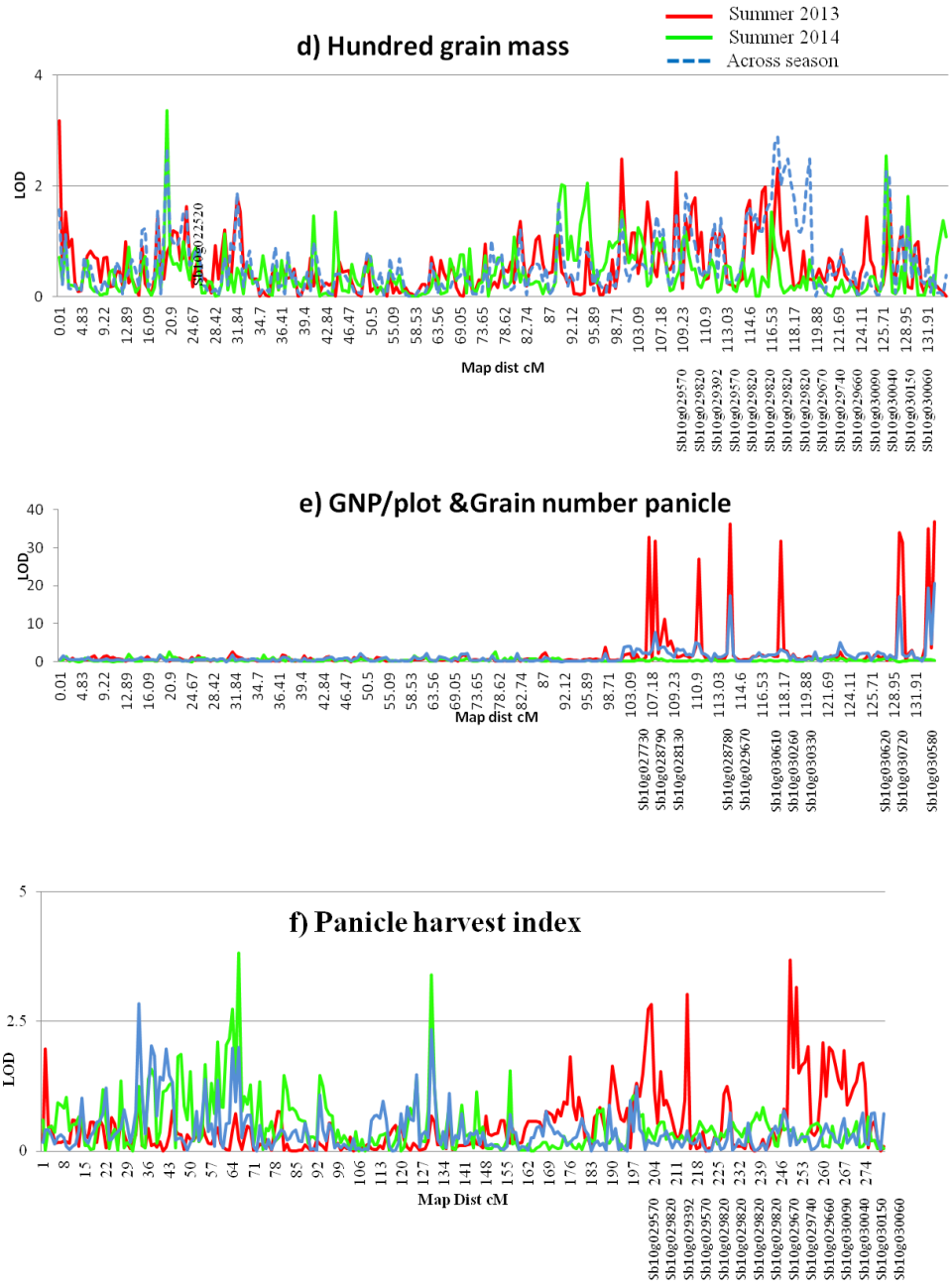


Fig .31 Contd.. QTL LOD graphs for Yield related traits with candidate genes

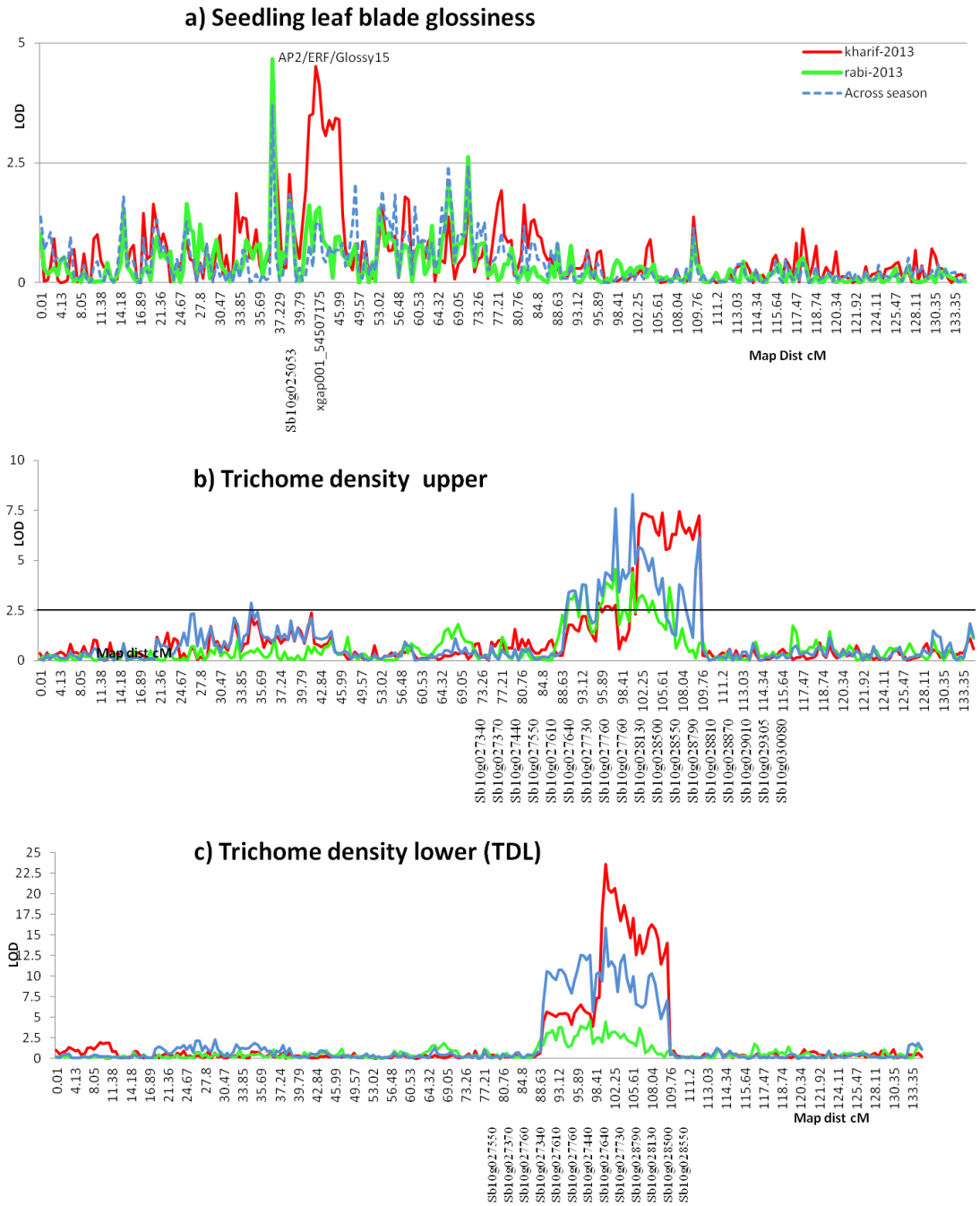


Fig .32 QTL LOD graphs for shoot fly morphological traits with candidate genes underlying

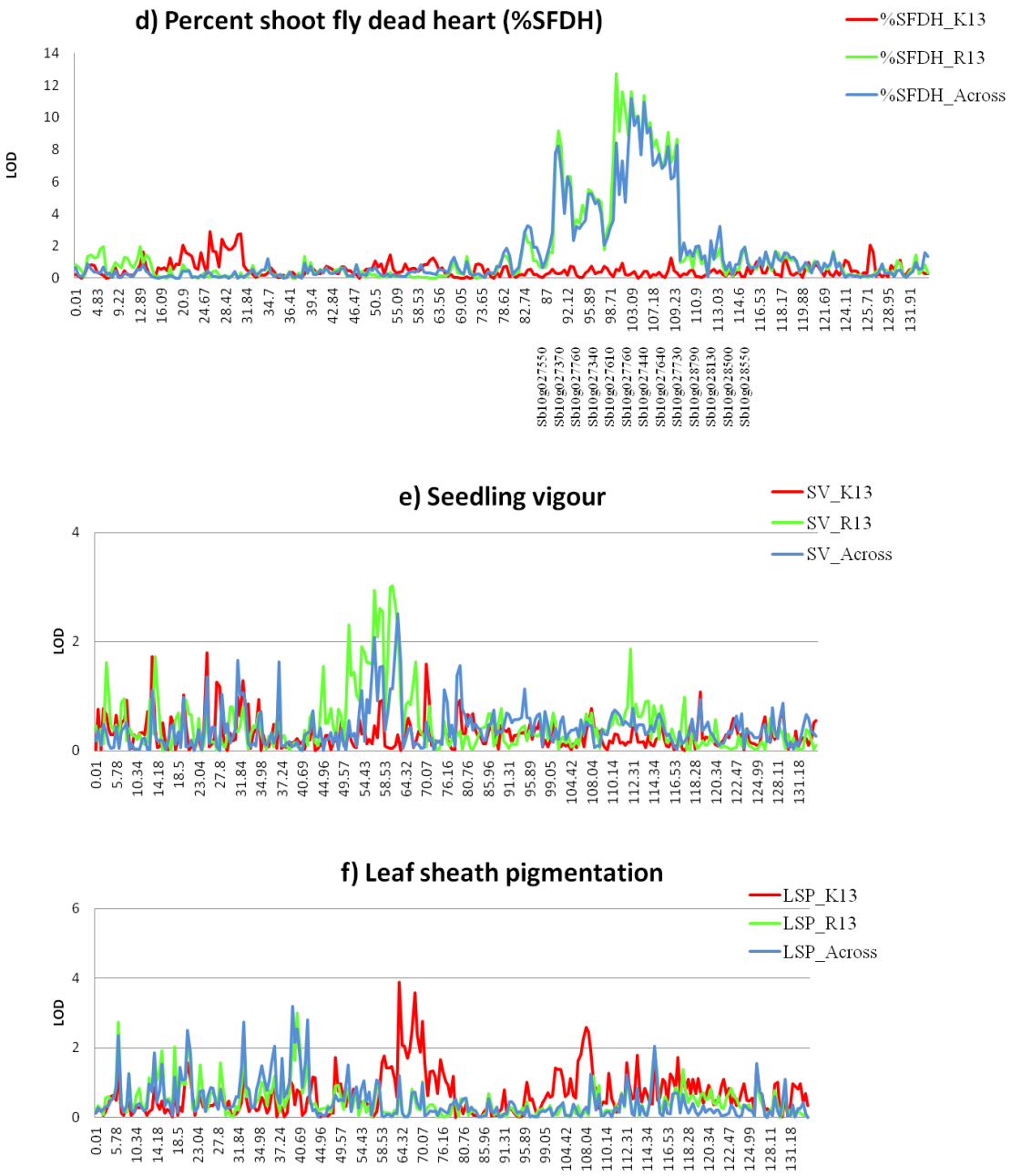


Fig .32 contd.. QTL LOD graphs for shoot fly morphological traits with candidate genes underlying

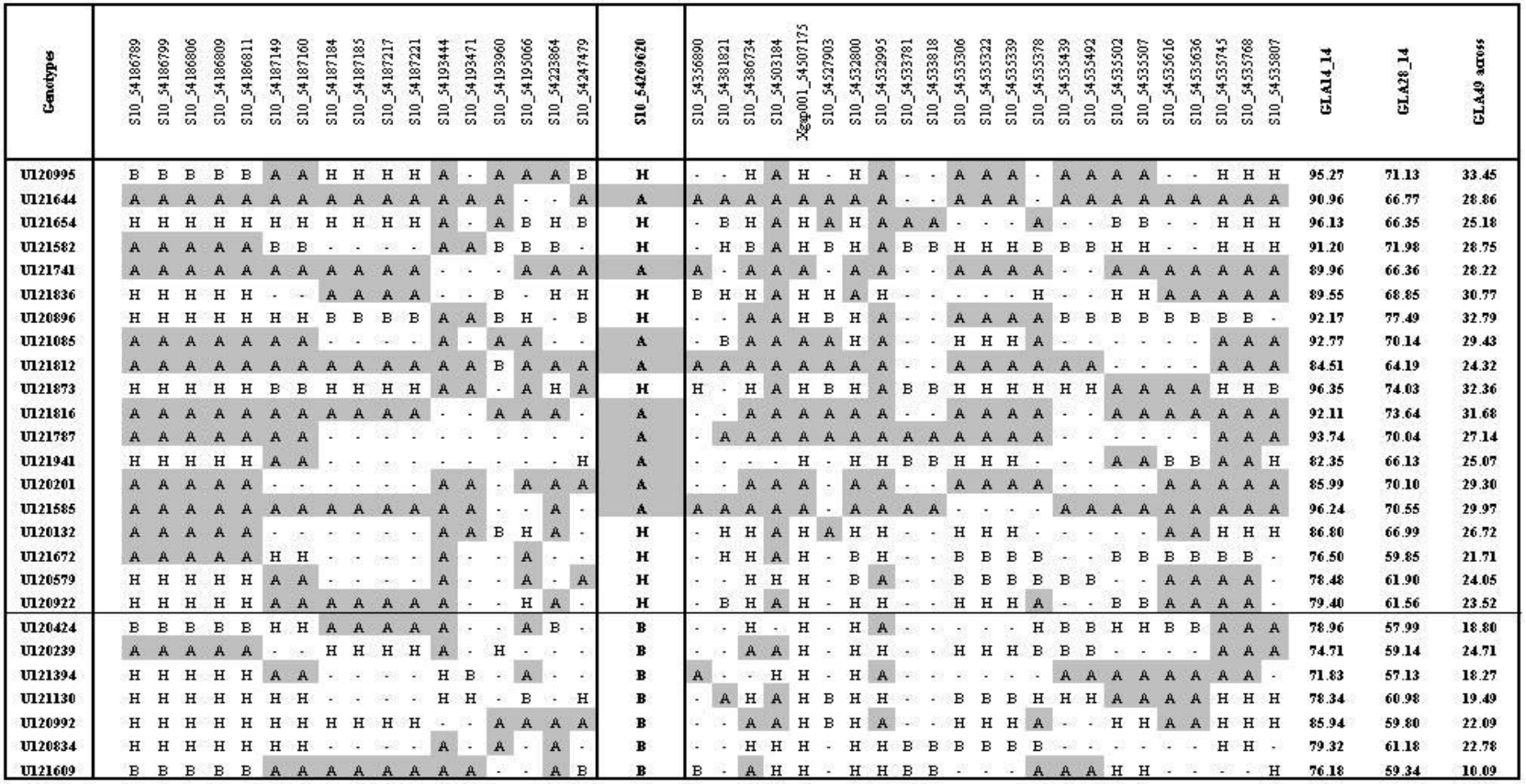


Fig .33 cQTLstg10.1 fine mapping

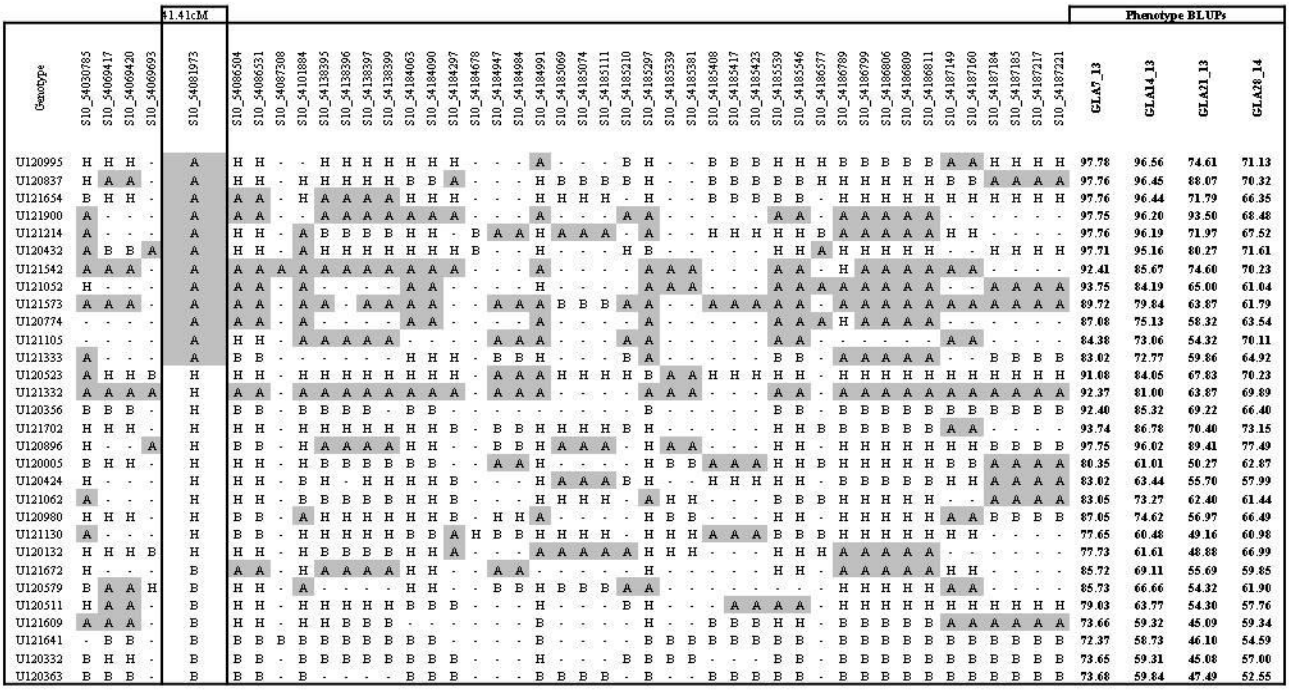


Fig .34 cQTLstg10.2 Fine mapping

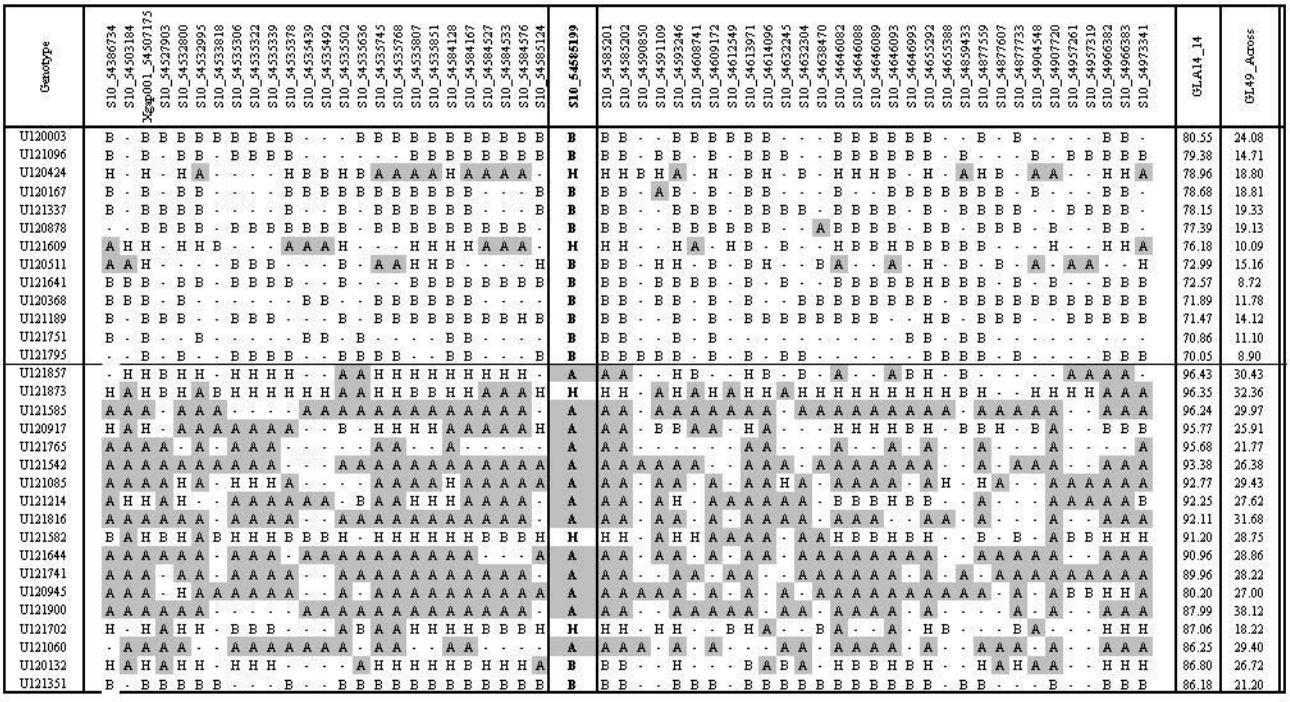


Fig .35 cQTLstg10.3 Fine mapping

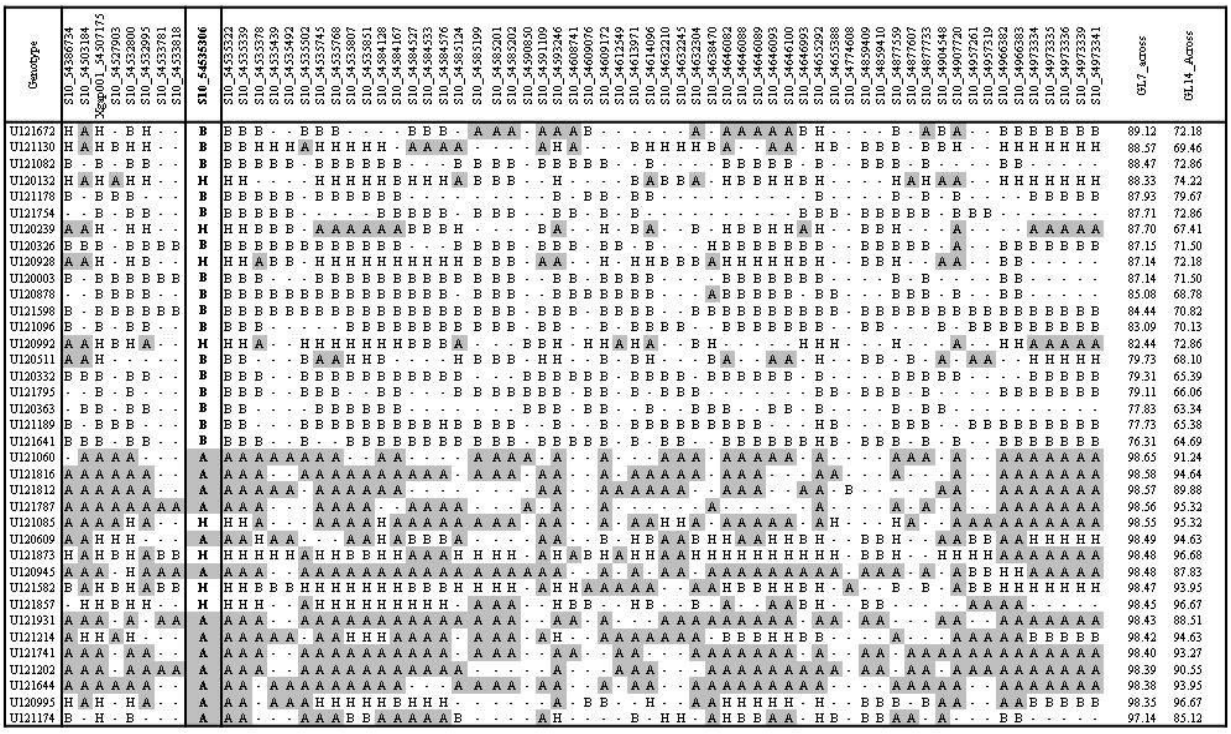


Fig .36 cQTLstg10.4 Fine mapping

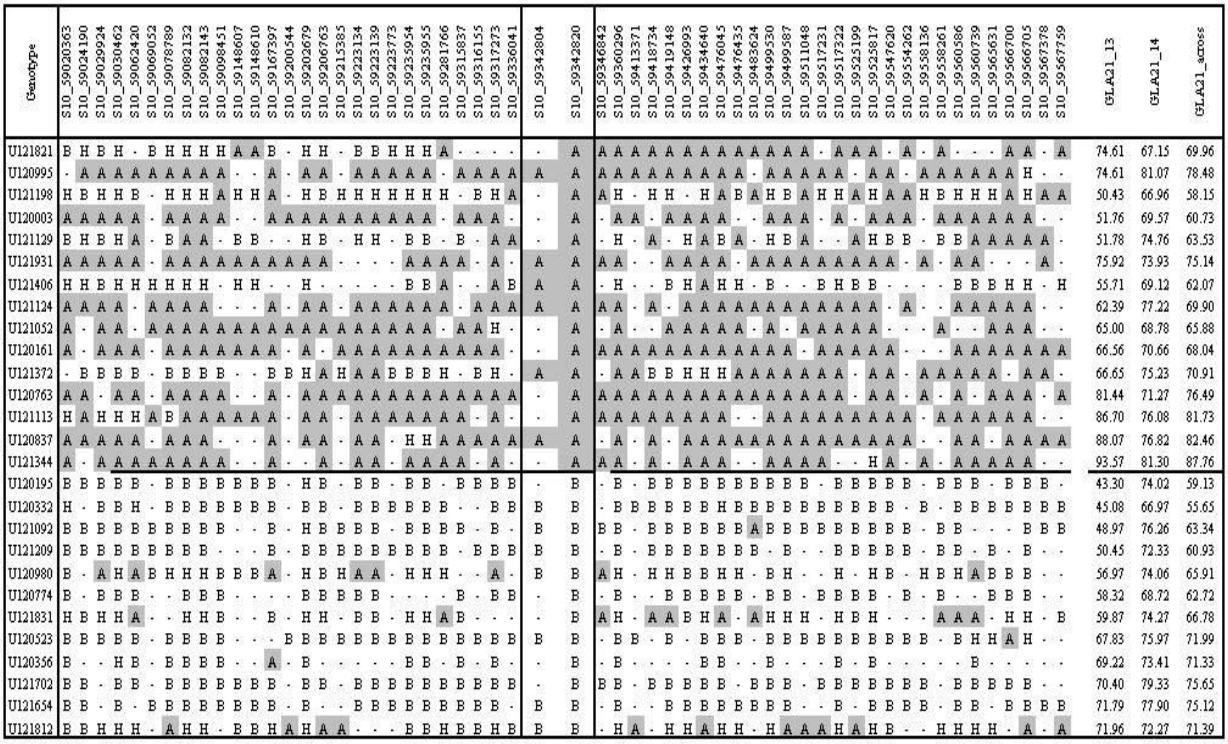


Fig .37 cQTLstg10.5 Fine mapping

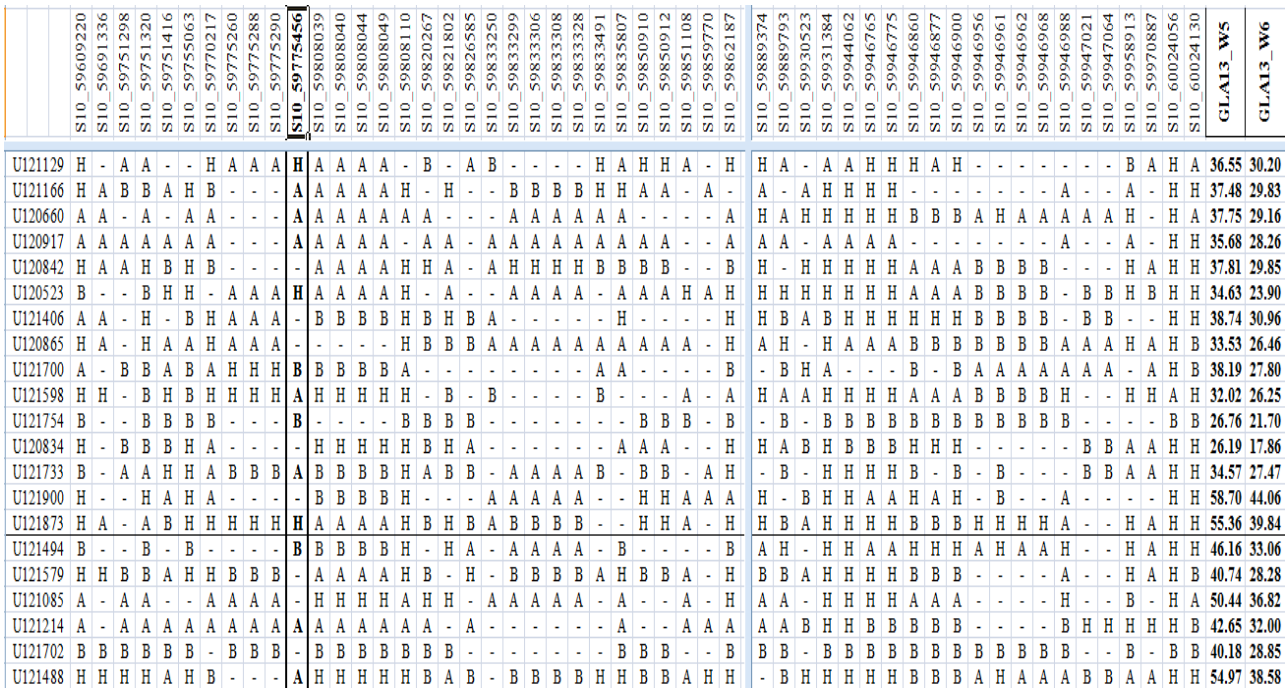


Fig .38 cQTLstg10.6 Fine mapping

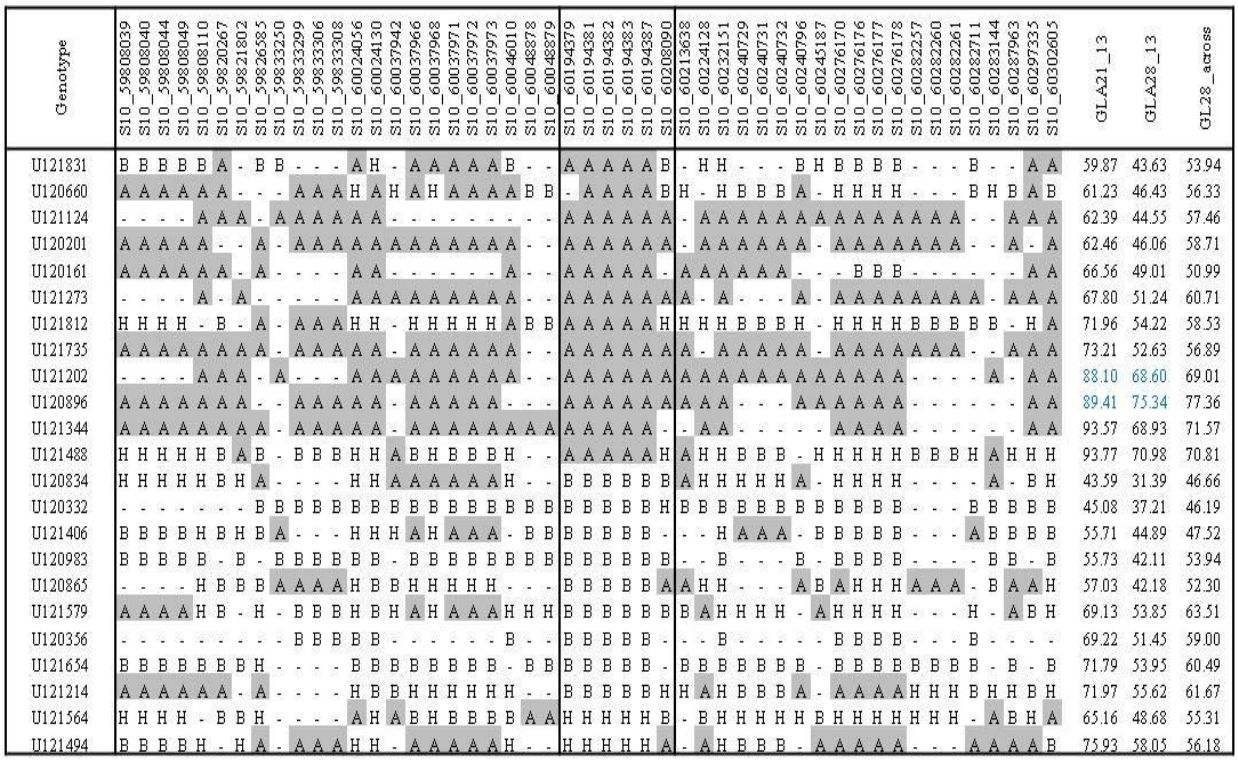


Fig .39 cQTLstg10.7 Fine mapping

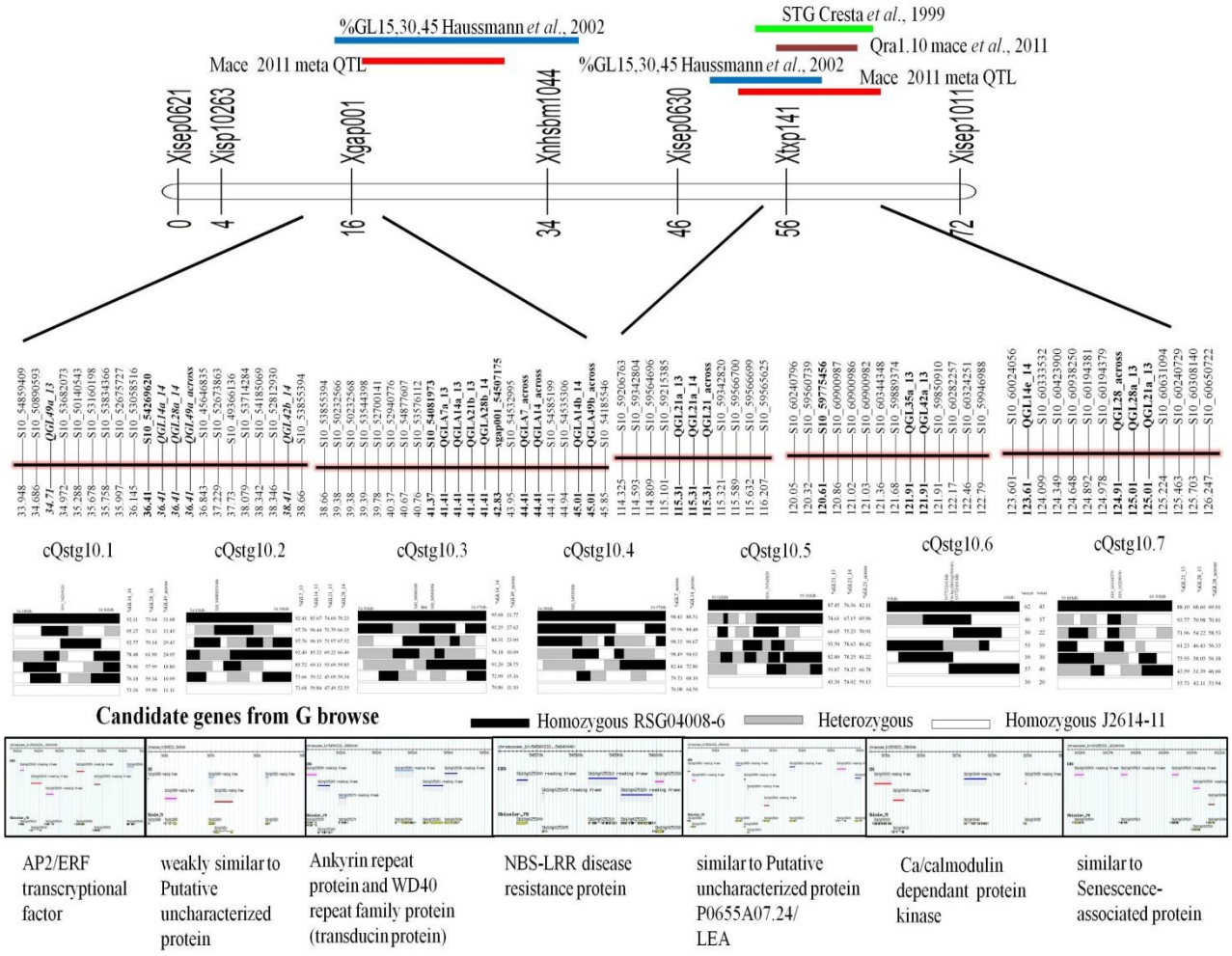


Fig .40 Fine mapping of stay-green QTL clusters fine mapping with candidate genes

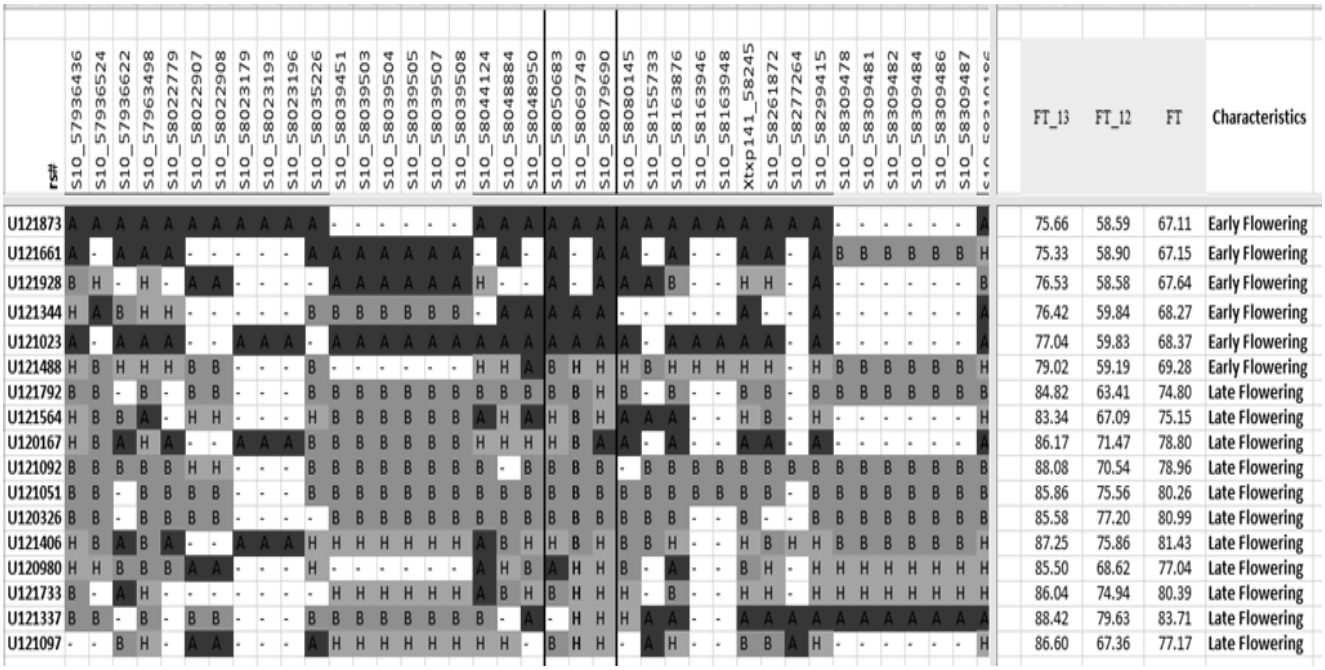


Fig 41: Fine mapping Flowering time region on SBI-10

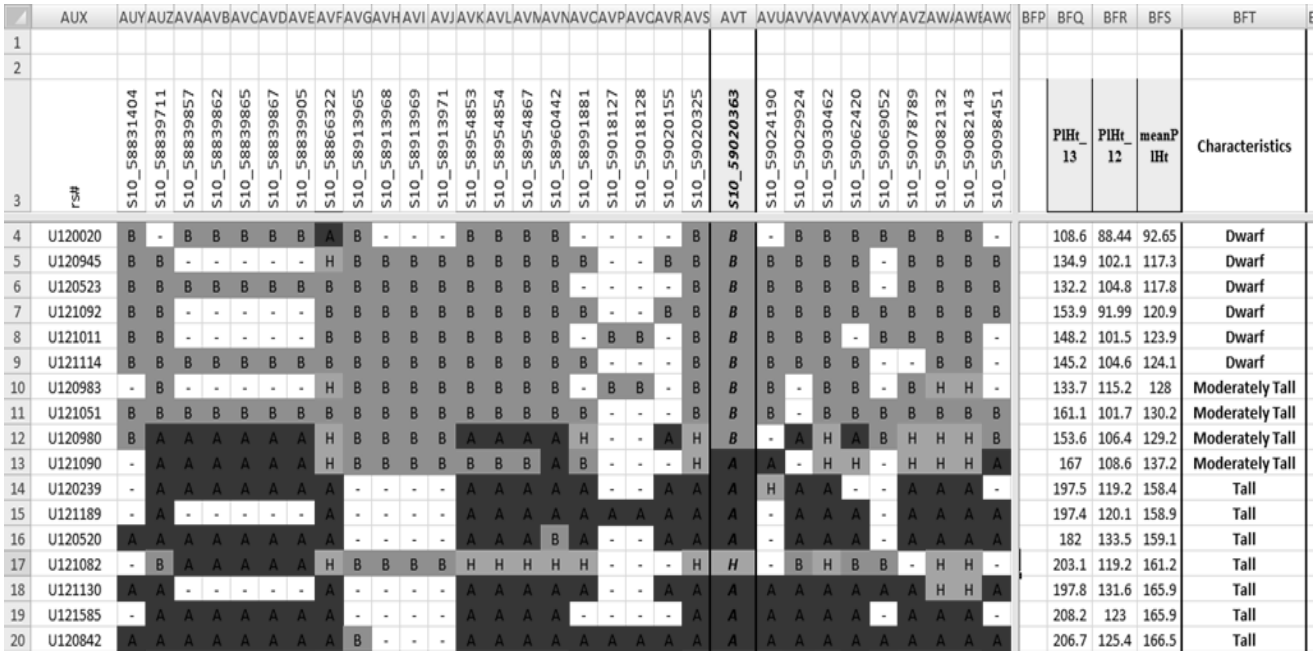
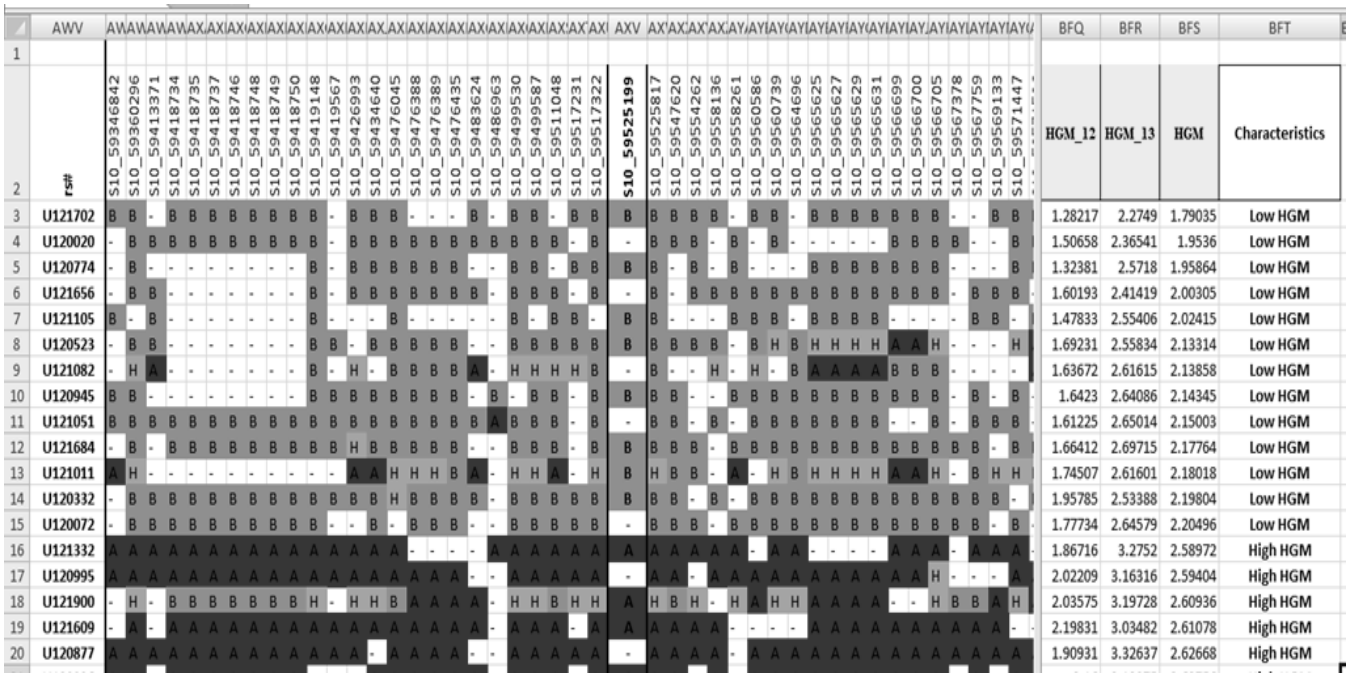
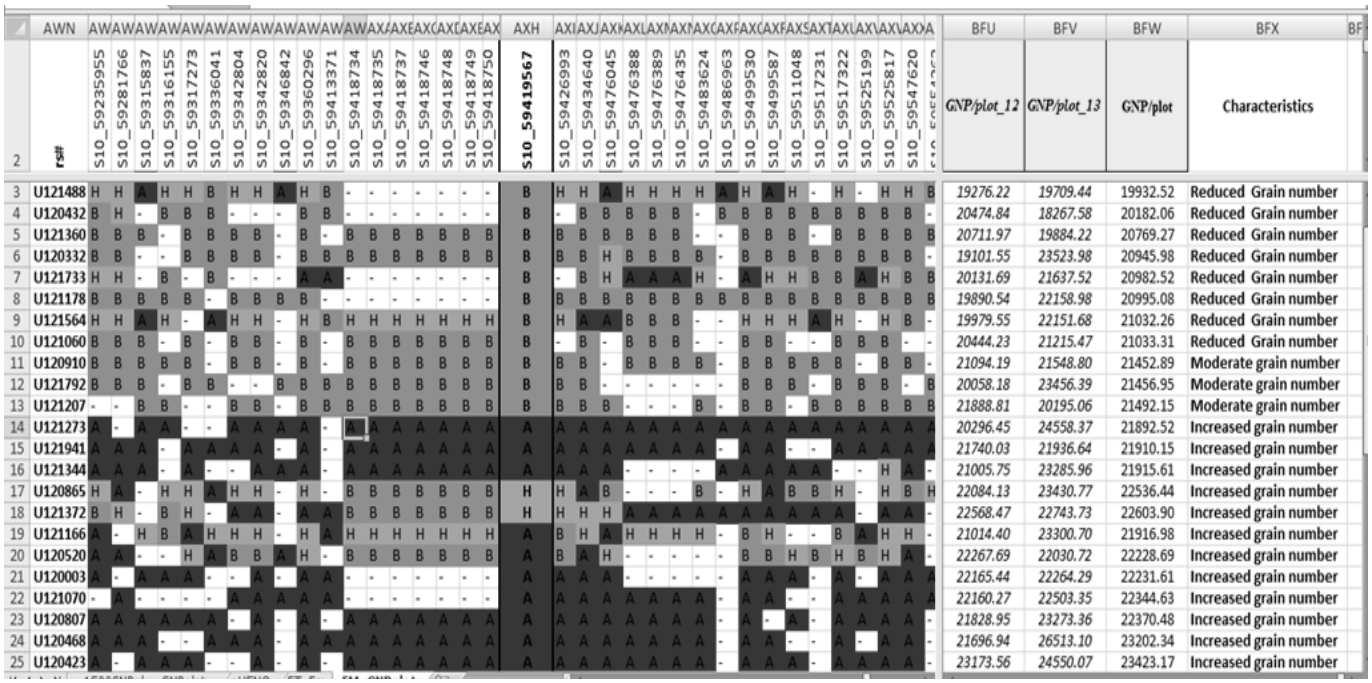


Fig 42: Fine mapping Plant height region on SBI-10



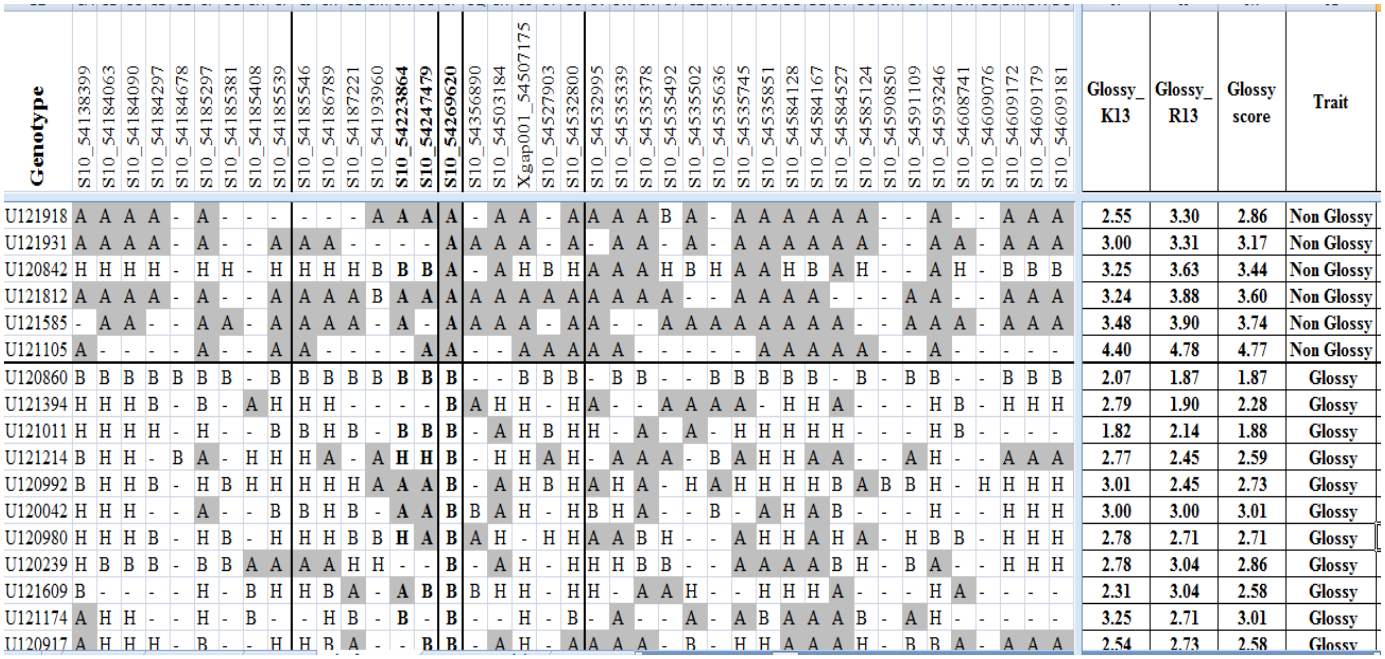
rs#	HGM_12	HGM_13	HGM	Characteristics
U121702	1.28217	2.2749	1.79035	Low HGM
U120020	1.50658	2.36541	1.9536	Low HGM
U120774	1.32381	2.5718	1.95864	Low HGM
U121656	1.60193	2.41419	2.00305	Low HGM
U121105	1.47833	2.55406	2.02415	Low HGM
U120523	1.69231	2.55834	2.13314	Low HGM
U121082	1.63672	2.61615	2.13858	Low HGM
U120945	1.6423	2.64086	2.14345	Low HGM
U121051	1.61225	2.65014	2.15003	Low HGM
U121684	1.66412	2.69715	2.17764	Low HGM
U121011	1.74507	2.61601	2.18018	Low HGM
U120332	1.95785	2.53388	2.19804	Low HGM
U120072	1.77734	2.64579	2.20496	Low HGM
U121332	1.86716	3.2752	2.58972	High HGM
U120995	2.02209	3.16316	2.59404	High HGM
U121900	2.03575	3.19728	2.60936	High HGM
U121609	2.19831	3.03482	2.61078	High HGM
U120877	1.90931	3.32637	2.62668	High HGM

Fig 43: Fine mapping HGM region on SBI-10



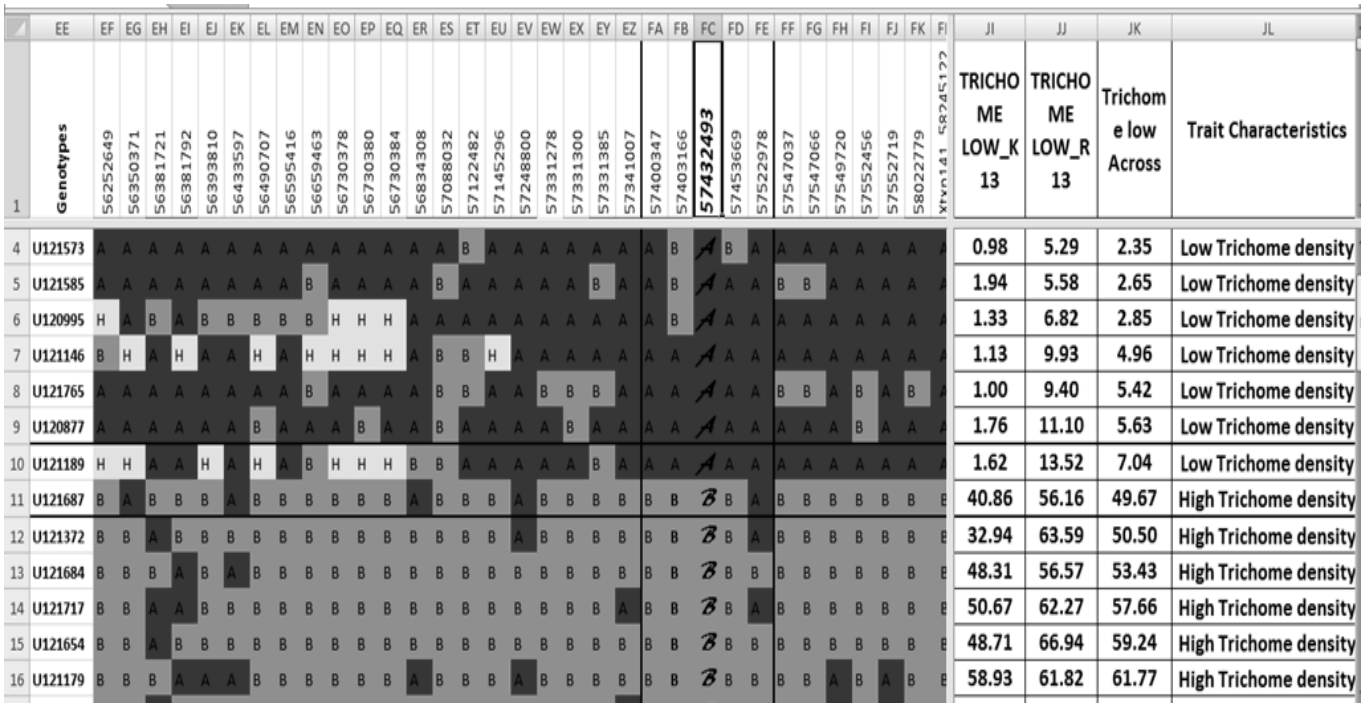
rs#	GNP/plot_12	GNP/plot_13	GNP/plot	Characteristics
U121488	19276.22	19709.44	19932.52	Reduced Grain number
U120432	20474.84	18267.58	20182.06	Reduced Grain number
U121360	20711.97	19884.22	20769.27	Reduced Grain number
U120332	19101.55	23523.98	20945.98	Reduced Grain number
U121733	20131.69	21637.52	20982.52	Reduced Grain number
U121178	19890.54	22158.98	20995.08	Reduced Grain number
U121564	19979.55	22151.68	21032.26	Reduced Grain number
U121060	20444.23	21215.47	21033.31	Reduced Grain number
U120910	21094.19	21548.80	21452.89	Moderate grain number
U121792	20058.18	23456.39	21456.95	Moderate grain number
U121207	21888.81	20195.06	21492.15	Moderate grain number
U121273	20296.45	24558.37	21892.52	Increased grain number
U121941	21740.03	21936.64	21910.15	Increased grain number
U121344	21005.75	23285.96	21915.61	Increased grain number
U120865	22084.13	23430.77	22536.44	Increased grain number
U121372	22568.47	22743.73	22603.90	Increased grain number
U121166	21014.40	23300.70	21916.98	Increased grain number
U120520	22267.69	22030.72	22228.69	Increased grain number
U120003	22165.44	22264.29	22231.61	Increased grain number
U121070	22160.27	22503.35	22344.63	Increased grain number
U120807	21828.95	23273.36	22370.48	Increased grain number
U120468	21696.94	26513.10	23202.34	Increased grain number
U120423	23173.56	24550.07	23423.17	Increased grain number

Fig 44: Fine mapping gnp/Plot region on SBI-10



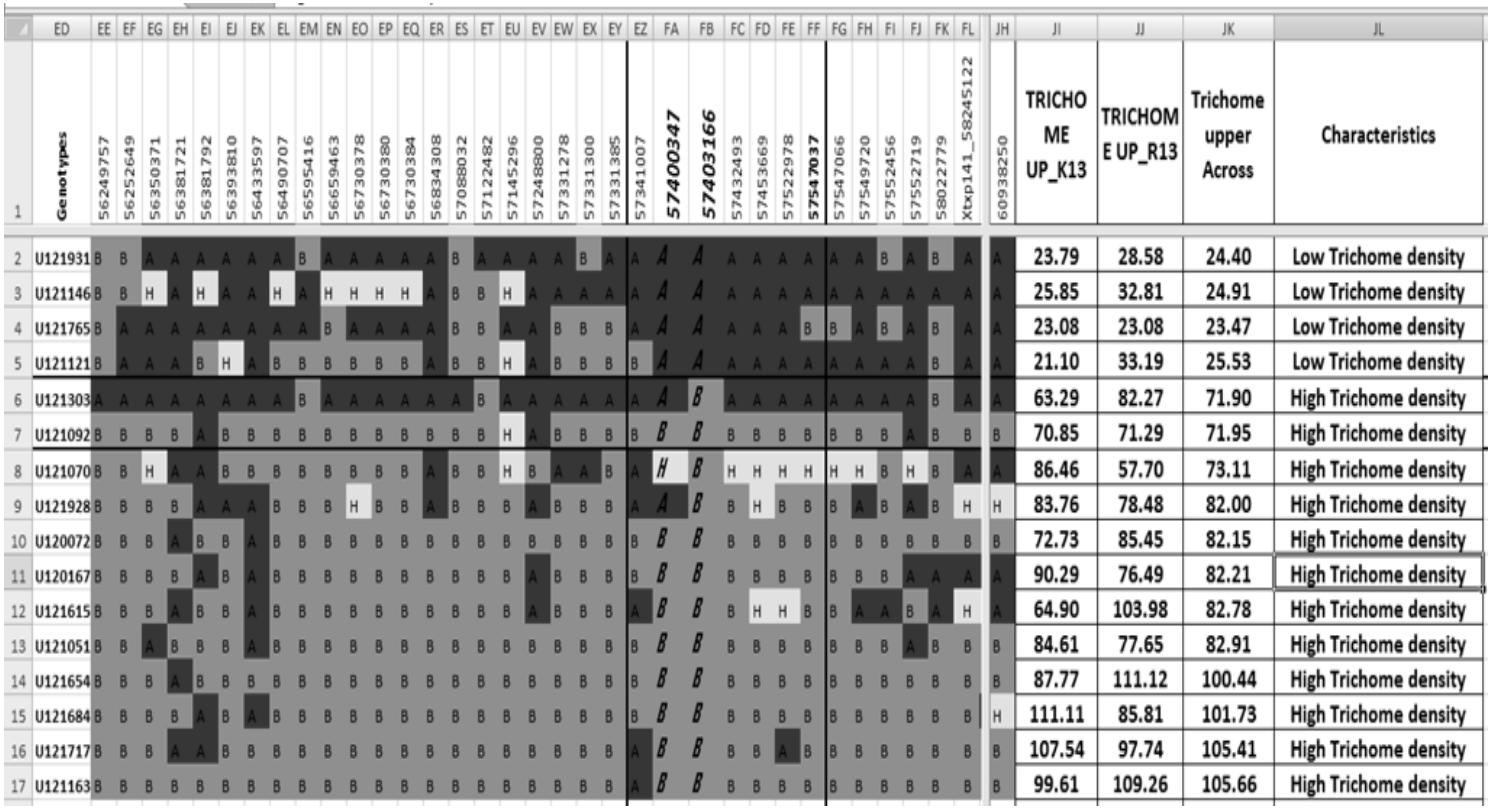
Genotype	S10_54138399	S10_54184063	S10_54184090	S10_54184297	S10_54184678	S10_54185297	S10_54185381	S10_54185408	S10_54185539	S10_54185546	S10_54186789	S10_54187221	S10_54193960	S10_54223864	S10_54247479	S10_54269620	S10_54356890	S10_54503184	Xgap001_54507175	S10_54527903	S10_54532800	S10_54532995	S10_54535339	S10_54535378	S10_54535492	S10_54535502	S10_54535636	S10_54535745	S10_54535851	S10_54584128	S10_54584167	S10_54584527	S10_54585124	S10_54590850	S10_54591109	S10_54593246	S10_54608741	S10_54609076	S10_54609172	S10_54609179	S10_54609181	Glossy_ K13	Glossy_ R13	Glossy score	Trait
U121918	A	A	A	A	-	A	-	-	-	-	-	-	A	A	A	A	-	A	A	-	A	A	A	A	B	A	-	A	A	A	A	A	A	-	-	A	-	-	A	A	A	2.55	3.30	2.86	Non Glossy
U121931	A	A	A	A	-	A	-	-	A	A	A	-	-	-	-	A	A	A	A	-	A	-	A	A	-	A	-	A	A	A	A	A	A	-	-	A	A	-	A	A	A	3.00	3.31	3.17	Non Glossy
U120842	H	H	H	H	-	H	H	-	H	H	H	H	B	B	B	A	-	A	H	B	H	A	A	A	H	B	H	A	A	H	B	A	H	-	-	A	H	-	B	B	B	3.25	3.63	3.44	Non Glossy
U121812	A	A	A	A	-	A	-	-	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	-	-	A	A	A	A	-	-	-	-	A	A	-	A	A	A	3.24	3.88	3.60	Non Glossy
U121585	-	A	A	-	-	A	A	-	A	A	A	A	-	A	-	A	A	A	A	-	A	A	-	-	A	A	A	A	A	A	A	A	-	-	-	A	A	A	-	A	A	A	3.48	3.90	3.74	Non Glossy
U121105	A	-	-	-	-	A	-	-	A	A	-	-	-	-	A	A	-	-	A	A	A	A	A	-	-	-	-	-	A	A	A	A	A	-	-	A	-	-	-	-	-	4.40	4.78	4.77	Non Glossy
U120860	B	B	B	B	B	B	B	-	B	B	B	B	B	B	B	B	-	-	B	B	B	-	B	B	-	-	B	B	B	B	B	-	B	-	B	B	-	-	B	B	B	2.07	1.87	1.87	Glossy
U121394	H	H	H	B	-	B	-	A	H	H	H	-	-	-	-	B	A	H	H	-	H	A	-	-	A	A	A	A	-	H	H	A	-	-	-	H	B	-	H	H	H	2.79	1.90	2.28	Glossy
U121011	H	H	H	H	-	H	-	-	B	B	H	B	-	B	B	B	-	A	H	B	H	H	-	A	-	A	-	H	H	H	H	H	-	-	-	H	B	-	-	-	-	1.82	2.14	1.88	Glossy
U121214	B	H	H	-	B	A	-	H	H	H	A	-	A	H	H	B	-	H	H	A	H	-	A	A	A	-	B	A	H	H	A	A	-	-	A	H	-	-	A	A	A	2.77	2.45	2.59	Glossy
U120992	B	H	H	B	-	H	B	H	H	H	H	H	A	A	A	B	-	A	H	B	H	A	H	A	-	H	A	H	H	H	H	B	A	B	B	H	-	H	H	H	H	3.01	2.45	2.73	Glossy
U120042	H	H	H	-	-	A	-	-	B	B	H	B	-	A	A	B	B	A	H	-	H	B	H	A	-	-	B	-	A	H	A	B	-	-	-	H	-	-	H	H	H	3.00	3.00	3.01	Glossy
U120980	H	H	H	B	-	H	B	-	H	H	H	B	B	H	A	B	A	H	-	H	H	A	A	B	H	-	-	A	H	H	A	H	A	-	H	B	B	-	H	H	H	2.78	2.71	2.71	Glossy
U120239	H	B	B	B	-	B	B	A	A	A	A	H	H	-	-	B	-	A	H	-	H	H	H	B	B	-	-	A	A	A	A	B	H	-	B	A	-	-	H	H	H	2.78	3.04	2.86	Glossy
U121609	B	-	-	-	-	H	-	B	H	H	B	A	-	A	B	B	B	H	H	-	H	H	-	A	A	H	-	-	H	H	H	A	-	-	-	H	A	-	-	-	-	2.31	3.04	2.58	Glossy
U121174	A	H	H	-	-	H	-	B	-	-	H	B	-	B	-	B	-	-	H	-	B	-	A	-	-	A	-	A	B	A	A	A	B	-	A	H	-	-	-	-	-	3.25	2.71	3.01	Glossy
U120917	A	H	H	H	-	B	-	-	H	H	B	A	-	-	B	B	-	A	H	-	A	A	A	A	-	B	-	H	H	A	A	A	H	-	B	B	A	-	A	A	A	2.54	2.73	2.58	Glossy

Fig 45: Fine mapping Glossy region on SBI-10



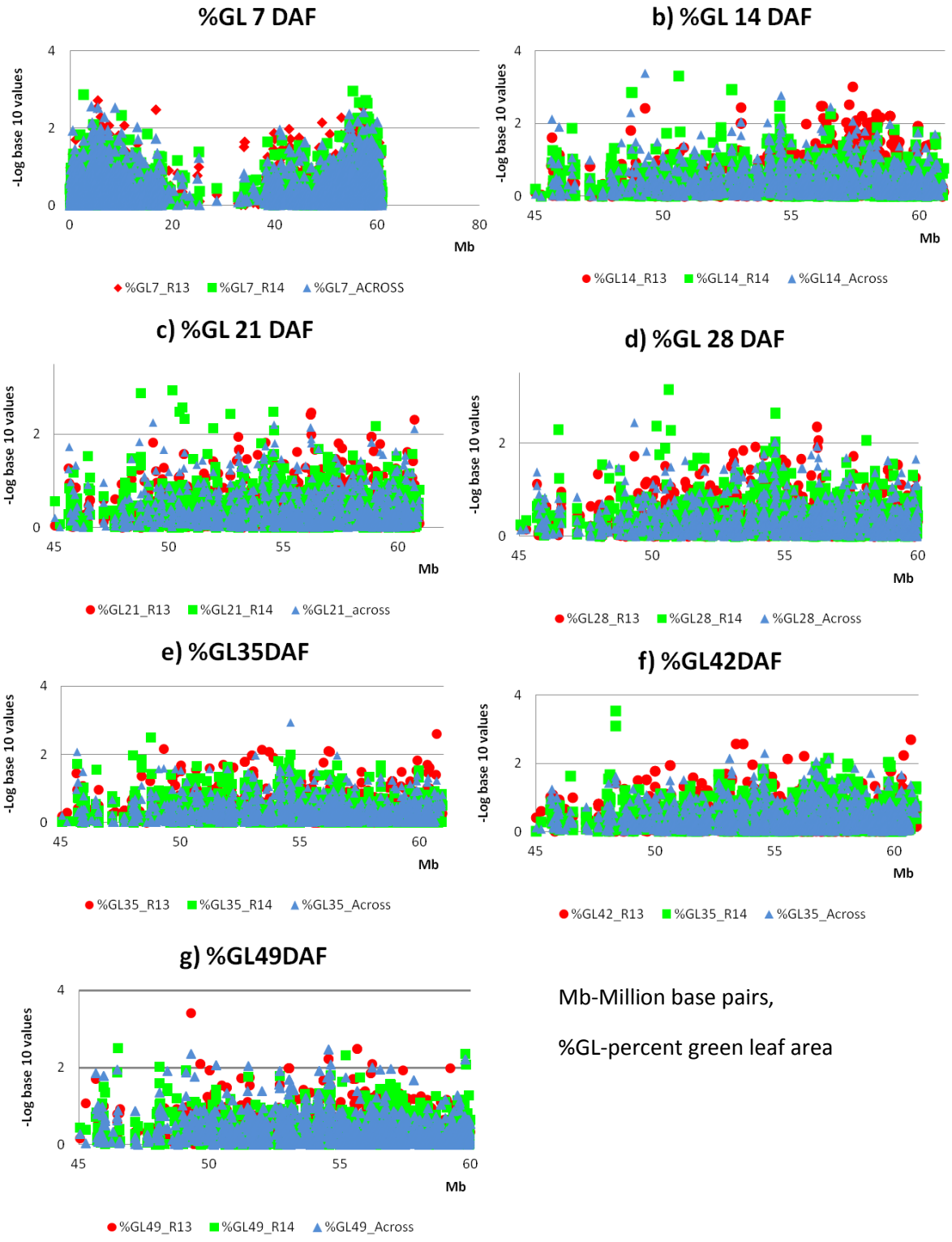
	Genotypes	56252649	56350371	56381721	56381792	56393810	56433597	56490707	56595416	56659463	56730378	56730380	56730384	56834308	57088032	57122482	57145296	57248800	57331278	57331300	57331385	57341007	57400347	57403166	57432493	57453669	57522978	57547037	57547066	57549720	57552456	57552719	58022779	TRICHOME LOW_K 13	TRICHOME LOW_R 13	Trichome low Across	Trait Characteristics
4	U121573	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	B	A	B	A	A	A	A	A	A	A	0.98	5.29	2.35	Low Trichome density
5	U121585	A	A	A	A	A	A	A	A	B	A	A	A	A	B	A	A	A	A	A	B	A	A	B	A	A	A	B	B	A	A	A	A	1.94	5.58	2.65	Low Trichome density
6	U120995	H	A	B	A	B	B	B	B	B	H	H	H	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	1.33	6.82	2.85	Low Trichome density
7	U121146	B	H	A	H	A	A	H	A	H	H	H	H	A	B	B	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	1.13	9.93	4.96	Low Trichome density
8	U121765	A	A	A	A	A	A	A	A	B	A	A	A	A	B	B	A	A	B	B	B	A	A	A	A	A	A	B	B	A	B	A	B	1.00	9.40	5.42	Low Trichome density
9	U120877	A	A	A	A	A	A	B	A	A	A	B	A	A	B	A	A	A	A	B	A	A	A	A	A	A	A	A	A	B	A	A	A	1.76	11.10	5.63	Low Trichome density
10	U121189	H	H	A	A	H	A	H	A	B	H	H	H	B	B	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	1.62	13.52	7.04	Low Trichome density
11	U121687	B	A	B	B	B	A	B	B	B	B	B	B	A	B	B	B	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	40.86	56.16	49.67	High Trichome density
12	U121372	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	32.94	63.59	50.50	High Trichome density
13	U121684	B	B	B	A	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	48.31	56.57	53.43	High Trichome density
14	U121717	B	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	A	B	B	B	B	B	B	50.67	62.27	57.66	High Trichome density
15	U121654	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	48.71	66.94	59.24	High Trichome density
16	U121179	B	B	B	A	A	A	B	B	B	B	B	B	A	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	A	B	A	B	58.93	61.82	61.77	High Trichome density

Fig 46:Fine mapping trichome lower region on SBI-10



	ED	EE	EF	EG	EH	EI	EJ	EK	EL	EM	EN	EO	EP	EQ	ER	ES	ET	EU	EV	EW	EX	EY	EZ	FA	FB	FC	FD	FE	FF	FG	FH	FI	FJ	FK	FL	JH	JI	JJ	JK	JL
1	Genotypes	56249757	56252649	56350371	56381721	56381792	56393810	56433597	56490707	56595416	56659463	56730378	56730380	56730384	56834308	57088032	57122482	57145296	57248800	57331278	57331300	57331385	57341007	57400347	57403166	57432493	57453669	57522978	57547037	57547066	57549720	57552456	57552719	58022779	Xtxp141_58245122	60938250	TRICHOME UP_K13	TRICHOME UP_R13	Trichome upper Across	Characteristics
2	U121931	B	B	A	A	A	A	A	A	B	A	A	A	A	A	B	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	B	A	B	A	A	23.79	28.58	24.40	Low Trichome density
3	U121146	B	B	H	A	H	A	A	H	A	H	H	H	H	A	B	B	H	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	25.85	32.81	24.91	Low Trichome density
4	U121765	B	A	A	A	A	A	A	A	A	B	A	A	A	A	B	B	A	A	B	B	B	A	A	A	A	A	A	B	B	A	B	A	B	A	A	23.08	23.08	23.47	Low Trichome density
5	U121121	B	A	A	A	B	H	A	B	B	B	B	B	B	A	B	B	H	A	B	B	B	B	A	A	A	A	A	A	A	A	A	A	B	A	A	21.10	33.19	25.53	Low Trichome density
6	U121303	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	B	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	B	A	A	63.29	82.27	71.90	High Trichome density
7	U121092	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	H	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	70.85	71.29	71.95	High Trichome density
8	U121070	B	B	H	A	A	B	B	B	B	B	B	B	B	A	B	B	H	B	A	A	B	A	H	B	H	H	H	H	H	H	B	H	B	A	A	86.46	57.70	73.11	High Trichome density
9	U121928	B	B	B	B	A	A	A	B	B	B	H	B	B	A	B	B	B	A	B	B	B	A	A	B	B	H	B	B	B	A	B	A	B	H	H	83.76	78.48	82.00	High Trichome density
10	U120072	B	B	B	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	72.73	85.45	82.15	High Trichome density
11	U120167	B	B	B	B	A	B	A	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	90.29	76.49	82.21	High Trichome density
12	U121615	B	B	B	A	B	B	A	B	B	B	B	B	B	B	B	B	B	A	B	B	B	A	B	B	B	H	H	B	B	A	A	B	A	H	A	64.90	103.98	82.78	High Trichome density
13	U121051	B	B	A	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	84.61	77.65	82.91	High Trichome density
14	U121654	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	87.77	111.12	100.44	High Trichome density
15	U121684	B	B	B	B	A	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	H	111.11	85.81	101.73	High Trichome density
16	U121717	B	B	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	A	B	B	B	B	B	B	B	B	107.54	97.74	105.41	High Trichome density
17	U121163	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	99.61	109.26	105.66	High Trichome density

Fig 47:Fine mapping trichome upper region on SBI-10



Mb-Million base pairs,

%GL-percent green leaf area

Fig .48 GWAS for stay-green

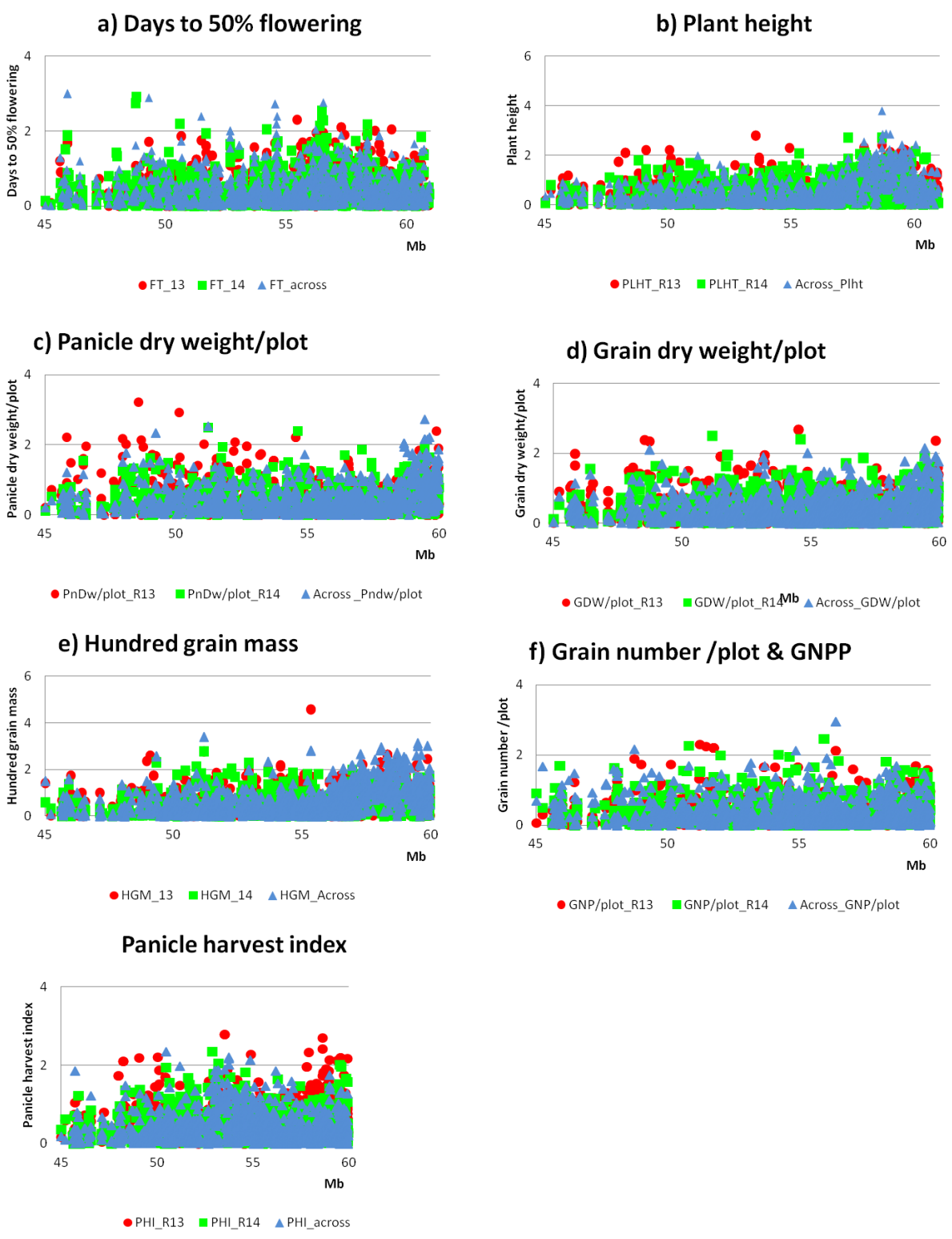


Fig .49 GWAS of Agronomic traits

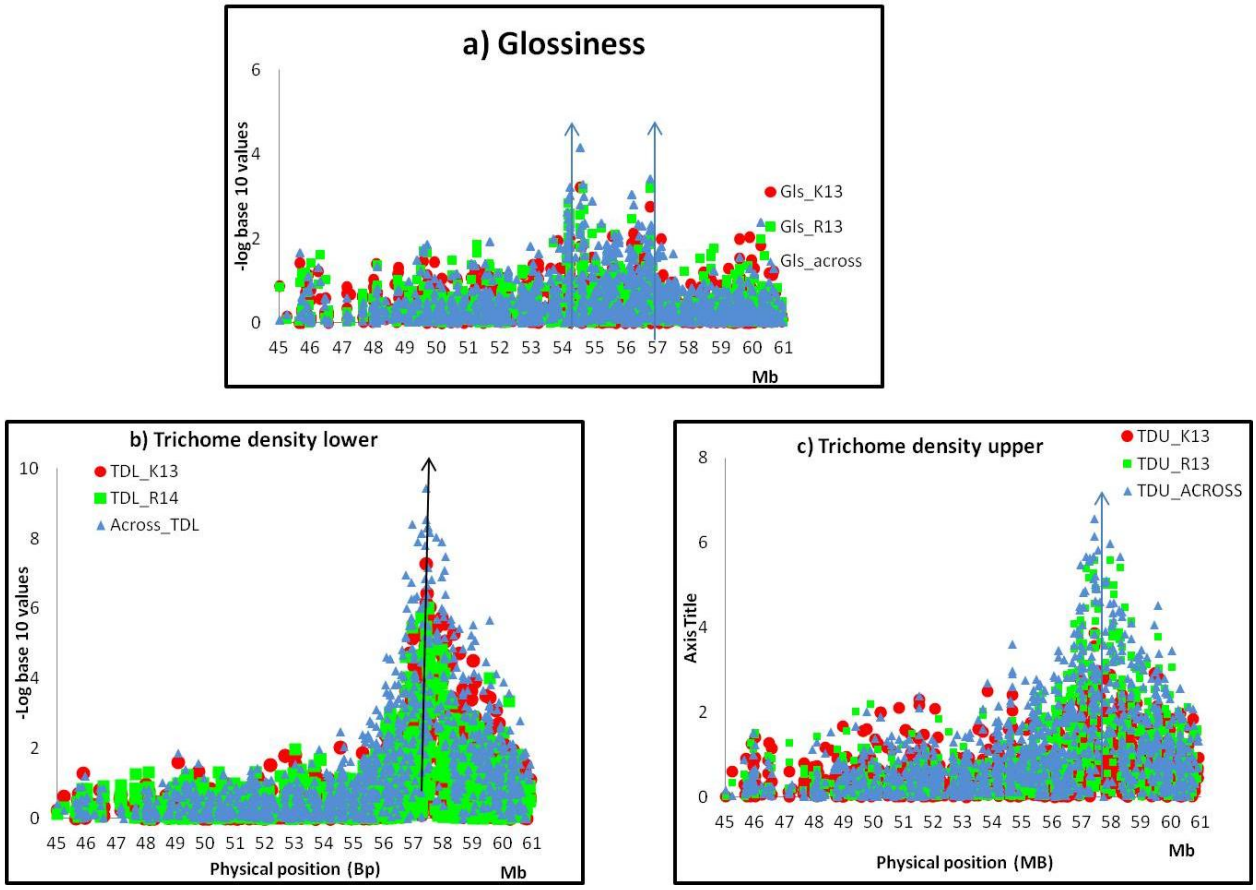


Fig .60 GWAS for SFR component traits

5. DISCUSSION

Present discussion is based on the results obtained from the focused fine genetic mapping of stay-green and shoot fly resistance QTLs observed on *Sorghum bicolor* chromosome SBI-10 from introgression line cross RSG04008-6 (moderately stay-green and shoot-fly susceptible) × J2614-11 (moderately shoot-fly resistant and moderately senescent). Fine mapping was carried out using F_2, $F_{2:3}$ and $F_{2:4}$ phenotyping and construction of high density linkage map with GBS SNPs, and QTL mapping, Fine mapping and GWAS analysis identified probable candidate genes based on functional annotation and discussed below in detail. As the parents (RSG04008-6 from Kassahun, 2006 and J2614-11 from Jyothi, 2010) are introgression lines derived from different MABC programs of ICRISAT, Patancheu, there would be a chance of unexpected cross-contamination of seed or DNA samples. Care was taken to ensure the parental seed material and experimental parents (RSG04008-6 and J2614-11) were compared to their grandparents with the help of molecular markers in the target genomic region. Confirmed true heterozygote F_1 hybrids were selfed to produce F_2 fine mapping population seed material. Since both the parents are introgression lines, they reduce the linkage drag and more advantageous than the pure lines for fine mapping or genetic dissection of particular trait studies.

5.1. F_2 and F_3-derived informative F_3 and F_4 progenies

In order to reduce the cost effect of genotyping, we used 5 SSR markers for identification of recombination break point in 1894 F_2 fine mapping populations and based on molecular marker data, 384 F_2 double recombinant genotypes were selected. Selected double recombinant F_2 s were screened with the additional 3 new markers and then selfed to produce F_3 seed which was replicated for seed multiplication in order to validate the shoot fly morphological traits and stay-green characters. Breeding to increase recombination and marker based selection of genotypes with right recombination rate results in increased response to

selection (McClosky *et al.,* 2013). Selective genotyping approach is advantageous and it reduces time and cost of genotyping by selection of desired recombinants from the high resolution population (Hillel *et al*., 1990, Tanskley, 1993, Darvasi, 1997).

5.1.1. Parental variation and mean performances of parents, F_2 and F_4 populations

Parents varied in the initial stages but both were showing moderate stay-green levels at the end of the grain filling stage. A graph was plotted between both parental mean values as well as progeny mean values that showed better performance of $F_{2:4}$ progeny than both the parents, inferring the contribution of stay-green alleles from both the parents. Shoot fly morphological traits in F_2 populations like seedling leaf blade glossiness and trichome density looked like that of parents. In case of agronomic traits both the parents varied much for plant height, flowering time, and hundred grain mass. Traits like PnDw/Plot, GDW/Plot, GNP/Plot and GNPP for RSG04008-6 and J2614-11 showed low yield in summer 2013 when compared to summer 2014 due to high levels of stress in summer 2013 due to the delay in sowing.

5.1.2. Frequency distribution

Transgressive segregation was observed for trichome density lower and upper. In case of F_4 stay-green observations, % GL 7 and % GL 14 completely skewed towards female parent and % GL 21, 28, 35, 42, 49 showed near to normal distribution pattern for both the seasons with transgressive segregation. Agronomic traits like flowering time, plant height, PnDW/Plot, GBW/Plot, GNP/Plot, GNPP, PHI and HGM exhibited near to normal distribution which indicates a polygenic inheritance (Thoday *et al.,* 1976).

5.1.3. Phenotypic correlation coefficient

5.1.3.1. Shoot fly traits association

Glossiness is positively correlated with % SFDH, TDL and TDU where increased glossy values (non-glossiness) lead to shoot fly susceptibility and positively associated with seedling vigour and leaf sheath pigmentation. This indicates glossy leaves are erect and show plant vigour in early stages of plant. Both TDU and TDL were highly negatively correlated with shoot fly dead hearts since the trichomes prevent shoot fly infestation. As the leaf glossiness, seedling vigour, leaf sheath pigmentation, trichome density, oviposition are associated with shoot fly resistance/susceptibility, these traits can be utilized as morphological markers in screening shoot fly resistance and while breeding for the trait (Riyazaddin *et al.,* 2015).

5.1.3.2. Stay-green and agronomic traits association

When the correlation results were compared with Haussmann *et al.,* (2002b), their two RIPs were positively correlated but no effect of GL was noticed on grain yield for RIP1. Two different seasons stay-green phenotyping displayed many overlapping or co-localized QTLs as well as new and unique QTLs. Common correlations among varying environments were rarely seen. But in present study all the stay-green traits are positively correlated with each other in both the environments. In case of correlation with yield it was negative during *rabi* 2012/2013 whereas it was positively correlated during *rabi* 2013/2014 which has been supported by Jordan *et al.,* (2012) also. During *rabi* 2012/2013, seeds were sown very late and the crop experienced heavy stress during anthesis when compared to *rabi* 2013/2014. The strength of correlation between % GL and grain yield (GY) (GDW/plot and PnDW/Plot) depends on environmental conditions and genetic background as pointed out by Jordan *et al.,* (2012) and Gregersen *et al*., (2013). Stay-green data were positively correlated with GDW/plot, PnDW/plot, GNP/plot and GNPP for summer 2014 whereas negative correlation was observed for summer 2013. Our correlation results were also in agreement with the results obtained by Jordan *et al.,* (2012). Haussmann *et*

al.,(2002b) mapped stg QTLs on LG-G (SBI-10L) between flanking markers Xgap001 and Xtxp141. Stay-green QTLs were mapped beyond Xtxp141 (Mace and Jordon 2011; Mace *et al*., 2012). So, for identifying those previously mapped stg QTLs in the present introgression line cross, $F_{2:4}$ populations and its fine mapping may generate data that can help to understand the delay in senescence. Haussmann *et al.*, (2002b) showed 5-7% phenotypic variance for the individual QTLs which was almost similar but slightly better phenotypic variances (9.8%) was observed in the present study for individual seasons. During *rabi* 2013/2014, senescence data were positively correlated with grain yield (grain number, grain dry weight, panicle dry weight and panicle harvest index). This indicates that moderately stressed plants can with stand drought and can give high yields. FT shows significant positive correlation with plant height and HGM indicates positive association but negative correlation with % GL shows the photosynthetic activities require more nutrients for translocation from leaves to seed.

5.1.4. Impact of Population, size and field design

In our present study F_2 1894 fine mapping population was used for genetic dissection of traits. F_2 population can reduce number of generations and more advantageous when comes to recombination events due to meiotic divisions and the increased population size gives more frequency to identify desired recombinants. F_2 and F_3 populations are early generation populations mostly used for QTL mapping and fine mapping studies (Vales *et al.*, 2005). $F_{2:4}$ recombinant population studies are rarely conducted but with increased marker density we can reduce the number of generation leads to reduction of time, money and man power. As $F_{2:4}$ mapping population is 152 still it has its own significance but when compared to 1894 F_2 population it has less efficiency in identifying genomic regions but, increased maker density and field replicated data has reduced the negative effects of reduced population size. Alpha lattice design will minimize the experimental errors. Unbalanced ANOVA is preferred

for alpha lattice design to reduce the experimental errors. BLUPs were estimated for unbalanced ANOVA instead of predicted means in order to minimize errors

5.1.5. Molecular markers

Till now, sorghum RFLP (Pereira *et al.,* 1994, Xu *et al.,* 1994), SSRs (Brown *et al.,* 1996; Bhattramakki *et al.,* 2000; Kong *et al.,* 2000; Hausmann *et al.,* 2002a), AFLP, RAPD (Menz *et al.,* 2002) were rapidly used for mapping strategies. But, recent advancement in next generation sequencing technologies and improved computational advances were utilized for molecular marker data generation and analysis. DArT (Mace *et al.,* 2008, 2009) and SNPs are recent advanced marker systems where data generation and the cost are inversely proportional as the time frame is increasing. In the present study, SSR markers were used initially for recombinant selections and then selected recombinants were skimmed and sequenced by GBS methods. GBS was used extensively in sorghum for population diversity studies (Nelson *et al.,* 2011; Morris *et al.,* 2013a,b; Thurber *et al.,* 2013; Perez *et al.,* 2014; Lasky *et al.,* 2015; Zhang *et al.,* 2015). We have performed GWAS analysis also with GBS SNP and genotyped SSR markers for locating the variant genomic regions responsible for stay-green and agronomic as well as shoot fly morphological traits.

5.1.6. Genetic linkage map on total F_2 and selected F_2 population with SSR markers

In the present study, initial linkage map of 37 cM length was constructed using five SSR markers on F_2 population of 1,894 individuals derived from cross RSG04008-6 × J2614-11. In previous studies this target region was reported to be above 45 cM interval but now it is 37 cM (5 Mb), which indicates a reduction in map length most likely due to moderately large F_2 population size. After adding three additional flanking markers and reducing the population size to 369 (selected recombinants), the map length increased to 72 cM (15 Mb), partly due to double crossovers as the recombination frequencies were converted to map distance based on the Kosambi mapping function (Kosambi, 1943), but largely

due to the addition of flanking markers on both ends of the mapped interval. Mace and Jordan (2011) integrated different sorghum QTL mapping studies onto the physical map resulting in a QTL cluster in sorghum and thus many QTL clusters were identified. Similarly, a comparison has been made in the present attempt, for all the shoot fly resistance QTL mapping studies to delimit the glossy and trichome density QTL size on SBI-10L. Our results are in agreement with earlier studies which showed *Xgap001 – Xnhsbm1044* and *Xisep0630 –Xtxt141* intervals which need to be further studied in detail by utilizing high through put marker genotyping or SNP.

5.1.6.1. Genetic linkage map is highly informative for F_2 selected recombinants: Horseshoe effect and principle co-ordinate analysis (PCA)

GBS approach has been followed for increasing marker density in the target region. We have a different approach for the mapping or integrating GBS SNPs into the SSR genetic map of SBI-10L. As per Cheema and Dicks (2009), map estimation is an iterative process. We initially studied the marker genotyping data sets clearly and eliminated the disturbance and created marker sets from the SNP marker data set of SBI-10L. Cheema and Dicks (2009) discussed nearly 11 software methods for genetic mapping and concluded that the genetic mapping depends on the type of population, type of marker data and the principles of software. For developing a genetic linkage map, it needs to be studied clearly and one should approach the best method that suits the genotyping data for linkage mapping. Linkage mapping completely depends on the recombination fraction between two loci or LD decay observed between non-random associations of markers. In the present study, we selected double recombinants which can give wrongly expanded map distances that may lead to unwanted estimation of QTLs and error prone fine mapping. In order to overcome the errors, we utilized THREaD Mapper Studio which calculates inter marker distances using principle co-ordinate analysis (PCA). A total of 1515 SNPs were identified from 45-60 Mb physical intervals where we are suspecting the presence of stay-green QTL co-localization based on the results published by several workers (Haussmann *et al.,* 2002b; Mace and Jordon 2011 and Mace *et*

al., 2012). Due to large 1515 SNP markers, the online THREad mapper was unable to generate complete map but generated a distance matrix plot for 392 SNPs + 7 SSR markers. We cross checked the allelic composition of SSRs and compared them with SNPs and also the spurious (distracted) markers by PCA. PCA can be utilized in genetic marker analysis because the order of the marker data point along the curve that correspond to their linkage. These nearly linked markers were again recalculated for distance matrix which shows reduced disturbances in the distance matrix plot. PCA was used in the present study, since it has advantages like fast recalculation and identification of unreliable data points which can help to reduce the marker order uncertainty. The marker distribution of linkage map was compared with the physical map and they are near to similar with little noise in the marker order due to huge missing data in SNP markers.

5.1.6.2. Advantages of Ultra-high density map developed from GBS, SNP data

In sorghum, Mace and Jordan (2011) and Zou *et al.,* (2012) have carried out genetic and physical map integration studies. GBS and SNP studies have revealed an average marker density of 2.1 markers per centimorgan and this was found to be the high density map of sorghum. In the present study, average marker density of sorghum chromosome SBI-10L was 1.95 markers per centimorgan which shows the importance of the GBS SNP integrated SNP map of target region. Our study is the first fine mapping study in sorghum targeting stay-green and the results are near to similar to that of the results obtained by Haussmann *et al.,* (2002b). This could be because of the stay-green source E36-1 that was used for both the stay-green QTL mapping studies. This is first fine mapping study for seedling leaf blade glossiness and trichome density (upper and lower) where initially with F_2 fine mapping population 2.46 Mb and 730 kb regions were further fine mapped to 283 kb, 221 kb respectively by utilizing GBS, SNP data and F_4 phenotyping data. From the F_4 QTL mapping, it was clearly evident that glossy 15 in the 283 kb interval and O-methyl transferase and

EF-hand Ca^{2+}-binding protein CCD1 in 221 kb interval are the most probable candidate genes. As the population selected itself is a double recombinant population, the frequency of recombination was more when compared to the whole population which can lead to artefactual increase of the map distance. In order to nullify the double recombinants effect, SNPs have been filtered using horseshoe effect before constructing the high resolution genetic linkage map. Construction of genetic map with all the 1515 SNPs may lead to false QTLs/pseudo QTLs. Most of the distracted markers were removed before mapping. When the genetic map order was compared with physical map order, they have been found not exactly the same but few marker positions were misplaced. Physical positions were determined based on the reference genome and in the present study none of the parents were pure lines.

5.1.6.3. Segregation distortion

Segregation distortion was observed for all markers and this could be due to selective recombinants which increased heterozygous nature along with homozygous alleles distorted in their meiotic segregation. Population type and size of population, crosses and marker type, also affect the segregation distortions (Liang *et al.,* 2006). Low to moderate size of population shows more segregation distortion when compared to large population as the 1894 F_2 populations exhibited low segregation distortion when compared with 152 F_4 genotypes in our study. Segregation distortion is recognized as a powerful evolutionary force and affects the construction of linkage maps (Liu *et al*., 2010). Segregation distortion depends on the recombination frequencies and then impedes mapping precision and the linkage analysis of QTLs (Wu *et al.,* 2010). In the present study, the segregation distortion skewed towards male parent J2614-11 which indicates the presence of genes or markers influence segregation distortion towards the male parent J2614-11 (Tao *et al.,* 1998a).

5.1.6.4. Efficiency and essence of GBS, SNP map on SBI-10L

Annotated sorghum genome sequence was utilized for the functional analysis of the QTL mapped and fine mapped regions. In this QTL, known SAP, SAGs, NAC, WRKY, AP2/ERF (Guo and Gan, 2006; 2012) genes for stay-green fine mapped region and AP2/glossy15 and zinc finger (C2H2 type) protein, EF-hand Ca^{2+}-binding protein CCD1 and O-methyl transferase genes for glossy and trichome density mapped regions have been noticed. These results indicate the efficiency and essence of the GBS SNP map. SAP-senescence associated proteins involved in senescence mechanism have been found to be SEN1 and DIN (Oh, 1996). SAG-senescence associated genes which are activated under drought stress conditions belong to the categories SAS and NAC that have been reported to be involved in senescence mechanism in many studies of rice and *Arabidopsis* (Guo and Gan, 2006; Li *et al.,* 2012; Liang *et al.,* 2014), WRKY-transcription factors that play major role in overcoming drought and senescence-associated problems (Cai *et al.,* 2014), AP2/ERF transcription factors that are reported to be involved in drought tolerance (Guo and Gan, 2012; Licausi *et al.,* 2013; Mawlong *et al.,* 2015). Crasta *et al.,* (1999) identified stay-green QTLs on SBI-10 and Haussmann *et al.,* (2002b) identified stay-green QTLs (% GL 15, 30, 45) on SBI-10L between *Xgap001* and *Xtxp141* flanking markers using two different populations having common stay-green donor E36-1 which are overlapping with nodal root angle QTLs (Mace *et al.,* 2012, Singh *et al.,* 2012). The co-localization of the QTL as evident from the previous studies (Haussmann *et al.,* 2002b; Crasta *et al.,* 1999) as well as the QTL identified in the present study show the importance of the GBS-SNP map. In the present study, identified QTLs for stay-green were overlaying with the QTLs identified by Haussmann *et al.,* (2002b) earlier. Calcium/calmodulin-dependent protein kinase (Sb10g030040) has been identified in the present study, which plays a functional role in the identification of drought tolerant locus in the fine mapped regions as well as GWAS analysis (Franz *et al.,* 2011). QTL cQstg10.5 was stable across both the environments mapped at same location (S10_59342820) which encodes for LEA2 proteins (Sb10g029570) reported to play a role in late embryogenesis

and drought stress mechanisms. Thus, these results suggest that the GBS SNP map is of high quality efficient map and is suitable for QTL identification and gene mapping. GBS was vastly used by molecular breeders as this is an effective method for genotyping huge population with high efficiency.

5.2. QTL mapping advantages over SFR morphological traits

For breeding shoot fly resistance, pyramiding resistance component traits appear to be the best way to develop commercially usable levels of host plant resistance, with timely sowing (to avoid high population pressure of pest). Timely sowing provides the most eco-friendly method for the management of shoot fly pest. Combined effects of glossiness and trichome density reduces the severity of shoot fly infestation and plants with high levels of expression for both the traits show better resistance to this insect pest. These morphological traits are well studied (Sharma *et al.,* 2005; Dhillon *et al.,* 2005; Dhillon *et al.,* 2006; Kumar *et al.,* 2008, 2011), genetically mapped (Sajjanar, 2002; Folkertsma *et al.,* 2003; Deshpande, 2005; Mehtre, 2006, Satish *et al.,* 2009, 2012; Aruna *et al.,* 2011; Apotikar *et al.,* 2011) and further introgressed (Jyothi *et al.,* 2010) into to cultivated varieties in order to deploy insect pest resistance in combination with other economically important traits like high grain and stover yields and quality. Previously, these SBI-10 QTLs for trichomes and glossiness were detected in many studies, and summarized in Table 28. Except in the study of Aruna *et al.,* (2011) (IS2122), all other SFR QTL mapping studies used IS18551 donor for shoot fly resistance, but the mapping populations used varied in population size, type (segregating and recombinant inbred lines), environment and location. Regions of the sorghum genome contributing to insect resistance are mostly syntenic to maize, suggesting such regions are highly conserved. The glossiness QTL and possible trichome density QTL identified in the present study were detected earlier by Sajjanar (2002); Deshpande (2005); Mehtre (2006); Jyothi (2010); Aruna *et al.,* (2011) and Satish *et al.,* (2009; 2012). However, the present work shows evaluation of ‘Gls’ and ‘Td’ QTLs in the SBI-10 over different environments (late *rabi* 2011/12 and *kharif* 2012), across the seed generations

(F_2 and $F_{2:3}$), different population sizes (1894 and 369), different mapping methods (QTL cartographer for F_2 and PLAB QTL for the selected subset of F_2 and its derived $F_{2:3}$) and mapping approaches (traditional fine mapping) which resulted in consistent QTLs.

5.2.1. QTL mapping of seedling leaf blade glossiness and trichome density in total F_2 and $F_{2:3}$ population with initial SSR linkage map

Due to large F_2 population, many recombination events have been found within the introgressed genomic segment originally introduced to BTx623-background from IS18551 by marker-assisted backcrossing (MABC) that affects the shoot fly reaction phenotype. The background of the parents varies from the introgressed segment and the F_2 progeny with increased number of recombination. This may affect the QTL detection power when compared to recombinant inbred lines. QTL analysis can also be affected by the size of the early-generation (F_2 and F_3) and high populations can result in detection of large numbers of QTLs including minor effect QTLs (Vales *et al.,* 2005). However, F_2 and F_3 QTL mapping results, based on *rabi* and *kharif* season evaluations respectively, were found similar for glossiness. Leaf glossiness characterized by deposition of less wax or alteration in quantity and quality of epicuticular wax accumulation on leaves may be controlling the leaf smoothness of the surface and could be responsible for leaf blade erectness (Li *et al.,* 2013). A single gene may not be solely responsible for the glossy phenotype as other genomic regions influence the up- and/or down-regulation of wax synthesis, and at least four glossiness-associated QTLs have been detected in sorghum. Key transcription factors responsible for glossy phenotypes were consistently reported in the mapped QTL region between Xisp10263, Xgap001and Xnhsbm1044. This target glossy QTL (QGls10) was detected in both screening environments and also reported in previous studies (Sajjanar, 2002; Folkertsma *et al.,* 2003; Deshpande, 2005; Mehtre, 2006; Satish *et al.,* 2009, 2012; Jyothi, 2010; Apotikar *et al.,* 2011, and Aruna *et al.,* 2011). Genomic recombination events were searched by traditional fine mapping, and *Xgap001* showed clear association with glossiness, and

glossy15 (Sb10g025053) gene is just 237 kb away from *Xgap001* within the mapped QTL region. Thus, glossy15 (Sb10g025053) could be a likely candidate gene for 'QGls10' as it is known to control transcriptional regulation of glossy phenotype expression in maize (Moose and Sisco, 1994). This suggests that 'QGls10' needs to be studied further in the fine mapping approach with higher density markers in this, and other possible candidate genes in the target interval as well as further narrowing down the QTL. In the search for recombination events in the support interval, the 'QTd10' QTL have been found to be highly associated with *Xtxp141* and *Xisep0630*. Precise microscopic field observations of trichome density may resolve the location of its controlling genomic regions. But, these were not practical due to large number individuals observed in the full F_2 population. Presence of 'QTd10' within the same support interval (*Xisep0630-Xtxp141*) showed the consistency of the QTLs in sorghum molecular mapping of component traits for shoot fly resistance.

5.2.1.1. F_2 and $F_{2:3}$ QTL mapping on selected 369 individuals

It is concluded that one QTL for glossiness score (with the glossy allele originating from donor parent IS18551) is present in the SBI-10L target region. QTLs for trichome density were mapped differently in the *rabi* and *kharif* seasons, but within support intervals sharing a common marker, Xtxp141. To clearly differentiate these F_2 and $F_{2:3}$ 'QTd10' QTLs, increased marker density and more efficient phenotyping is required. Fine mapping of these QTLs will improve our understanding of the molecular basis of seedling leaf blade glossiness and trichome density traits (important morphological component traits contributing to sorghum shoot fly resistance). In F_2 subset, the rate of recombination has increased due to selected recombinants with heterozygous nature, which will increase the recombination fraction and this could affect the QTL detection power and may increase the rate of false discovery rate (FDR) of QTLs. Sometimes, missing marker data and segregation distortion in early generation population like F_2 may lead to disturbance in estimation of QTL position and its effects. Since F_2 selected informative recombinants are highly

distorted from the normal Mendelian segregation, increased heterozygosity may increase the dominance effect of the detected QTL, which may be due to over dominance effect or the pseudo over dominance effect of the QTL. Segregating populations (F_2 and $F_{2:3}$) have heterozygous variant regions which complicate the gene action during linkage repulsion phase of two dominant alleles. This may result in over dominance or pseudo over dominance. When both the loci are dominant, it may result in over dominance as in the case of trichome density. The statistical analysis methods, experimental designs and the phenotyping technique variations could also affect the dominance and over dominance effects of the detected QTL (Schnable and Springer, 2013). QTLs from resistant parent express dominance or over dominance; but if they segregate in the next generation they may not be detected due to less trait variation or other genomic regions might have more influence in phenotype expression. This could also be due to environmental effect on trichome density levels which may lead to less phenotypic variation and cannot separate the genomic regions responsible for the phenotypic variation in the target QTL region (SBI-10L).

5.2.1.2. $F_{2:4}$ QTL mapping on selected 152 genotypes

A total of 39 QTLs were detected for six component traits of shoot fly resistance - seedling leaf blade glossiness (Gls-4), trichome density lower (Tdl-8) and trichome density upper (Tdu-7). Out of 39 QTLs, 20 are major QTLs and remaining 19 are minor QTLs. When the fine map QTLs were compared with earlier results, QGls10 was consistently mapped from F_2, F_3 generations and now F_4 mapping resulted into a single gene glossy15 which was predicted earlier (Satish *et al.,* 2009, 2012; Aruna *et al.,* 2011; Kiranmayee *et al.,* 2016). In case of trichome density lower, it is clearly evident that O-methyl transferase is mapped with high phenotypic variance during *kharif* with 48% phenotypic variance and 23 LOD. This could be the appropriate candidate gene that exhibited consistency across seasons but the same QTL was observed with 8% phenotypic variance during *rabi*. Percent shoot fly dead heart *rabi* QTL was also mapped at same position where trichome density lower was mapped with high

LOD and 12% phenotypic variance. This implies that trichome density lower has relation with shoot fly dead heart percentage and indicated that % SFDH and trichome density lower have interrelations. The level of shoot fly infestation and the level of trichome density lower affected needs to be studied alongside *O-methyl transferase* encoding gene and *cyclin dependant kinase CDKB2;1gene*. Thsese genes must be cloned and expressed in the plants to find out its exact function.

5.2.1.3. Comparison of identified shoot fly component trait QTLs with previous SFR QTL

Our fine genetic mapping revealed *glossy15* (Sb10g025053) could be the probable candidate gene as revealed by QTL analysis during the two seasons and across season data mapped at SNP S10_54269620. When comparing the identified QTL regions with previous studies, the mapped QTLs were overlying with glossiness and trichome density QTLs detected by Sajjanar (2002), Deshpande (2005), Mehtre (2006), Satish *et al*., (2009, 2012) and Aruna *et al*., (2011).

5.2.2. Stay-green (post flowering drought tolerance)

In the present study, we aimed to develop a high resolution genetic map with newly developed SNP integrated with SSR markers for increasing the marker density of SBI-10L. This would be further helpful for dissecting the stay-green QTLs under terminal drought stress conditions and for estimating genomic regions that control the onset of senescence. Usually senescence is a regular developmental process in plant life cycle. But in few genotypes, senescence is delayed by the effect of environmental factors or mutations or alteration in the genomic regions. These changes in turn may alter the availability of specific nutrients (C, N). Stay-green was well studied (Borrell *et al*., 2014a, b) and various stay-green QTLs have been mapped in different genetic backgrounds (Borrell *et al.,* 2000; Xu *et al.,* 2000, Subudhi *et al.,* 2000; Kebede *et al.,* 2000; Crasta *et al.,* 2000; Haussmann *et al.,* 2002b, Harris *et al.,* 2007; Kassahun *et al.,* 2010; Mace and Jordon, 2011; Mace *et al.,* 2012; Naga raja Reddy *et al.,* 2014).

Interestingly, all the previous studies on stay-green QTLs were co-localized with one or the other studies irrespective of genetic background and the stay-green donors which show the conserved stay-green regions in sorghum. Our present study focuses on the mapped (Haussmann *et al.,* 2002b), introgressed (Kassahun, 2006) reliable stay-green QTLs on sorghum chromosome SBI-10L.We fine mapped the % GL 7, 14, 21, 28, 35, 42 and 49 QTLs, of which few were identified by Haussmann *et al.,* (2002b) earlier. Co-localization of QTLs may be due to tight linkage of the genes or pleiotropic effect of the locus that results in clustering of QTLs over generations. Stay-green QTLs were consistent over environments and in different genetic backgrounds also.

5.2.2.1. Stay-green QTL co-localization/comparison with previously identified stay-green QTLs

The studies of Crasta *et al.,* (1999), Haussmann *et al.,* (2002b) and the present study identified different QTLs that have been found to be co-localized (Fig .40). Few completely new QTLs were also identified (Table 17). E36-1 was the source of drought tolerance as has been revealed by the studies of Haussmann *et al.,* (2002b) and the present studies also. Haussmann *et al*., (2002b) mapped the Stg QTLs between *Xgap001* and *Xtxp141* SSR markers. So, this has been utilized in the present study, along with few other SSR markers developed by others (Ramu *et al*., 2010). The QTLs identified in our present study can be roughly compared with QTLs discovered by Haussmann *et al.,* (2002b). All the 33 identified QTLs along with 6-7 QTL clusters were located within the QTLs mapped by Haussmann *et al.,* (2002b) which are overlying with the STG QTLs developed by Crasta *et al.,* (1999). But, we could not conclude if the QTL clusters identified in the present study are the same as have been identified earlier. To date, there were no reports of fine mapping studies related to stay-green in sorghum but attempts were made by Borrell *et al.,* (2009) and Harris *et al.,* (2007) to study another popular stay-green source B35-1 but on chromosomes other than SBI-10. Therefore, this is the first stay-green fine mapping report in sorghum which generated probable candidate genes related to delay in the onset of senescence.

With the genes identified in the present study, one can perform positional cloning experiments to validate the major gene effects in stay-green phenotype. Our fine mapping analysis shows that gene as well as QTL cluster was influencing the stay-green trait but not a solitary gene. However, it is very difficult to conclude that which genomic region is responsible for which pathway. Knock out mutation experiments may reveal the functional role(s) of these genes during drought stress tolerance. BSA with a larger population may reveal about the genes responsible for delayed senescence, or as distinct from stay-green phenotypes. Identification of genes from this region that are differentially expressed between parents under drought stress conditions is vital for future studies. But, there was no clear evidence that a single gene is responsible for the delay in senescence in these lines. Identification of the net-work of genes that act simultaneously and modulate the down-stream genes is necessary to develop stay-green phenotypes and drought tolerant lines in sorghum.

5.2.2.2. Advantages of stay-green fine mapping using progeny testing or haplotype analysis

With the help of haplotype analysis/progeny test, stay-green genomic regions have been narrowed down into genes. As the identified stay-green QTLs were co-localized, QTL clusters for stay-green were identified and further fine mapped to important regions where it ended up with single genes for each stay-green QTL clusters. This helped us to identify candidate genes underlying the stay-green traits.

5.3. Candidate genes in sorghum SBI-10L showing both QTL and GWAS evidences for agronomic and yield traits:

5.3.1. Flowering time

QTL mapping studies for flowering time identified 2 major QTLs with 12% PVE for each QTL. Earlier studies identified ma1, ma2, ma3, ma4 and ma5 QTLs on SBI-06, 07, 01, 10, 2 (Quinby and Karper, 1945; Quinby, 1966, 1967; Rooney

and Aydin, 1999; Crasta *et al.,* 1999; Mace and Jordan2011) and our results revealed nine QTLs for days to 50% flowering on SBI-10. These data may be similar to ma4 and another major QTL similar to QDTFL5_10 (Crasta *et al.,* 1999; Mace and Jordon, 2011) and Qdf_dsr.2 (Naga raja Reddy *et al.,* 2013). Nearly 24 genes were identified in the mapped flowering time QTLs on SBI-10L. Out of these, pentatricopeptide (PPR) repeat-containing protein (Sb10g023920, cytochrome P_450 (photosynthesis) (Sb10g022450), type III chlorophyll a/b-binding protein (Sb10g023930), ankyrin repeat-containing protein (Sb10g025310), putative leucine zipper (Sb10g024190), putative uncharacterized protein (Sb10g023430, Sb10g025010, Sb10g024920, Sb10g025010), *glossy15*/AP2 (Sb10g025053), NBS-LRR (Sb10g025283) have been identified as the major contributors of flowering time in the present fine genetic mapping study. GWAS studies identified xyloglucan endotransglycosylase (Sb10g028550) and zinc finger POZ domain unique genes. Ankyrin repeat protein (Sb10g025310)and NBS-LRR (Sb10g025283) were located in both the analysis, but further studies are necessary to carry out the identified genes and QTLs on SBI-10L.

5.3.2. Plant height

In the present study, plant height QTL mapping identified 7 QTLs with combined phenotypic variance of 21%.Previous studies identified Dw1 on SBI-09, Dw2 on SBI-06 and Dw3 on SBI-07 (Quinby, 1974; Mace and Jordan, 2011; Zou *et al.,* 2012; Thurber *et al*., 2013; Higgins *et al.,* 2014). Squamosa promoter-binding-like protein 12 (Sb10g029190), O-methyl transferase ZRP4 (Sb10g027340, Sb10g027640), development and cell death domain = N-rich protein, putative, expressed (Sb10g024460), AdoMet_Mtases (involves in the direct methylation of oleic acid esterified as a component of phospholipids/directly interacts with DNA, RNA also) (Sb10g029820), hypothetical protein, K03355 anaphase-promoting complex subunit 8 (Sb10g025960), MADS box transcription factor (Sb10g029810), catalytic domain of protein kinases (Sb10g029180) are few important candidate genes

identified by QTL mapping and GWAS analysis. SPB protein has a role in dwarfing (Preston and Hileman, 2013) and may be one of the most probable candidate genes on SBI-10L.

5.3.3. Grain dry weight/plot and panicle dry weight per plot

For both the traits, two QTLs were commonly located at position 105.61 (*Xtxp141*) and 117.31 (S10_59418734). GDW/Plot marker trait associations identified using GWAS and QTL analysis are similar to PnDW/Plot are Exo70 exocyst complex subunit (Sb10g030660, Sb10g030710), transcription termination factor Rho; provisional (Sb10g029670), leucine rich repeat domain like (Sb10g030730), putative polycarboxypeptidase isoform 1 (Sb10g025540), putative uncharacterized protein (Sb10g028550, Sb10g030080, Sb10g029010), meiotic serine proteinase (Sb10g028870) and ADP binding protein (Sb10g021860). Few unique MTAs observed are putative uncharacterized proteins like P0548E04.1.9 (Sb10g030610) for PnDW/plot and NBS-LRR disease resistance protein (Sb10g025283), calcium-dependent protein kinase CPK1, adapter protein 2 (Sb10g030150) and putative uncharacterized proteins (Sb10g022900, Sb10g028550, Sb10g030080, Sb10g029010) for GDW/Plot.

5.3.4. Hundred grain mass(HGM)

Candidate genes located in HGM QTL interval are MATE efflux (SB10g029392), MADS box transcription factors near to S10_59525199 SNP and calcium/calmodulin-dependant kinase (SB10g030040) and few uncharacterized proteins, of which squamosa promoter–binding proteins are reported to be involved in 100 seed weight QTL *GW8* in rice (Wang S *et al.*, 2012).

5.3.5. Grain number per plot (GNP/Plot) and grain number per panicle (GNPP)

As grain number per panicle is derived from grain number per plot, they are mapped at the same locations. MTAs identified putative prolylcarboxypeptidase

isoform 1 (Sb10g025540), serine/threonine protein kinase (Sb10g022730), basic leucine zipper (Sb1024190), zinc binding family protein (Sb10g024575), polyphenol oxidase (Sb10g024220) and *Arabidopsis* response regulator 10-Myb like DNA binding domain. Candidate genes identified in QTL interval are mostly putative uncharacterized proteins (Sb10g028790, Sb10g029670, Sb10g030610, Sb10g30580), mitogen activated protein kinase (Sb10g028780) and cell division protease ftsH homolog (Sb10g030720).

5.3.6. Panicle harvest index (PHI)

Candidate genes underlying QTL interval are Exo70 exocyst complex subunit, transcription termination factor Rho (Sb10g029670) and RF4; DNA polymerase sigma (Sb10g026810). Five MTAs found in GWAS analysis revealed the presence of basic leucine zipper transcription factor (Sb10g024190), zinc finger POZ domain (Sb10g026760), F-box domain (Sb10g027760), TLP-PA; allergenic/antifungal thumatin like protein (Sb10g030230) and prolylcarboxy peptidase isoform1 (Sb10g025540). PHI is affected by FT, plant height, plant biomass and grain yield. Increasing PHI can be an alternative to increased seed yield in sorghum as pointed out by Luo *et al.,* (2015).

5.4. QTL co-localizations

Significant correlations and associations have been observed between trait results in co-localisation of QTLs. Where common genomic regions influence different traits indicates pleiotropism and linkage of the traits. It is difficult to distinguish the pleiotrophy with linkage. As in the present study, stay-green (cQstg10.4), flowering time QTLs (QFT.3_14) were mapped at 44.1 cM which encode for ankyrin repeat protein. Such a protein has been reported in flowering mechanism as well as in drought tolerance (Xu *et al.,* 2007). FT 2_14 was mapped at 36.91 and stay-green QTL cluster (cQstg10.1) at 36.41 where the interval of the mapped QTLs regions are near to AP2 transcription factor. AP2 type of transcription factors are also involved in flowering mechanism (Jofuku *et al.,* 1994) and drought tolerance (Licausi *et al.,* 2013). As expected, % SFDH QTL and trichome density lower QTLs of both *rabi* and *kharif* 2013 seasons were

falling at S10_57432493 (99.61 cM) which are near to *O-methyl transferase* protein and *cyclin dependant kinase CDKB2;1* (Sb10g027670). Both these genes may have a role to play in trichome density and percent shoot fly dead heart according to the QTL data, fine mapping and GWAS analysis. GNP/Plot and GNPP QTL were mapped near to starch branching enzyme indicating their role in grain quantity increase. More grain number in general indicates more flowering. Interestingly, at 82.71 cM (S10_56433597), percent shoot fly dead heart QTL (Q%SFDH1_R13) and stay-green QTL (QGL14b_13) were co-mapped which encode a tubulin beta-2/beta-3 chain protein. At this region, a grain dry weight QTL (QGDW/Pot.1_14) was observed which is very near to the cluster at 82.18 cM (S10_56205739) that encodes zinc finger POZ domain protein. Many of the identified genomic regions appear to affect multiple traits. Single gene or multiple gene clusters present in the mapped regions could be responsible which should be validated by cloning and overexpressing the probable candidate genes. As expected, the co-localized QTLs for the traits in the present study are supported by significant correlation among co-localized traits.

5.5. GWAS results support the fine mapping results

Association mapping can also be a powerful tool for fine mapping of quantitative traits (Flint-garcai *et al.,* 2003). Our data on trichome density indicate that this population is highly variant for the trait. A total of 10 SNPs of the MTAs identified were over-lapping with QTL map and fine mapped candidate genes. This shows the importance of GWAS studies.

5.5.1. Major component traits of shoot fly resistance and their candidate genes

5.5.1.1. Glossiness

Leaf glossiness trait has multiple functions in biotic (shoot fly resistance) and abiotic stress tolerance (drought, salinity, high temperature). Cuticular waxes on leaf could be the reason for the glossy phenotype. Water sprinkling method on leaves differentiates non-glossy leaves with glossy leaves by adherence and non-

adherence of water droplets respectively (Tarumoto *et al.*, 1980). Scanning electronic microscopic observations show increased number of wax crystals on leaf surface of non-glossy leaves when compared to glossy leaves (Tarumoto *et al.*, 1981). The mapped QTL region was searched for candidate genes and several wax synthesis-related genes were found. One of the candidate genes related to wax synthesis and deposition of wax present in the QTL region has a C2 calcium lipid-binding domain (Sb10g025040), which is involved in plant stress signal transduction, and this C2 domain was able to bind membrane lipid ceramides (De Silva *et al.*, 2011). These wax-deficient mutant loci in maize, *Brassica* and sorghum are defined as '*glossy*' loci and in *Arabidopsis* and barley, they are named as ceriferum (*cer*) mutant loci (Kunst and Samuels, 2003, 2009). One of the candidate genes, *glossy15* (Sb10g025053), encodes an APEPETAL2 (AP2)-like transcription factor involved in the transition from juvenile leaf epidermis to adult leaf epidermis characteristics, and is expressed after second leaf growth stage (Moose and Sisco, 1994, 1996). AP2/ERF transcriptional factors have been reported to be involved in wax biosynthesis (Tiwari *et al*., 2012). Recently, Go *et al*., (2014) reported that AP2/ERF (Sb10g025053) acts as a bi-functional transcriptional factor which down regulates the wax biosynthetic pathway by interacting with promoter regions of wax synthesis proteins. A MYB transcription factor (Sb10g024950) present in the mapped glossy QTL region has been reported to be involved in activation of AP2/ERF transcription factors associated in the wax biosynthesis (Cominelli *et al.*, 2008).

5.5.1.2. Trichome density andcandidate genes

Trichomes are non-glandular, cellular appendages that protrude above the epidermis (Maiti and Gibson, 1983). Trichomes act as physical barriers between the insect pests and the leaf blade epidermis that inhibit egg laying and/or larval movement, which leads to reduction in 'dead heart' formation. Trichome density is genetically controlled and negatively correlated with oviposition, and dead heart incidence (Maiti and Gibson, 1983; Dhillon *et al.*, 2005). A MYB transcription factor gene homolog (Sb10g027280) was observed in the trichome

density QTL region. Liang *et al.,* (2014) showed that in *Arabidopsis*, a WD40, HLH, and MYB transcriptional factors regulate the trichome initiation process programmed by cell development. These transcriptional factors recognize the specific DNA motifs in gene regulatory regions to activate or repress transcription, mostly by interacting with other proteins like Armadillo repeats, Specicle Poz-like proteins, F-box domain proteins, WRKY proteins, MYB transcription factors, ethylene zinc finger proteins, EF-hand Ca^{2+}-binding proteins, and thumatin-like proteins. In *Arabidopsisthaliana*, TRANSPARENT TESTA GLABRA2 (TTG2) encodes a WRKY transcription factor and is expressed in young leaves, trichomes, seed coats, and root cells which are not involved in root hair production. During epidermal cell differentiation, MYB transcription factors and HLH transcription factors regulate TTG2, which modulates Glabra2 expression in trichomes (Eulgem *et al.,* 2000; Johnson *et al.,* 2002; Ishida *et al.,* 2007). One additional WRKY transcription factor gene homolog (Sb10g025600) is present in the target trichome density QTL region; and this may be one of the probable candidate genes. An ethylene zinc finger protein gene homologous with Sb10g027550 has also been observed to play a key role in regulating trichome development in *Arabidopsis* (Zhou *et al.,* 2013). It appears that ZFP5 and ZFP6, the zinc finger proteins, necessary for gibberellic acid and cytokinin signalling regulate trichome cell differentiation (Zhou *et al.,* 2013). Cyclin dependant kinase CDK (Sb10g027670) has also been reported to be involved in trichome density (Pattanaik *et al*., 2014). CDKB2;1 is a B type cyclin dependant kinase and is unique to plants and are involved in G2-to-M phase mitotic events which may control the development of tissues such as trichomes (Kono *et al*., 2003). In the process of trichome development, cells undergo endoreduplication where CDK was not expressed properly resulted in plants with few trichome or less trichomes (Schnittger *et al*., 2003). An Armadillo repeat protein gene that appears to be homologous to Sb10g027680 regulates both the gene expressions and cell-cell adhesion. Patra *et al.,* (2013) demonstrated that ubiquitin protein ligase3 (upl3) N-terminal domain has Armidallo repeats that interact with the C-terminal domain of Glabra3/Enhanced

Glabra3 for trichome development in *Arabidopsis*. An F-box domain protein homologous to Sb10g027730 has Armadillo repeats that may act as transcriptional factor and involved in plant developmental processes (Coates, 2008). An EF-hand Ca^{2+}-binding protein gene homolog (Sb10g027680) is one of the candidate genes underlying the putative QTd10 trichome density QTL. Kinesin-like calmodulin is EF-hand Ca^{2+}-binding protein that interacts with a microtubule motor protein and regulates trichome morphogenesis. Over expression of KIC inactivates kinesin-like calmodulin binding protein (KCBP) by disrupting its interaction with microtubules and its participation in trichome morphological complex resulting in trichomes with less branches/no branches (Reddy *et al.,* 2004). Jakoby *et al.,* (2008) mentioned Speckle type POZ proteins (homologous to Sb10g026780) which were also expressed in trichomes (Table 29).

In case of glossiness QTL, higher score value indicates the non-glossiness, and lower the score, more preferred the trait (glossy). Glossiness is inherited from resistant parent where the moderately large F_2 population had more dominance effect due to higher population size and better scores which could influence the dominance nature of the detected QTL. In both the generations, glossiness QTL was detected within the same support interval. This confirms that single glossiness QTL is located in the target marker interval. Further fine mapping and focused gene expression studies can be carried out utilizing this high resolution cross. This should reveal which of the underlying candidate gene/s is responsible for the observed variation and its functional role. In contrast, the putative QTL for trichome density on the lower surface of seedling leaf blades, thought to have been introgressed from grandparent IS18551 into BTx623-background line J2614 as reported by Jyothi *et al.,* (2010), was detected in the full F_2 population and the subset of 369 informative recombinants selected from this under lower-temperature, short-day length *rabi* conditions. But it was not clearly detected in the F_3 progenies when the same were evaluated in the *kharif*. This warrants considerable further study, starting with phenotyping of the same F_3 progenies for lower leaf blade trichome density during the *rabi* season using available

remnant seed. Expression of this QTL only under *rabi* verses *kharif* conditions would warrant further studies to understand the environmental regulation of this QTL for the trait. Based on F_2 genotyping data of 7 co-dominant SSR markers and $F_{2:3}$ phenotyping data, a further reduced subset of 182 most informative recombinants were selected, and corresponding F_3 progenies were selfed to produce F_4 seed which will go for field trials and can be used for further studies to restrict the genomic region that appears to contribute to the control of sorghum seedling leaf blade glossiness and lower surface trichome density.

5.5.2. Genes related to delay in senescence and the identified stay-green QTL regions

Leaf senescence is a complex trait which involves 1000 up- and down-regulated genes termed as senescent associated genes (SAGs) and senescence associated proteins (SAP) based on the environmental factors, physiological, chemical, developmental stages (Guo *et al.,* 2004; Gepstein *et al.,* 2004), degenerative process, developmentally regulated programmed cell death (PCD) mechanisms. Senescing leaf products such as nitrogen, phosphorus, metal ions, minerals and nutrients are translocated from leaves to the grain filling sites (Lim *et al.,* 2003, 2007; Buchanan *et al.,* 2003). Studies on *Arabidopsis* have generated information on different mechanisms involved in autophagy, chlorophyll catabolism and nutrient remobilization during senescence (Sakuraba *et al.,* 2014, Luo *et al.,* 2013).

5.6. Transcriptional factors, hormones and signal transduction proteins associated with senescence mechanism and present in the identified QTL regions

In the present study, identified stay-green QTLs when searched for candidate genes, 2 NAM (Sb10g027100, Sb10g030770) transcriptional factors were located at 21.8 kb near to the identified Q10GL28a_13, Q10GL28a_across, Q10GL21c_13 QTLs detected during drought stress conditions. It has been reported earlier that increased expression of NAC transcription factor leads to drought stress tolerance and acts as a negative regulator of drought and increases

grain nitrogen concentration in wheat (Distelfeld *et al.,* 2014; Zhao D *et al.,* 2015). Senescence-associated NAC transcription factors have been described in *Arabidopsis thaliana* (Lee *et al.*, 2012; Guo and Gan, 2006), *Oryza sativa* (Liang *et al.,* 2014), *Bambusa emeiensis* (Chen *et al.,* 2011), barley (Christiansen and Gregersen, 2014; Distelfield *et al.,* 2014) and maize (Mao *et al.,* 2015). Another SNP S10_54532995, identified to encode NBS-LRR (Sb10g025283) reported to be a defense response protein (known for biotic stress) might be influenced during stress and this leads to increased levels of SA, ABA and cytokinin. NBS-LRR mutant leads to early senescence; dwarfism and flower sterility. Therefore, it appears that it might play a role in senescence (Sarazin *et al.,* 2015). Sb10g025053 (S10_545269620) gene encodes for AP2 transcription factor and is involved in the regulatory pathways of many plant biological mechanisms. Plants adapt to water deficient stress by initiating a series of molecular, biochemical and physiological changes. Over expression of AP2/ERF family genes have been shown to improve stress tolerance under water defect conditions (Guo and Gan, 2012; Licausi *et al.,* 2013; Mawlong *et al.,* 2015).

Nearly four SNPs S10_54966383, S10_55002960, S10_55315031, S10_57432493 have been detected that encode WRKY type of transcription factors (Sb10g025600, Sb10g025990, and Sb10g027640). Their over expression lead to drought tolerance in rice (Cai *et al.,* 2015) and maize (Thirunavukarasu *et al.,* 2014). MADS box transcription factors (Sb10g029810) are influenced under drought stress conditions and their alterations may lead to stay-green which is involved in many developmental processes (Shore and Sharrocks, 1995). Exocyst subunit (Sb10g030620) has been found to be involved in auto phagosome/autolysis associated in plant cell death process or programmed cell death. As senescence is a natural mechanism of cell death process, autolysis enzymes and proteins are involved in senescence (Lim *et al.,* 2003, 2007). Sb10g031030 gene encodes for AGO1 argounates which is a part of gene silencing complex. AGO1 transcriptional activity increases under ABA and drought treatments suggesting that a transcriptional activation of MIR168a is

required for stabilising AGO1 during stress response (Li *et al.,* 2012; Bologna and Voinnet, 2014; Zhao Y *et al.,* 2015). Ankyrin repeat protein (Sb10g025310) is reported to be involved during flower senescence and a detailed study may reveal its role in leaf senescence (Xu *et al.,* 2007). Transducin family protein/WD40 is also reported to be involved in senescence mechanism (Guo and Gan, 2012). Pentatricopeptide repeat protein (PPR) (Sb10g026180 SNP S10_55532236, Sb10g029530- SNP S10_59316155) is involved in chlorophyll degradation mechanism by stabilizing RNA in chloroplasts. PPR could be involved in chlorophyll catabolism during senescence and alteration in PPR may lead to stay-green phenotype (Yagi *et al.,* 2013; Manna *et al.,* 2015). Squamosa binding protein family transcriptional factors (SBP) (Sb10g026200, SNP S10_55599913) have been reported to be involved in leaf, flower, fruit and vegetative phase developmental stages (Preston and Hileman, 2013). Zinc finger (Sb10g028300- 58163876) proteins are also reported to be involved in senescence and few MYB transcription factors are known to modulate the process of senescence (Guo and Gan, 2012; Griffith *et al.,* 2014). CGA1 (CYTOKININ-RESPONSIVE GATA FACTOR 1; Sb10g022580) protein is known to be involved in chloroplast metabolism (Guo and Gan, 2012; Hudson *et al.,* 2013). GA_3 (Sb10g022520) and ethylene (C2H2 ZN finger) are the major phytohormones that enhance the transcriptional factors to trigger senescence (Guo and Gan, 2012).

5.7. Shoot fly resistance and stay-green studies in improving productivity under stress

As the shoot fly attacks the plants during early stages and damages the crop, molecular breeders are interested in developing shoot fly resistant cultivars utilizing molecular marker assisted breeding strategies. Improving yield under water limiting condition is another major challenge present in front of plant breeders. Molecular breeders are heading towards their goal by developing drought tolerant cultivars/stay-green cultivars utilizing molecular marker assisted breeding strategies. Genetic and genomic studies of crop plants helped us to

identify important genes involved in stay-green mechanism and the F_4 plants with more stay-green activity with high agronomic performance along with shoot fly resistance were further selfed to homozygosity and can be released as a drought tolerant high yielding variety.

5.7.1. Selection of superior F_4 genotypes based on genotyping and phenotyping data

Based on phenotypic BULP values of the F_4 selective genotyping population, nearly 20 genotypes were selected and based on genotypic data within the phenotypic selection, up to 7 plants were preferred as breeding materials for "3-gene cassette" unless selfed until homozygosity. These lines can be used as a donor in marker assisted breeding programs of multiple resistant varieties or could be released as varieties after multi-location trials.

5.7.2. Conventional breeding approach

Double selected recombinants have the favourable alleles at all three loci (for a high level of glossiness, good green leaf area retention, and high trichome density), and hence recombinants could be used as donors of the "cassette" of these three genes in applied marker-assisted breeding programs targeting the *rabi* sorghum production where both shoot fly resistance and terminal drought tolerance are essential traits. In the course of producing such a recombinant from the cross of the BTx623-background, shoot fly resistance QTL (J2614-11) and the R16-background stay-green QTL introgression lines (RSG04008-6), these regions were fine mapped and the underlying genes for all three of these components of the cassette were found out. Based on increased marker density and reduced genomic regions of shoot fly component traits, stay-green, yield and agronomic traits, probable candidate genes underlying the QTLs and their involvement for the trait have been found out. Marker assisted selection strategy can be applied for reduced errors in selection as MAS is time-efficient, non-destructive, cost-insensitive and reduces linkage drag of the target genomic region with unfavourable alleles from their common donor parent. Multi-location field evaluations of the identified QTLs across environments and generations are

vital to find out the QTL stability. QTL mapping and their fine mapping as well as transfer of resistant genomic regions and pyramiding them is critical for developing a stress tolerant line alongside utilizing the next generation sequencing technologies and GBS. GBS has been successfully used in GWAS, genomic diversity study, genetic linkage analysis, molecular marker discovery, genomic selection under large scale plant breeding programs (He *et al.,* 2014).

In this study, the stay-green genomic regions have been delimited from 15 Mb to 8 genes, which are co-localized with a few genome-wide association study (GWAS) marker-trait associations (MTAs). As we have performed GWAS for the weekly % GL scores, 32 candidate genes for stay-green traits have been identified, of which WD40 repeat family protein (Sb10g025320), AGO1 (Sb10g031030), GA_3 (Sb10g022520) and NAC transcription factor (Sb10g030770) are crucial candidate genes for creating delayed senescent phenotypes. QTL mapping in F_2, $F_{2:3}$, F_4 replicated data clearly show the glossy is highly associated with glossy15 (Sb10g025053) which has been reported in maize for glossiness. The present population displayed high variation for trichome density lower and upper and the percent shoot fly dead heart QTLs that overlapped with the trichome density lower indicate a close correlation of trichome density trait in shoot fly resistance. Many genes could be responsible for the stay-green trait as well as glossy and trichome density characters. It is interesting to note that the large effect stay-green QTL previously mapped to SBI-10L appears to be very complex than the mapped seedling leaf blade glossiness and trichome density upper and lower. Not all of its components from donor parent E36-1 may necessarily be economically desirable, so in the long term, detailed dissection and reconstruction studies may be required to form a superior cassette of favourable alleles across this chromosome arm that can be easily manipulated in sorghum breeding program targeting enhanced stability of the characters. But, it is necessary to validate the genes located in the QTL regions. It is concluded from the study, that marker assisted back cross programs can be widely used with the help of advanced next generation sequencing

technologies combined for breeding resistant lines. A crop plant with resistance to two different stresses can be overcome and a multiple resistant variety with high agronomic performance can result super 3-gene cassette crop sorghum. We have preferred HPR where the shoot fly infestation rate can be reduced with increased trichome density on both abaxial and adaxial leaf surfaces which prevent shoot fly from laying eggs and crawling of larvae on the leaf surface. In future, further isolation and characterization of *glossy15* (Sb10g025053) on SBI-10 needs to be studied in detail.

Conclusions

Our study reveals that advances in genomics, molecular breeding and NGS can help to dissect the stay-green character and also shoot fly resistance in sorghum. Genes responsible for stay-green, leaf blade glossiness and trichome density need to be cloned and their introgression and expression level studies should be made available in sorghum in order to enhance the genetic architecture. The parents are introgressed lines, they have different genetic backgrounds, but the background noise for the interested traits has not been reduced substantially. None-the-less, genotypes identified have a combination of RSG04008-6 stay-green (drought tolerance) trait with glossiness and trichome density. Homozygous lines with these characters can lead to the development of a variety in sorghum with multiple resistances.

SUMMARY

Sorghum [*Sorghum bicolor* (L.) Moench, 2n = 2x = 20] is the fifth most important cereal crop globally, and is grown primarily in arid and semi-arid conditions. Major biotic and abiotic constraints hampering sorghum production include shoot fly infestation during early stages of crop development (seedling establishment and early growth stages; but only present in the eastern hemisphere) and terminal drought stress during post-flowering growth stages. These two stresses can devastate the crop. Hence developing sorghum varieties with resistance for these two stresses is critical. In order to understand the genetic basis of sensitivity to these two stresses and to genetically dissect host plant resistance to shoot fly and tolerance to terminal drought stress, the following objectives were proposed for the present study:

- to develop an introgression-line cross-based fine-mapping population for morphological components of shoot fly resistance and for the stay-green mechanism of terminal drought tolerance previously mapped to sorghum chromosome SBI-10L;
- to fine-map the target traits by combining genotyping and phenotyping datasets of a selected recombinant sub-set of the fine-mapping population;
- to annotate functionally and characterize candidate genes identified in the target region; and
- to identify recombinant progenies with pyramided traits of interest.

Genotyping-by-sequencing (GBS) and identification of single-nucleotide polymorphism markers (SNPs)

At ICRISAT-Patancheru, Hyderabad, favourable alleles for shoot fly resistance (SFR) and stay-green (STG) quantitative trait loci (QTLs) have been introgressed into more elite backgrounds by pedigree selection and/or marker-assisted backcrossing (MABC) programs. QTL mapping of these traits suggests that the introgressed STG QTL from E36-1 (available in introgression line RSG04008-6) overlaps with SFR QTLs detected in IS18551 (available in introgression line

J2614-11) on the long arm of sorghum chromosome 10 (SBI-10L). This genomic region is flanked by simple sequence repeat markers (SSRs) *Xisep0621* and *Xisep1011,* which is a 72 cM interval (45-60 Mb physical distance). An F_2 fine-mapping population with 1,894 plants was generated by crossing introgression-line RSG04008-6, which is moderately stay-green but shoot fly susceptible, with introgression-line J2614-11, which is moderately senescent, but shows reasonable SFR, followed by selfing of a single true F_1 hybrid from this cross. A total of 361 F_2 recombinants in the target region harbouring these QTLs were identified by screening the full F_2 population with 7 polymorphic SSRs mapping to the target interval on SBI-10L. From these 361 recombinants, DNA samples from 182 were sent for skim-sequencing. Using the skim-sequencing data for GBS genotyping with the TASSEL pipeline resulted in identification of 32,836 SNPs. A total of 1,515 SNPs were found in the SBI-10L target region. Subsequent curation of the SNP data identified a total of 260 high quality SNPs, which were integrated with the base map of 7 SSRs distributed across the target region. The high-resolution genetic map constructed with five SSRs and these 260 SNPs, spanned a length of 136 cM. Out of 182 F_2 recombinants used for GBS, 152 progenies with sufficient seed were advanced to the F_3 and F_4 generations, which were phenotyped for the target traits. Phenotyping was carried out during the post-rainy seasons (*rabi*) of 2013 and 2014 for STG and agronomic trails, and during the rainy season (*kharif*) of 2013 and *rabi* season of 2013 for morphological traits contributing to SFR. Best linear unbiased predictors (BLUPs) were calculated, within and across seasons, from these observations and then used for further trait mapping analyses.

Stay-green (STG) trait and QTLs

Observations for the STG trait were scored as percent green leaf area (%GLA) at weekly intervals (i.e., %GLA07, %GLA14, %GLA21, %GLA28,%GLA35, %GLA42, and %GLA49). These STG observations and those for other agronomic and yield-related traits like flowering time, plant height, panicle dry weight per plot, graindry weight per plot, grain number per plot, grain number

per panicle, hundred-grain mass and panicle harvest index, were collected from both the 2013 and the 2014 post-rainy season trials. F_4 progeny frequency distributions showed transgressive segregation for most of the observed traits, indicating their polygenic inheritance. Statistical analysis of these data demonstrated high heritabilities for the weekly stay-green scores. Stay-green scores from the two years trials were shown to be positively correlated ($r \geq 0.90^*$) with each other, and negatively correlated with flowering time ($r = -0.78^*$) and plant height ($r = -0.21^*$), across seasons. Panicle dry weight per plot, grain dry weight per plot and grain number per plot were negatively correlated with %GLA scores during r*abi* 2013 (when plants severely drought stressed with high temperatures during grain filling) and positively correlated with %GLA scores during *rabi* 2014 (when plants were moderately stressed with low temperatures during grain filling). These data indicate that the recombinant fraction of the introgression-line fine-mapping population better withstands terminal drought stress under low temperature conditions. QTL analysis with the combined trial data set resulted in the identification of 33 putative QTLs for stay-green-related %GLA observations accounting for from 8 to 32% of their corresponding observed phenotypic variances, and 10 major QTLs for agronomic traits, accounting for 21 to 53% of their observed phenotypic variances, mapping to the SBI-10L target region.

QTL clusters for the stay-green trait

From 33 stay-green QTLs detected, 19 QTLs were clustered into seven groups on SBI-10L and named as *cQstg10.1* (3 QTLs) at 36.41 cM (SNP S10_54269620), *cQstg10.2* (4 QTLs) at 41.41 cM (SNP S10_54081973), *cQstg10.3* (2 QTLs) at 44.41 cM (SNP S10_54585199), *cQstg10.4* (2 QTLs) at 45.01 cM (SNP S10_54535306), *cQstg10.5* (3 QTLs) at 115.31 cM (SNP S10_59342820), *cQstg10.6* (2 QTLs) at 120.6 cM (SNP S10_59775456) and *cQstg10.7* (3 QTLs) at 125.01 cM (SNP S10_60194379).

Shoot fly resistance morphological component traits and their QTLs

Morphological component traits of SFR, including seedling leaf blade glossiness score (Gls), trichome density on the upper leaf blade surface (TDU), and trichome density on the lower leaf blade surface (TDL), as well as seedling vigour score (SV) and shoot fly dead-heart percentage (%SFDH), showed transgressive segregation in the phenotyped portion of the recombinant population. Glossiness score and trichome density values showed high heritabilities (66-87%) across seasons, and were correlated (r = 0.16 and r = -0.66*, for Gls and TDL, respectively) with %SFDH values. This confirms that increased trichome density on the lower surface of sorghum seedling leaf blades reduces the severity of shoot fly damage. QTL mapping for SFR-related morphological component traits detected 39 QTLs, of which 20 were major QTLs. A single QTL for glossiness score was consistently mapped to SNP S10_54269620, which falls in the *glossy 15* gene region on SBI-10L. For TDL, a QTL at SNP S10_57432493 was consistently mapped in both seasons, as well as in the across-season analysis, explaining 76% of the observed phenotypic variance for this trait, and thereby identifying this as a major QTL for trichome density on the lower leaf blade surface (TDL). This SNP is ~100kb upstream to *O-methyl transferase* (*Sb10g027640*) and a *cyclin-dependant kinase* (*Sb10g027670*) genes.

Genome-wide association mapping (GWAS)

GWAS for weekly %GLA scores resulted in 207 stay-green-associated SNPs (*P*-values 1.15×10^{-2} to 3.00×10^{-4}) present in the vicinity of 32 candidate genomic regions, among which a leucine-rich repeat protein (*Sb10g022060*), WRKY (*Sb10g025320*), AGO1 (*Sb10g031030*), GA_3 (*Sb10g022520*) and NAC transcription factor (*Sb10g030770*) genes appear to be crucial candidate genes for delaying senescence. Sixty-four candidate genes were identified by CIM-based QTL mapping and thirty-four were identified by GWAS, of which 10 were common candidate genes identified by both analyses. In summary, stay-green genomic regions from 15 Mb were delimited to 7 QTL clusters on SBI-10L by

CIM, and these overlapped with 8 genes, which were also co-localized with marker-trait associations (MTAs) identified by GWAS. The identified candidate genomic regions can be utilized in drought tolerance breeding programs using marker assisted selection. SFR GWAS analysis indicated that the SNP S10_57432493 is associated with the trichome density lower (TDL) at the highest *P* value of 3.67×10^{-10}. Nearly 480 marker-trait associations that are in congruence with the QTL mapping results were identified for Gls, TDU and TDL traits, with *P* values ranging from 3.67×10^{-10} to 9.91×10^{-3}.

Haplotype analysis for fine mapping the target genomic regions

Seven QTL clusters were identified for the stay-green-related traits and subsequent haplotype analysis of 7 sub-regions identified 8 trait-associated SNPs present in the fine-mapped region spanning intervals 54-54.5 Mb (54081973-54585199, 503 kb) and 59.3-60 Mb (59342820 – 60194379, 851 kb). The first four STG-QTL clusters (*cQTL10.1*, *cQTL10.2*, *cQTL10.3*, and *cQTL10.4*) fell within a 503 kb region and the remaining three QTL clusters (*cQTL10.5*, *cQTL10.6*, and *cQTL10.7*) fell within an 851 kb region, having 11 and 8 QTLs, respectively. The following SNPs were identified in these candidate genomic regions:

- *cQTLstg10.1* overlaps an AP2/ERF transcription factor family gene (SNP: S10_54269620; Gene Id: *Sb10g025053*);
- *cQTLstg10.2* contains a putative uncharacterized protein gene (SNP: 54081973; Gene Id: *Sb10g024920*).
- *cQTLstg10.3* contains ankyrin-repeat protein gene (SNP: S10_54585199; Gene *Id: Sb10g025310) and a W*D40 repeat protein gene (SNP: S10_54593246; Gene id: *Sb10g025320*);
- *cQTLstg10.4* overlaps a NBS-LRR protein gene (SNP:54081973; Gene Id: *Sb10g025283*);
- *cQTLstg10.5* contains a LEA2 protein (SNP: S10_59342820; Gene Id: *Sb10g029570*).

- *cQTLstg10.6* contains a calcium/calmodulin-dependant protein kinase gene(*S*NP:59775456; Gene Id: *Sb10g030040)*,
- *cQTLstg10.7contains a* senescence-associated protein gene (SNP: S10_60194379; GeneId*: Sb10g030520*),

Fine-mapping of seedling leaf blade glossiness score (Gls), trichome density {(lower surface (TDL) and upper surface (TDU)} QTLs reduced their intervals from 2.46 Mb and 800 kb, respectively, to 347 kb (54185546-54532800 Mb) and 221 kb (57331385-57552719 Mb), respectively. It is also evident from the QTL mapping that *glossy*15 (*Sb10g025053*) in the 347 kb interval is the most probable candidate gene for the Gls QTL. Further, *O-methyl transferase* (*Sb10g027640*),*cyclin dependant kinaseCDKB2:1* (*Sb10g027670*), an ARM repeat super family protein gene (*Sb10g027680*) and EF-hand Ca^{2+} binding protein CCD1 (*Sb10g027610*) in the 221 kb interval are the most probable candidate genes for the trichome density (TDU and TDL) QTLs. A total of 44 SNPs potentially associated with glossiness score (347 kb) and 37 SNPs potentially associated with trichome density (221 kb) were observed in these fine-mapped regions.

Useful recombinants

Finally, seven useful double-recombinant segregants from the phenotyped fine-mapping population were identified based on their genotypic data and phenotypic expression of the desirable trait combination, which can be further inbred until they attain homozygosity, at which point they can be utilized as donors for pyramided two-component shoot fly resistance combined with the stay-green trait in genetic backgrounds with superior agronomic performance. These high-performance genotypes with combined shoot fly resistance and stay-green traits also can be evaluated in multi-location trials and/or observation nurseries in both *rabi* and *kharif* seasons and can be further validated by expression analysis of their introgressed traits. Thus these selected progenies are

potentially valuable resources for sorghum breeding programmes in regions where sorghum shoot fly ordinarily limits sowing dates for this crop.

Conclusions

- Most of the target trait QTLs detected were consistently detected over environments, showing that they are highly reliable and stable. Stable QTLs were identified for seedling leaf blade glossiness and trichome density lower along with %GL14, %GL21 and %GL42 QTLs of stay-green in *Sorghum bicolor* on SBI-10L, and these results identified in our study are in agreement with previous reported QTLs and can be useful in improving SFR and drought resistance in sorghum through MAB and can be directly utilised in molecular breeding programs..
- Next-generation sequencing data coupled with GBS-SNP analysis resulted in the identification of genomic regions that contain candidate genes associated with the stay-green character and shoot fly resistance. In addition, this identified a huge number of SNPs that can be useful for genomics-assisted sorghum breeding. The complete set of candidate genes identified for target traits reported in this study could be useful in shoot fly resistance and stay-green mechanism of sorghum.
- Further cloning and expression studies of the significantly associated candidate genes may reveal the appropriate role in the biological processes associated with sorghum shoot fly resistance and the stay-green (component of both terminal drought tolerance and ruminant livestock feeding value of sorghum stover).
- After attaining homozygosity, the seven selected recombinants identified as having the favourable alleles desired for a "3-trait cassette" (stay-green + glossy seedling leaf blades + high seedling leaf blade trichome density) can be released for commercial use as potentially valuable parents for breeding commercial hybrids. These lines also can be used by sorghum breeders as donors for the "3-gene cassette" in both conventional and genomics-assisted sorghum breeding programmes.

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APPENDIX

- **Composition of solutions and Reagents**

Shoot fly Resistance screening reagents

Interlard fish meal

Moistened fishmeal kept in plastic bags in the interlards to attract shoot flies

Acetic acid: Alcohol (2:1)

Acetic acid 100 ml

Alcohol 50 ml

Mix well to prepare 150ml of Acetic acid : Alcohol solution for clearing chlorophyll

Lactic Acid (90%)

Lactic Acid 90 ml

Distilled water 10 ml

- **Solutions and Reagents for molecular work**

SDS buffer (2%, 1 lit, pH=8)

Tris buffer (1M, pH=8) 100ml

NaCl sol. (5M) 20 ml

EDTA (0.5M, pH=8) 100ml

SDS 20g

Adjust pH=8 and then makeup volume to 1000ml.

Proteinase K (10mg/ml stock)

100mg proteinase K dissolved in 10ml of SDW.

$T_{10}E_1$

Tris (Trizma) (1M, pH=8): 5ml

EDTA (0.5M, pH=8): 1ml

Make upto 500ml using DDW.

$T_{50}E_{10}$

Tris (1M, pH=8) 50ml

EDTA (0.5M, pH=8) 10ml

Make upto 500ml using DDW

5M NaCl

292.2 g NaCl in 750ml of SDW

Makeup the volume to 1 lit.

Ethanol (70%)

Absolute 70ml alcohol

Distilled water 30ml

RNase (50ml of 10mg/ml)

RNase 500 mg

Tris (1M, pH=8) 1ml

NaCl (5M) 150 ul

Make up to 50 ml using SDW and keep in boiling water for 10-15 minutes to dissolve

3M Sodium Acetate (pH=5.2, 250ml)

Sodium acetate 102.6g

Dissolve in 100 ml of SDW.

Adjust the pH to 5.2 using Acetic acid.
And finally makeup to 250ml using SDW.

T10E1	**1lit**
Trizma base	1.21g
EDTA	0.372 g

3% CTAB of 1lit

CTAB	30 g
NACL	81.8 g
EDTA	7.45 g
Tris.HCL	12.1g
Make up to	1000 ml
PH	8

Chloroform: isoamyl alcohol (24:1).

Chloroform	240 ml
Isoarnylalcohol	10 ml

Store in dark at room temperature

Make up and dispenses the solution in a fumed cupboard

Phenol: Chloroform: Isoamyl alcohol (25:24:1)

Mix equal volumes of buffered Phenol and Chloroform and isoamyl alcohol (25:24:1).

Store at 4˚C.

10X Tris-Borate Buffer (TBE) (per lit)

Tris buffer	108g (Tris base)
Boric Acid	55g
EDTA	9.3g

Add deionised water and make upto 1lit.

PH	8.3

.**6X gel loading buffer (0.25% Bromophenol blue,40% sucrose) (10ml)**

Sucrose	4 g
Bromophenol Blue	2.5 g
dH_2O	10 ml

store at 4˚C.

Ethidium bromide (10 mg/l)

Dissolve 100 mg of ethidium bromide in 10 ml of distilled water.

Wrap tube in aluminum foil and store at room temperature

100 base pair ladder (50 ng/ul)

100bp ladder (stock conc.1 ug/ul) - 50 ul

ANNEXURE

Annexure 1: Total SSR markers screened in 15Mb region

S No	* Marker_name	*Physical position	* Primer_Sequences_Forward	* Primer_Sequences_Reverse	* Motif	*Polymorphism information(P)
1	**Xisep0621**	**46.5478**	**CAGTCGCGGTGGTAGACAT**	**GCCGAGTCGTCAGAAGAAGA**	**(GCG)4**	**P**
2	SvPEPCAA	47.1317	GCAGCTCAGGGACAAATAC	CTGCTTCAGGTAAGGATCG	(AT)10	P
3	Xisep0625	48.098	CTAGCAGCAGCAGCAGTCAC	GCCTTTGCTTGCTTTGATTT	(TCC)4	P
4	Xtxp290	49.2964	CACGACGTTGTAAAACGACGCCGTTCTCCTCTC	TAAAACCAGTGGCAAAACTA	(CT)17	PA
5	**Xisp10263**	**49.6729**	**TATCTTCTCCGCCCTTTC**	**TAAGNGCCAAGGGAATG**	**CA/CTG**	**P**
6	Xiabtp131	53.7667	TCGGTTACGAGAGCAGACCTG	CTCTCCGAGTGCTGGATGTC	NN	PA
7	Xisep0634	54.4266	GCATAGCCACCAGATCTTCC	AATCATGCTTGCACACTTGC	(CAG)5	P
8	Xiabtp294	54.4296	GAAGCAGGCAGAGAGGTGAC	GCTGCTTCCTCCTCCTCTTTC	NN	PA
9	**[1]Xgap001**	**54.5072**	**TCCTGTTTGACAAGCGCTTATA**	**AAACATCATACGAGCTCATCAATG**	**(AG)16**	**P**
10	Xiabtp410	54.7746	CATACCAGAGTGCTCGCAAA	AGCGAGCGAGAGAGACAGAG	NN	NA
11	**[2]Xnhsbm1008**	**54.7809**	**TGAATGGCAATGTGTTTGGT**	**ACGTGTTCCCGTAGGTTGTC**	**(TCTA)18**	**PA**
12	**[3]Xnhsbm1011**	**54.9085**	**TGGGATGCCATATTCTTTTTG**	**GTTCCTGGTGTTCGTTTGCT**	**(TTC)17**	**P**
13	**[4]Xisep0643**	**55.0106**	**CTCACCTTGGGAGCTGAATC**	**GGAGGACCTAGCAAGCAAGA**	**(TC)7**	**P**
14	Xnhsbm1013	55.044	GCAACTCGTGACACCAGAGA	TGCCGATTCATCTTCCAAAT	(GT)13	PA
15	**[5]Xiabtp389**	**55.1128**	**GCACGAGAACAGCACGATTA**	**AATCCATCGCACACATCAGTA**	**NN**	**P**
16	XmSbCIR262	55.3239	GCACCAAAATCAGCGTCT	CCATTTACCCGTGGATTAGT	(CATG)3.25	M
17	Xtxp320(PhyB)	55.3811	TAAACTAGACCATATACTGCCATGATAA	GTGCAAATAAGGGCTAGAGTGTT	(AAG)20	PA
18	Xiabtp502	55.4724	ATCTAACACTGGGCCCTGAC	CGAACGTACATACTCATGTCTCC	NN	M
19	Xiabtp152	55.4982	CCGTCCGAGAAGGACTACTG	CGTAGCCAGCTGATCCAGA	NN	M
20	Xisep0639	55.6679	TCGGACGGAGTCATCAGATA	GCCTTCGTGTCTTCTGTCCT	(TCT)6	M
21	Xnhsbm1033	55.9881	GGCCTTTTGGTTATGATTGC	GGGTCTATTGTGCCTTGACG	(GA)19	NA
22	Xnhsbm1043	56.886	TTTCTCATCGCGACTCACAC	TGGATGAGACATCGACCTTG	(AGAT)13	PA
23	**[6]Xnhsbm1044**	**56.9659**	**GCGCACCAGAGTCATATTGTT**	**GCCCTTTTGCAACGTCTAAA**	**(TATG)16**	**P**
24	[7]Xiabtp340	57.1881	CATTGCTCACTGCTCAGTTCA	CCATCGATCGAGCTCTCTG	NN	P
25	Xiabtp203	57.1901	AACTGTCGAAAGCGATGGTC	CATGGACATGCACCAAGAAC	NN	M
26	Xiabtp476	57.338	CTTCTTCCCGTGCCTTTTCTG	CACCACCTCCACCTCCTCTC	NN	P
27	Xiabtp117	57.3866	ACCAAAGCAAACGACATGC	GAGAGGAAGTCGGTGACGAG	NN	M
28	Xnhsbm1048	57.3932	CGAACCCCCTACTCCACTCT	CGCGATTTTCTTTCACACAA	(ACTCT)5	M

29	**[8]Xisep0630**	**57.4008**	**GATCGAGTCGTTC GTCGAGT**	**AAATCCATCGAC CAATCAGC**	**(GTC)5**	**P**
30	Xiabtp146	57.6243	AAAGGAGGCTTGTC GTGCTA	GTCCAGGCACCT GTCACTCTC	NN	M
31	Xiabtp488	57.6718	AAAAGGCACCACCT TCTCCTC	GCATCGCCATCTC TCTCTTC	NN	M
32	Xcup16	57.7692	TGCAGTGCTAGCTC ATGGTC	CTTTCCAGCCTCC CATATCC	(CTTTT)4	PA
33	Xiabtp267	57.9237	CTCGTTCCCGTAGC TGTCTC	AAGAGATCGGAG AAGGTCTCG	NN	M
34	Xiabtp264	58.0157	AAAAAGGCAACAG CAACACC	AGACTGGAGGGA GCAAGTGA	NN	M
35	Xgap325	58.1707	AGCGCAGGAGCGCG AA	TCATCCGCTACTA CCGTCAGAAA	(AAG)22	M
36	**[9]Xtxp141**	**58.2451**	**TGTATGGCCTAGC TTATCT**	**CAACAAGCCAAC CTAAA**	**(GA)23**	**P**
37	Xiabtp466	58.3103	AGCTCCCAGTGTTA GCTCCA	CGGAAGCCCACA GCTTATAC	NN	P
38	Xiabtp208	58.4112	CTACTCCAGCCTGT GGAACG	AGGTCCAGCTCC TGGTTGTA	NN	M
39	Xisep0646	58.6222	AGAGGAGGACGAG GAGGAAG	ACAGGGTGAGCT GGTTGGT	(GGA)5	M
40	Xcup07	60.5691	CTAGAGGATTGCTG GAAGCG	CTGCTCTGCTTGT CGTTGAG	(CAA)8	P
41	**Xisep1011**	**60.7464**	**GGAGAAGGAGGTG CAGGAG**	**CACTGACTGACC ACGAGCTT**	**(GT)5**	**P**

PA-poor amplification, P-polymorphic, M-monomorphic, NA- not amplified

Nine markers in red font are used for F1 heterozygosity confirmation between Xgap001- Xtxp141

Additional three markers added are in bold fonts

Annexure 2: Total GBS SNP count chromosome wise

Chromosome	SNP Count
1	5,920
2	5,102
3	3,534
4	2,848
5	2,372
6	2,719
7	2,215
8	2,387
9	2,349
10	**3,390**
Total	32,836

LIST OF RESEARCH PUBLICATIONS AND CONFERENCE PAPERS

Research Articles

- Kiranmayee KNSU, Kishor PBK, Hash CT, Deshpande SP (2016) Evaluation of QTLs for Shoot Fly (Atherigona soccata) Resistance Component Traits of Seedling Leaf Blade Glossiness and Trichome Density on Sorghum (Sorghum bicolor) Chromosome SBI-10L. Tropical Plant Biology 9: 12-28. doi:10.1007/s12042-015-9157-9
- Kiranmayee KNSU, Hash CT, Deshpandae SP, Varaprasad KVGK and Kavi kishor PB (2015) Biotechnological approaches to evolve sorghum drought stress tolerance and shoot fly resistance. Current Trends in Biotechnology and Pharmacy. Vol 9 (3):281-292.
- Genetic dissection and candidate gene identification of stay-green QTLs on sorghum chromosome SBI-10L using GBS SNPs and GWAS provides insights into breeding drought tolerance. Review process. New Phytologist.In Review
- Fine mapping of shoot fly resistance QTLs on sorghum chromosome SBI-10L. Manuscript under preparation targeting Plos One. In Review

Abstract submitted and conferences attended

- Usha kiranmayee K N S, Sharma H C, Kavi Kishor P B, Ramu P,Sivasubramani S, Munghate R S, Sakhale S A, Hash C T, Santosh P Deshpande (2015) Genome wide association studies (GWAS) for shoot fly resistance component traits and stay-green traits on sorghum chromosome SBI-10. Next Generation Genomic for Intergrated Breeding for Crop Improvement conference (NGGIBCI) – V (February 18-20, 2015), ICRISAT, Patancheru, India. Poster number NGGP-31.

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- K N S Usha kiranmayee, H C Sharma, P B KaviKishore, P Ramu, S Sivasubramani, R S Munghate , S Sakhale , C T Hash, Santosh P Deshpande (2015). Fine genetic mapping of combined shoot fly resistance (SFR) and stay green (STG) traits on sorghum chromosome SBI-10. Animal Genomes Conference- XXIII (January 10-14, 2015), Town & Country Convention Centre, San Diego, USA. Poster number P0562.

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Evaluation of QTLs for Shoot Fly (*Atherigona soccata*) Resistance Component Traits of Seedling Leaf Blade Glossiness and Trichome Density on Sorghum (*Sorghum bicolor*) Chromosome SBI-10L

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Abstract Shoot fly is a major insect pest of sorghum damaging early crop growth, establishment and productivity. Host plant resistance is an efficient approach to minimize yield losses due to shoot fly infestation. Seedling leaf blade glossiness and trichome density are morphological traits associated with shoot fly resistance. Our objective was to identify and evaluate QTLs for glossiness and trichome density using- i) 1894 F_2s, ii) a sub-set of 369 F_2-recombinants, and iii) their derived 369 $F_{2:3}$ progenies, from a cross involving introgression lines RSG04008-6 (susceptible)×J2614-11 (resistant). The QTLs were mapped to a 37–72 centimorgan (cM) or 5–15 Mb interval on the long arm of sorghum chromosome 10 (SBI-10L) with flanking markers *Xgap001* and *Xtxp141*. One QTL each for glossiness (*QGls10*) and trichome density (*QTd10*) were mapped in marker interval *Xgap001-Xnhsbm1044* and *Xisep0630-Xtxp141*, confirming their loose linkage, for which phenotypic variation accounted for ranged from 2.29 to 11.37 % and LOD values ranged from 2.03 to 24.13, respectively. Average physical map positions for glossiness and trichome density QTLs on SBI-10 from earlier studies were 4 and 2 Mb, which in the present study were reduced to 2 Mb and 800 kb, respectively. Candidate genes *Glossy15* (Sb10g025053) and ethylene zinc finger protein (Sb10g027550) falling in support intervals for glossiness and trichome density QTLs, respectively, are discussed. Also we identified a sub-set of recombinant population that will facilitate further fine mapping of the leaf blade glossiness and trichome density QTLs on SBI-10.

Keywords Shoot fly · F_2 · $F_{2:3}$ · Leaf blade glossiness · Trichome density · QTLs

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop globally. It is grown predominantly in semi-arid tropical conditions and used for food, feed, fodder and fuel (FAOSTAT 2010). Shoot fly, *Atherigona soccata* (Rondani) is one of the major insect pests of sorghum grown in Africa, Asia and Mediterranean Europe. In peninsular India, sorghum is cultivated during rainy and post-rainy seasons where shoot fly attacks the crop and damages early stages of crop growth, adversely affecting establishment and productivity (Sharma et al. 2003). Shoot fly infests sorghum seedlings from 7 days after emergence (DAE) to 30 DAE. The female shoot fly has just 30-days' life span and lays white, elongated cigar-shaped eggs singly on the abaxial (lower) surface of leaf blades parallel to the midrib (Dhillon et al. 2006). Eggs hatch into maggots following 1–2 days of incubation, and each larva/maggot enters the central leaf whorl of the seedling on which it hatched. The larva reaches and cuts the seedling growing point, and feeds on the decaying tissue,

resulting in drying of the central whorl causing a typical 'dead heart' symptom.

Among several components of integrated pest management practices used to minimize losses due to shoot fly infestation of sorghum, host plant resistance (HPR) and timely sowing remains the most preferred options as they are cost-effective, eco-friendly and easily adopted by farmers (Kumar et al. 2008). HPR to shoot fly is mediated by a number of morphological, biochemical and genetic factors. Shoot fly morphological component traits including seedling leaf blade glossiness (Maiti et al. 1984), seedling leaf blade trichome density (Maiti and Bidinger 1979), seedling vigor and leaf sheath pigmentation are positively associated with shoot fly resistance (SFR) (Tarumoto 2005). Further, these SFR component traits have been mapped, putative Quantitative Trait Loci (QTLs) identified for individual traits, and subsequently validated by marker-assisted backcrossing (MABC)-based introgression into genetic backgrounds highly susceptible to shoot fly (Usha Kiranmayee et al. 2015b). Using a sorghum recombinant inbred line (RIL) population derived from cross (BTx623×IS18551), Sajjanar (2002) and, Folkertsma et al. (2003) mapped SFR QTLs on SBI-01, SBI-03, SBI-05, SBI-07, SBI-09 and SBI-10. Similarly, using a (296B×IS18551)-based RIL population, Deshpande (2005); Mehtre (2006) and Satish et al. (2009, 2012) mapped SFR QTLs on SBI-01, SBI-03, SBI-04, SBI-05, SBI-06, SBI-09, SBI-07, and SBI-10. Aruna et al. (2011) mapped SFR QTLs on SBI-01, SBI-02, SBI-03, SBI-04, SBI-06, SBI-07, SBI-09, and SBI-10 using shoot fly resistance source IS2122. In a RIL population based on a reciprocal cross (IS18551×296B), Apotikar et al. (2011) found SFR QTLs on SBI-01 and SBI-03. Five putative QTLs for SFR component traits from IS18551 were then validated by MABC into the genetic backgrounds of elite shoot fly-susceptible hybrid seed parent maintainer lines 296B and BTx623 (Jyothi 2010). Probable candidate genes underlying the target QTLs for seedling leaf blade glossiness and trichome density have been reported by Satish et al. (2009, 2012) and Aruna et al. (2011). In the present study we attempted to refine QTL intervals for trichome density and glossiness QTLs on SBI-10 by comparing whole sorghum genome sequence (Paterson et al. 2009) annotation and a sequence-based physical map integrated with sorghum linkage maps (Ramu et al. 2010), with genetic and physical maps from different QTL mapping studies integrated based on whole genome sequence information (Mace and Jordan 2011). We also tried to compared earlier shoot fly resistance QTL mapping studies on sorghum chromosome SBI-10 with the present study based on genetic and physical maps.

Identification of genes, pathways and mechanisms involved in sorghum phenotypes for seedling leaf blade glossiness and trichome density have not yet been completed in sorghum. Most such studies have been done in model species like *Oryza sativa* (rice), *Arabidopsis* and *Zea mays* (maize). Wax deficient mutant loci in maize, *Brassica napus* and sorghum are defined as '*glossy*' loci, where as in *Arabidopsis thalina* and *Hordeum vulgare* (barley) they were named as *ceriferum* (cer) mutant loci (Kunst and Samuels 2003). In *Arabidopsis* many studies have reported shine (*shn*) mutants, which were isolated and characterized, determining that the *shn* gene encodes AP2/EREBP (ethylene responsive element binding protein) transcriptional factors that act in up and down regulation of lipid biosynthesis (Aharoni et al. 2004). More than 30 '*glossy*' loci have been identified and a few were cloned (*gl1*, *gl2*, *gl3*, *gl4*, *gl8*, *gl13* and *gl15*) in maize (Li et al. 2013) and their functional roles in glossiness have been reported. Similarly, for trichome density many studies have reported that WRKY and MYB transcription factors play important roles (Eulgem et al. 2000; Johnson et al. 2002; Ishida et al. 2007; Liang et al. 2014).

In order to understand the genomic regions responsible for seedling leaf blade glossiness and trichome density, several QTL mapping and validation studies have laid the foundation for using favourable alleles at the SFR QTLs in MABC programs. The reference genome sequence for sorghum is that of elite, shoot-fly susceptible sorghum maintainer line BTx623 (Paterson et al. 2009), which was one of the susceptible recurrent parents into which favorable SFR alleles from resistance source IS18551 were backcrossed for validation. When resistance QTL introgression lines (ILs) were field evaluated for shoot fly resistance performance (Jyothi 2010), one of the ILs viz., J2614-11 was identified as one of the better performing SFR introgression lines. Thus we used J2614-11 as the resistant parent in the present study, which focused on linked SFR component trait QTLs mapped to the long arm of chromosome SBI-10 (SBI-10L).

In the present study we have conducted experiments to re-evaluate the presence of QTLs for seedling leaf blade glossiness and trichome density as components of SFR on sorghum chromosome SBI-10L. We used a cross of non-glossy, shoot fly-susceptible, *rabi* adapted stay-green introgression line (RSG04008-6) with a drought-sensitive, glossy, shoot fly-resistant introgression line having high trichome density (J2614-11), to produce a high-resolution mapping population of 1,894 F_2 individuals. We further selected a sub-set of recombinant F_2-derived F_3 ($F_{2:3}$) progenies for refining the QTL interval of seedling leaf blade glossiness and trichome density QTLs on SBI-10L. The results of this study will contribute to fine mapping and cloning of genes underlying the confirmed QTLs.

Results

Development of High-Resolution Population

Parental Polymorphism and Confirming F_1s

Introgression line J2614-11 was the donor parent for seedling leaf blade glossiness and trichome density in the cross RSG04008-6×J2614-11. Parents were clearly differentiated visually for both glossiness and trichome density. In order to confirm their allelic composition, nine polymorphic SSR markers were assessed in pairs of parents (RSG04008-6 and J2614-11) and grandparents (R16 and E36-1 for RSG04008-6; BTx623 and IS18551 for J2614-11) across the target genomic region (marker interval *Xgap001-Xtxp141*). Marker alleles for each parent - grandparent pair of E36-1 - RSG04008, and J2614-11 - IS18551 were monomorphic across this SBI-10 target region, but these marker alleles were polymorphic between the two pairs of parents and the two donor grandparents, confirming that the introgressed parental target regions under study were derived from their respective grandparent donors. A total of seven plant×plant crosses were executed and seed from a single plant×plant cross was sown with one seed per hill. From a single plant×plant cross involving RSG04008-6×J2614-11 during *rabi* season 2010 (with plant no. U1000019) twelve putative F_1 seeds were produced. All 12 putative F_1 plants were screened for heterozygosity with a total of 9 polymorphic co-dominant SSR markers distributed across the target interval (*Xgap001-Xtxp141*). High quality grade 1 (Kanyika et al. 2015) marker allele profiles were obtained for all markers (Fig. S1). Eleven were confirmed to be true F_1s having heterozygous parental alleles, whereas one plant was homozygous for the seed-parent alleles, and was discarded.

Developing F_2s and Selection of Informative $F_{2:3}$-Progenies

All 11 of the true F_1s were selfed to produce 11 F_2 seed lots. Out of these eleven, one seed lot derived from a single F_1 plant (U110055) with 1,958 seeds was selected for advancement during late *rabi* season 2011/12, and used as a high-resolution recombinant mapping population.

A total of 1,894 F_2 individuals (surviving after sowing) from the high resolution cross (HRC), along with its parental introgression lines RSG04008-6 and J2614-11, were genotyped with 5 SSR markers covering the target SFR QTL region on sorghum chromosome SBI-10L. The five markers were selected in particular for genotyping the population because the introgression line parent J2614-11 was bred using *Xgap001* and *Xtxp141* as flanking markers for transfer of a two-component shoot fly resistance QTL by MABC from donor IS18551 into recurrent parent BTx623 background. We genotyped the complete F_2 population of 1,894 individuals with 5 SSR markers (Table S1) of which two were flanking markers *Xgap001* and *Xtxp141* (Sajjanar 2002; Deshpande 2005; Sharma et al. 2005; Dhillon et al. 2006; Mehtre 2006; Jyothi 2010; Ramu et al. 2010); markers *Xnhsbm1044* (Satish et al. 2009) and *Xisep0630* (Ramu et al. 2010) were reported to be associated with trichome density that conferred shoot fly resistance in sorghum population 296B×IS18551 (Satish et al. 2009); and *Xiabt340* was a marker located between *Xgap001* and *Xtxp141* which was not previously associated with either of the two shoot fly resistance component target QTLs. We selected 369 homozygous and nearly homozygous recombinant F_2 plants based on these 5 SSR markers. Individuals showing complete homozygosity for- i) RSG04008 alleles, or ii) J2614 alleles, or iii) complete heterozygosity across this region were not given preference. The 369 selected informative F_2 recombinant individuals were genotyped with 3 additional markers to extend the flanking regions for detection of the exact location of the target QTL regions and selection of a sub-set for fine mapping (Table S1). All 369 selected informative recombinant F_2 individuals were selfed to produce F_3 seed during the late *rabi* season 2011/12 sowing.

Trait Variation and Correlation of Seedling Leaf Blade Glossiness and Trichome Density Scores

The parental introgression lines differ significantly with each other for both glossiness and trichome density scores (Fig. 1 and Table 1). Among the F_2 population and their recombinant F_3 progenies, glossiness scores ranged from 0.09 to 4.95 and

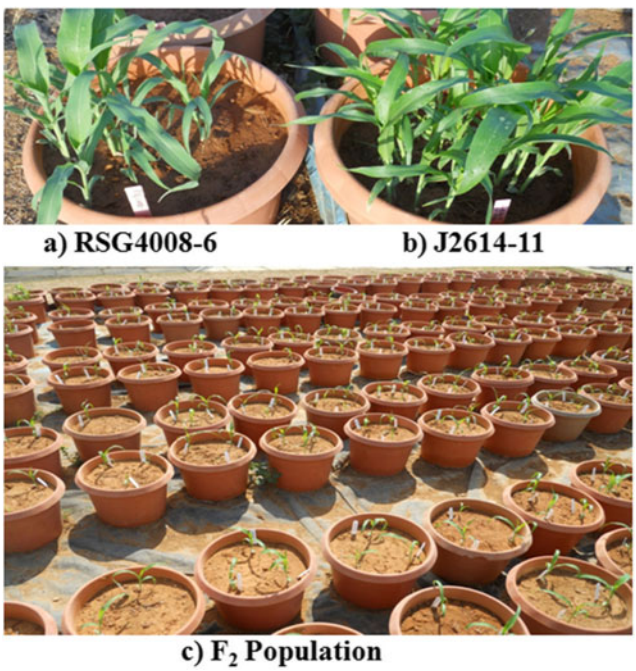

Fig. 1 **a** RSG4008-6 parent showing non-glossy leaves **b** J2614-11 parent showing glossy leaves **c** F2 population sown in pots

Table 1 Descriptive statistics and correlations of seedling leaf blade glossiness score and trichome density score in the complete F_2 population and F_3 progenies derived from 369 selected informative recombinant F_2 individuals

Trait	P1 (RSG)	P2 (J2614)	Min	Max	Mean ± SE	h^2 (%)	CV (%)	Correlations	
								Gls	Td
F_2Gls	5.00	1.00	1.00	4.96	1.94±0.09	97.75	13.7	1	
F_2Td	2.00	4.00	0.00	5.08	2.66±0.11	89.22	11.9	−0.0097*	1
F_3Gls	5.00	1.00	1.02	4.94	2.10±0.24	97.99	11.9	1	
F_3Td	2.00	5.00	0.27	4.90	3.63±0.25	91.95	11.7	−0.0065*	1

*correlation significant at $P<0.05$, F_2 1,894 individuals, F_3 selfed progeny of 369 selected informative recombinant F_2 individuals, h^2 heritability, *CV* coefficient of variation, *Gls* glossiness, *Td* trichome density

trichome density scores ranged from 0.00 to 5.00. In both the F_2 population and its derived F_3 progenies, glossiness and trichome density scores were negatively correlated with each other, indicating that a high trichome density score was associated with a low glossiness score and therefore that high trichome density is associated with a high degree of glossiness. Heritability estimates were very high (≥0.90) for both glossiness and trichome density scores (Table 1). The statistical *Z* test results showed (significant $P<0.05$) genetic variation for glossiness score (*Gls*) and trichome density (*Td*) indicating that data is suitable for QTL mapping.

Seedling Leaf Blade Glossiness

We scored 1,894 individual F_2 plants for the morphological component traits of shoot fly resistance. The trait glossiness was scored visually and the results were divided into two categories- (i) glossy and (ii) non-glossy (Satish et al. 2009). The complex glossiness trait was characterized by narrow, erect, pale, shiny green leaves and 1,457 F_2 individuals (76.92 %) exhibited glossy leaves (Fig. 1a and b). A total of 437 F_2 individuals (23.08 %) with non-glossy leaves were characterized by broad, dull, droopy leaves. For this trait, the phenotypes of F_2 individuals followed Mendelian genetics and segregated in a 3:1 ratio ($\chi^2=0.99$; Fig. 2a), with the glossy phenotype of shoot fly-resistant introgression line parent J2614-11 being dominant.

Seedling Leaf Blade Trichome Density

We observed substantial variation in trichome density score in the F_2 population (Fig. 2b). Very low trichome density scores ranging from 0.0 to 1.0 were observed in 285 F_2 individuals (15.05 %), and low trichome density scores between 1.0 and 2.0 were observed for 260 F_2 individuals (13.72 %). Likewise, under categories medium trichome density score 124 individuals (6.54 %), high trichome density score 863 individuals (45.56 %) and very high trichome density score 362 individuals (19.11 %) were noted (Fig. 2b.).

Genetic Linkage Map

The entire F_2 population of 1,894 individuals were genotyped with 5 linked SSRs (*Xgap001*, *Xnhsbm1044*, *Xiabt340*, *Xisep0630*, *Xtxp141*) spanning the introgression target region for SBI-10, resulting in a map distance of 37 cM (Fig. 3a). Based on marker arrangement, genotyping data were categorized into different classes having homozygotes of RSG04008-6, homozygotes of J2614-11, heterozygotes and near-homozygotes with different recombinations. Based on genotyping data across this target region, 369 informative recombinant F_2 individuals were selected for advancement to the F_3 generation. The selected informative recombinant F_2 individuals, were genotyped with three additional markers (*Xisep0621* and *Xisp10262* above *Xgap001*, and *Xisep1011* below *Xtxp141* on SBI-10L) to more

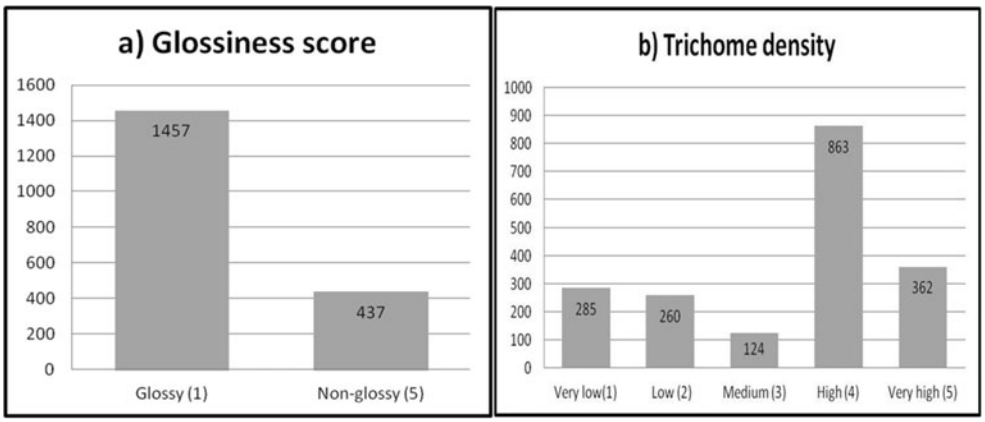

Fig. 2 Trait segregation among 1,894 F_2 individuals for **a** Glossiness score, and **b** trichome density score

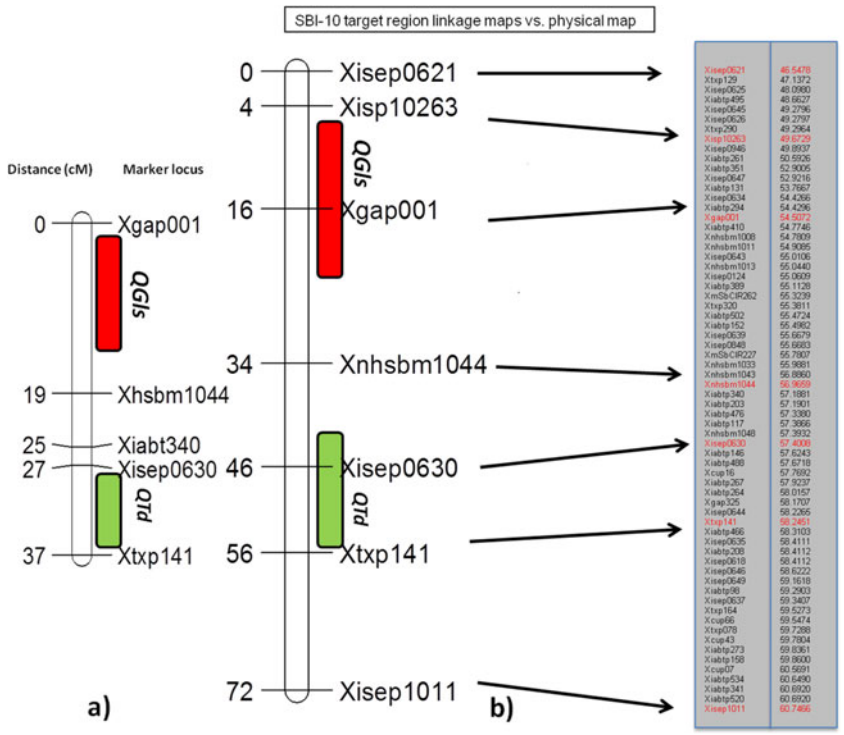

Fig. 3 Genetic linkage maps of target region **a** with 5 SSRs on F2 population of 1,894 individuals **b** with 7 SSRs on 369 selected informative recombinant F2 individuals, and linkage map vs. physical map

fully encompass the '*Gls*' and '*Td*' genomic regions. The linkage map constructed for these selected recombinant F_2 individuals had an expanded total length of 72 cM with 7 SSRs (*Xgap001*, *Xnhsbm1044*, *Xisep0630*, *Xtxp141*, *Xisep0621*, *Xisp10262* and *Xisep1011*) instead of eight and linkage map marker order was similar to the physical map (Fig. 3b). Marker *Xiabt340* was excluded from the linkage map of the recombinant sub-set as it had a large portion of missing data in the selected 369 recombinants when compared to the full population of 1, 894 F_2 individuals.

QTLs Detected in Complete F_2 Population

Composite interval mapping (CIM) analysis identified two QTLs for shoot fly resistance component traits on SBI-10, one each for leaf glossiness and trichome density in the F_2 population of 1,894 individuals (Table 2). The QTL for seedling glossiness score (*QGls10*) was mapped at LOD 24 (Fig. 4a) between markers *Xgap001* and *Xnhsbm1044* with an R^2 value of 6.23 % (indicating it is a relatively minor QTL but mapped with high confidence), with homozygosity for the J2614 allele from grandparent IS18551 reducing glossiness score (but increasing glossiness) by circa 1.36 units compared to the RSG04008 allele from grandparent E36-1. The seedling leaf blade trichome density score QTL (*QTd10*) was mapped between *Xisep630* and *Xtxp141* at LOD 8.11 with an R^2 value of 2.88 % (indicating unexpectedly that it too is a minor QTL) (Fig. 4b). The glossiness QTL and trichome density QTL were found within the intervals of 0–10 cM and 25–37 cM, respectively, on the map of the *Xgap001-Xtxp141* interval on SBI-10L of the F_2 high resolution mapping population (Table 2). F_2 QTL mapping resulted in incomplete confidence intervals for both '*QGls*' and '*QTd*' within the *Xgap001* and *Xtxp141* marker interval. In order to more exactly locate flanking genomic

Table 2 Shoot fly resistance component trait QTLs detected on SBI-10 using QTL Cartographer with data from a large F_2 population of 1,894 individuals derived from cross RSG04008-6×J2614-11

QTL	Pos (cM)	Marker interval	Supp. IV (cM)	LOD	R^2 %	Add[a]	Dom*
QGls10	1.0	*Xgap001-Xnhsbm1044*	0–10	24.13	6.23	−0.69	0.01
QTd10	31.6	*Xisep0630-Xtxp141*	25–37	8.11	2.88	0.31	−0.09

QTL Quantitative trait locus, *Pos* Position of QTL in cM, *LOD* Logarithm of odds, *R^2%* Percentage of phenotypic variance, *Add* Additive, *Dom* Dominance

[a] Genetic effects for the identified QTLs as per description in the QTL Cartographer Ver. 1.17 manual (page no.13. For trait *Gls* in this study, the lower trait value (1-glossy) is preferred over higher trait value (5-non-glossy), the negative additive effects for *QGls10* are contributed by J2614-11. For *QTd10*, the positive additive genetic effects are contributed by J2614-11

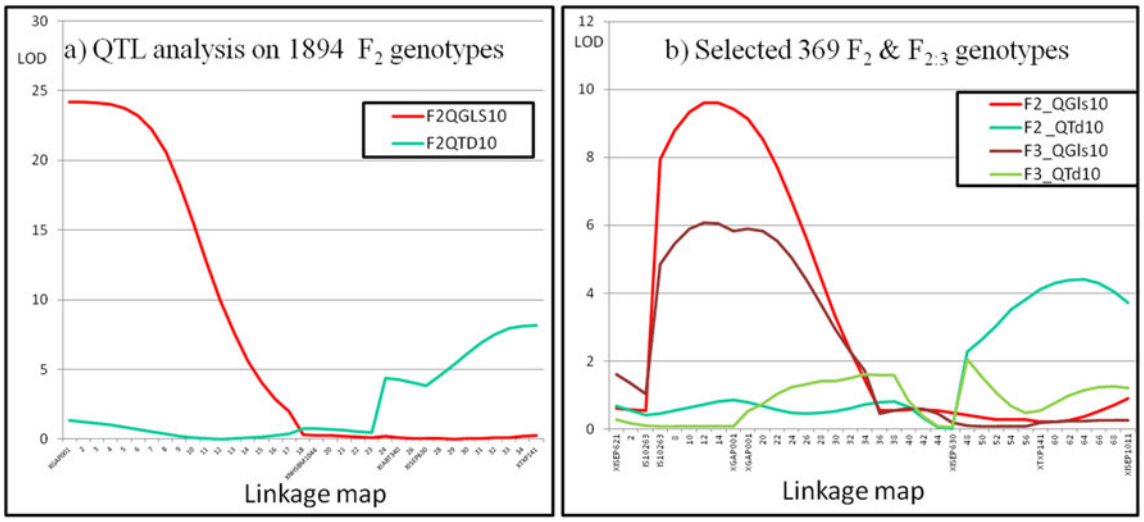

Fig. 4 **a** Map for glossiness score and trichome density score QTLs on SBI-10L among 1,894 F_2 individuals evaluated in *rabi* season of 2010–2011, **b** QTL confirmation among 369 selected informative recombinant F_2 individuals evaluated in *rabi* season of 2010–2011 and their derived $F_{2:3}$ progenies evaluated in a late *kharif* season 2012 sowing

regions contributing to the observed variation, three more polymorphic markers (*Xisep0621*, *Xisp10263* and *Xisep1011*) were added to the linkage map for the sub-set of selected informative F_2 individuals.

Mapping of Glossiness and Trichome Density Using the F_2 Total Population with Physical Map Positions

A moderately large segregating population with 1,894 F_2 indiviuals was developed from cross RSG04008-6×J2614-11 and was used in further mapping of the SBI-10 '*QGls*' and '*QTd*' region using physical positions. By aligning genotype and phenotype of all the 1,894 F_2s (Table S1) recombinant haplotypes were identified and their phenotypic variant locations were tabulated in Table 3. Marker *Xgap001* showed clear allelic association with glossy phenotype value. In case of trichome density, the marker interval *Xisep630-Xtxp141* might contain trichome coding region/genes, but the evidence is not clear.

QTLs Detected Among Selected F_2 Individuals and Their $F_{2:3}$ Progenies

A sub-set of 369 F_2 recombinants and their $F_{2:3}$ progenies were also utilized for QTL analysis. At LOD 5.95 the leaf blade glossiness score QTL (*QGls10*) was mapped between *Xisp10263* and *Xgap001* with R^2 of 11.37 % for the selected F_2 recombinant individuals and R^2 of 6.60 % for their $F_{2:3}$ progenies, with LOD values of 9.67 and 5.95, respectively (Table 4). This shows that there was indeed a glossiness QTL in the target region of sorghum chromosome SBI-10L. Further, it was expressed in both the post-rainy season (*rabi*, wherein the F_2 population was evaluated) and the rainy season (*kharif*, wherein the $F_{2:3}$ progenies were evaluated). The trichome density QTL (*QTd10*) for the selected 369 F_2 recombinants mapped between *Xtxp141* and *Xisep1011*, whereas that for their derived $F_{2:3}$ progenies mapped between *Xisep0630* and *Xtxp141*, within the same support interval. The portion of phenotypic variation accounted for by this trichome density QTL for the 369 selected informative $F_{2:3}$ progenies was just 2.29 % and for the selected recombinant subset of the F_2 population was 3.70 % with LOD values of 2.32 and 4.40, respectively (Fig. 4b, Table 4). The sub-set of 369 F_2 and F_3 progenies were sown in different environmental conditions and the F_2 has many heterozygous loci might have showed effects on QTL detection.

Mapping of QGls and QTd in $F_{2:3}$ Selected Recombinants

From the complete F_2 population, 369 recombinants were selected and further genotyped with *Xisep0621*, *Xisp10263* and *Xisep1011*, and the combined marker data was used for locating QTL-flanking markers for both glossiness and trichome density. Genotype marker data and their F_3 phenotye data were aligned for recombinant identification. Various recombination groups and their mean values were aligned and the F_2 data results were combined with F_3 results, as well as QTL mapping results. This clearly indicates a glossiness QTL region near *Xgap001* i.e., 54–55 Mb (interval *Xisp10263-Xgap001*). There is weaker evidence for a trichome density QTL region at 57–58 Mb (*Xisep0630-Xtxp141*). Nearly 2.0–2.5 Mb regions were reduced to 800 Kb regions for each QTL (Table 5).

Table 3 Genotype and phenotype of homozygous recombinants selected from the F_2 population

Physical pos (Mb)	54.51	56.97	57.19	57.40	58.25	BLUPs		
F_2 recombinant	*Xgap001*	*Xnhsbm1044*	*Xiabt340*	*Xisep0630*	*Xtxp141*	Glossiness	Trichome density	No.
RSG04008-6	A	A	A	A	A	4.00	2.00	60
Rec1	A	A	B	A	A	4.91	0.00	1
Rec2	A	A	–	B	B	4.96	3.27	2
Rec3	A	B	–	B	B	4.93	4.91	2
Rec4	A	B	–	A	B	4.93	3.10	1
Rec5	A	B	–	B	A	4.96	1.46	1
Rec6	A	A	–	B	A	4.93	1.75	4
Rec7	B	A	A	A	A	1.05	3.00	16
Rec8	B	A	–	B	B	1.03	3.19	11
Rec9	B	B	–	A	B	1.02	4.00	6
Rec10	B	B	–	B	A	1.02	1.29	1
J2614-11	B	B	B	B	B	1.26	3.00	114

A and B are homozygotes for marker genotypes of RSG04008-6 and J2614-11, respectively

No. is the number of recombinants with the same genotype

BLUPs Best linear unbiased predicted means

Preliminary Identification of Candidate Genes

Annotation of the sorghum genome sequence was utilized along with the UNIPROT database to identify probable candidate genes and their functional roles in controlling glossiness and trichome density of sorghum seedling leaves. In the total 15 Mb target region nearly 780 predicted genes are present (Table S2), of which probable candidate genes for the target traits based on their function are listed in Table 6. The *Xgap001-Xnhsbm1044* interval on SBI-10 L is about 2 Mb long, and contains 179 predicted genes. In the trichome density marker interval *Xisep0630-Xtxp141* 94 predicted genes are present (Table S2). Based on functional annotation *Glossy15*/AP2 transcription factor (Sb10g025053), MYB transcription factor (Sb10g024950), Calcium lipid binding domain (Sb10g025040) and cytochrome P450 (Sb10g025110) were the most likely candidate genes for the glossiness QTL as they are involved in different wax synthesis and transport mechanisms directly and indirectly. In case of the trichome density QTL, MYB transcription factor (Sb10g027280), ethylene zinc finger protein (Sb10g027550), Armadillo repeat protein (Sb10g027680), F-box domain (Sb10g027730), EF hand Ca+2 binding protein (Sb10g027610) and a key transcription factor WRKY (Sb10g025600) reported in many *Arabidopsis* trichome initiation studies all appear to be good candidates and have been previously reported in model plant *Arabidopsis* which shows further study is necessary to decode these glossy and trichome density regions.

Discussion

For breeding shoot fly resistance, pyramiding resistance component traits appears to be the best way to develop commercially usable levels of host plant resistance, which with timely sowing (to avoid high population pressure of pest) provides

Table 4 F_2 and $F_{2:3}$-based QTL mapping on SBI-10 results obtained using PlabQTL with data from the selected 369 recombinant F_2 individuals

QTL	Pos (cM)	Marker interval	Supp. IV (cM)	LOD	R^2 %	Add*	Dom[a]
F_2QGls10	14	*Xisp10263-Xgap001*	6–20	9.67	11.37	0.69	−0.01
F_3QGls10	12	*Xisp10263-Xgap001*	6–20	5.95	6.60	0.70	0.36
F_2QTd10	58	*Xtxp141-Xisep1011*	48–70	4.40	3.70	−0.32	0.56
F_3QTd10	48	*Xisep0630-Xtxp141*	46–54	2.32	2.29	0.03	0.01

QTL Quantitative trait loci, *Pos* Position of QTL in cM, *LOD* logarithm of odds, *R^2%* Percentage of phenotypic variance, *Add* Additive, *Dom* Dominance

[a] genetic effects for the identified QTLs as per description in the PLABQTL manual (page no. 13); the additive effect is half the difference between the genotypic values of the two homozygotes

Table 5 $F_{2:3}$ phenotyping data alignment with F_2 genotype haplotypes for mapping '*Gls*' and '*Td*'

a) Haplotypes for glossiness							b) Haplotypes for trichome density						
dist in (cM)	0	4	16	34			dist in cM	–	46	56	72		
physical pos (Mb)	46.54	49.67	54.51	56.97	Pheno BLUP		physical pos	57.19	57.40	58.25	60.74	Pheno BLUP	
F_2 recombinant	*Xisep0621*	*Xisp10263*	*Xgap001*	*Xnhsbm1044*	Glossiness	No. of rec	F_2 rec	*Xiabt340*	*Xisep0630*	*Xtxp141*	*Xisep1011*	Trichome density	No. of rec
RSG04008-6	A	A	A	A	4.94	21	RSG04008-6	A	A	A	A	2.12	24
Rec1	A	A	A	B	4.94	8	Rec1	–	A	A	B	2.12	1
Rec2	A	A	B	A	1.02	3	Rec2	A	A	B	B	4.90	1
Rec3	A	A	B	B	3.00	2	Rec3	–	A	B	B	3.20	6
Rec4	A	H	B	A	1.02	2	Rec4	–	B	A	A	3.50	2
Rec5	B	B	B	A	1.02	3	Rec5	–	B	B	A	4.90	2
J2614-11	B	B	B	B	1.02	10	J2614-11	B	B	B	B	3.51	51

Table 6 Candidate genes in mapped intervals of seedling leaf blade glossiness and trichome density QTLs on sorghum chromosome SBI-10L

Marker interval: Trait	Sorghum gene ID	Description	Functional role	Reference
Xgap001-Xnhsbm1044 (2 Mb; *Gls*)	Sb10g025040	C2 calcium lipid-binding domain	C2 CaLB binds to membrane lipids and mediate signal transduction	De Silva et al. (2011)
	Sb10g025110	Cytochrome P450	Oxidoreductase activity in wax/cutin biosynthesis	Li-Beisson et al. (2009)
	Sb10g025053	glossy15/AP2/EF/ EREBP transcription factor	Controls juvenile epidermal leaf trait and epicuticular wax synthesis and cutin deposition in maize	Foerster et al. (2015)
	Sb10g024950	MYB transcription factor and DNA binding domain	Over expression of MYB transcriptional factor alters WIN1/SHN1 encodes AP2/EREBP family that encodes glossy	Cominelli et al. (2008)
Xisep0630-Xtxp141 (800 kb;*Td*)	Sb10g025600	WRKY40 transcription factor	Transparent Testa Glabra2 (TTG2) encodes WRKY transcription factor and control trichome out growth	Ishida et al. (2007)
	Sb10g026780	Speckle-type POZ protein	Expressed in Arabidopsis trichomes	Jakoby et al. (2008)
	Sb10g027280	MYB transcription factor	WD40-HLH-MYB complex regulates trichome development in Arabidopsis	Liang et al. (2014)
	Sb10g027550	C2H2 Zinc finger protein	C2H2 zinc finger protein regulates trichome cell initiation in arabidopsis	Zhou et al. (2013)
	Sb10g027610	EF-hand Ca2+−binding protein CCD1	Interacts with a microtubule motor protein and regulates trichomemorphogenesis	Reddy et al. (2004)
	Sb10g027680	Armadillo repeat protein	Sequence-specific DNA binding functional transcriptional regulator for plant development activity	Patra et al. (2013)
	Sb10g027730	F-box domain	Acts as transcriptional factors in developmental and degradation process	Coates (2008)

the most eco-friendly method for management of this pest. Combined effects of glossiness and trichome density reduce the severity of shoot fly infestation and plants with high levels of expression for both traits show better resistance to this insect pest. These morphological traits are well studied (Sharma et al. 2005; Dhillon et al. 2005, 2006; Kumar et al. 2008, 2011), genetically mapped (Sajjanar 2002; Folkertsma et al. 2003; Deshpande 2005; Mehtre 2006; Satish et al. 2009, 2012; Aruna et al. 2011; Apotikar et al. 2011) and further introgressed (Jyothi et al. 2010) into two cultivated varieties in order to deploy insect pest resistance in combination with other economically important traits like high grain and stover yields and quality. Previously these SBI-10 QTLs for trichomes and glossiness were detected in many studies, as summarized in Table 7. The average seedling leaf blade glossiness and trichome density QTLs detected were nearly 15 cM (4 Mb) in size for each trait. In the present study the size of the QTLs was reduced to 2 Mb and 800 kb for '*QGls10*' and '*QTd10*', respectively, which signifies the present study. Except for Aruna et al. (2011) (IS2122) all other QTL mapping studies, IS18551 was the donor for shoot fly resistance, but the mapping populations used varied in population size, type (segregating and recombinant inbred lines), environment and location. In the present study introgression line J2614-11 derived its SFR traits from IS18551. Regions of the sorghum genome contributing to insect resistance are mostly syntenic to maize genomic regions contributing to insect resistance, suggesting such regions were highly conserved. The glossiness QTL and possible trichome density QTL identified in the present study were detected earlier by Sajjanar (2002); Deshpande (2005); Mehtre (2006); Jyothi (2010); Aruna et al. (2011) and Satish et al. (2009, 2012). However, the present work shows evaluation of '*Gls*' and '*Td*' QTLs in the SBI-10 over different environments (late *rabi* 2011/12 and *kharif* 2012), across two seed generations (F_2 and $F_{2:3}$), different population sizes (1894 and 369), different mapping methods (QTL Cartographer for F_2 and PLAB QTL for the selected sub-set of F_2 and its derived $F_{2:3}$) and mapping approaches (traditional CIM and fine mapping) resulted in consistent QTLs.

In the present study, an initial linkage map of 37 cM length was constructed using five SSR markers on an F_2 population of 1,894 individuals derived from cross RSG04008-6×J2614-11. In previous studies this target region was reported to be above 45 cM interval but now it is 37 cM (5 Mb), which indicates a reduction in map length most likely due to population type and size. After adding three additional flanking markers and reducing the population size to 369 (selected recombinants) the map length increased to 72 cM (15 Mb), partly due to double crossovers as the recombination frequencies were converted to map distance based on the Kosambi mapping function (Kosambi 1943), but largely due to the addition of flanking markers on both ends of the mapped interval. When marker order was compared with physical map, the arrangement was the same (Fig. 3b). Mace and Jordan (2011) integrated different sorghum QTL mapping studies onto the physical map resulting in QTL clusters a in sorghum. Similarly we have compared all the shoot fly resistance QTL mapping studies in sorghum to delimit the glossy and trichome density QTL sizes on SBI-10L. The present study results are in agreement with earlier studies which shows *Xgap001 – Xnhsbm1044* and *Xisep0630 – Xtxt141* intervals need to be further studied in detail by utilizing high throughput marker genotyping or single nucleotide polymorphisms.

Due to large F_2 population, many recombination events have been found within the introgressed genomic segment originally introduced to BTx623-background from IS18551 by marker-assisted backcrossing (MABC) that affects the shoot fly reaction phenotype. The background of the parents vary for the introgressed segment and the F_2 progeny with increased number of recombinations may affect the QTL detection power when compared to recombinant inbred lines. QTL analysis can also be affected by the size of the early-generation (F_2 & F_3) and large populations can result in detection of large numbers of QTLs including minor effect QTLs (Vales et al. 2005).

In both the seasons, a single glossiness QTL (*QGls10*) was mapped near SSR locus *Xgap001*. In addition, in the post-rainy season (late *rabi* 2011/12) evaluation of the F_2 population, a QTL for leaf blade trichome density (*QTd10*) was mapped near *Xtxp141*. During rainy season (*kharif*) 2012 '*QTd10*' was mapped near to *Xisep0630*; but the QTL intervals for both the seasons were overlapping. However, F_2 and F_3 QTL mapping results, based on post-rainy and rainy season evaluations, respectively, were found similar for glossiness. Leaf glossiness characterized by deposition of less wax, or alteration in quantity and quality of epicuticular wax accumulation on leaves which may be controlling the leaf smoothness of the surface of the cuticle and could be responsible for leaf blade erectness (Li et al. 2013). A single gene may not be soley responsible for the glossy phenotype as other genomic regions influence the up- and/or down-regulation of wax synthesis, and at least four glossiness QTLs have been detected in prior studies that considered the whole sorghum genome. However, key transcription factors responsible for glossy phenotypes were consistently reported in the mapped QTL region between *Xisp10263*, *Xgap001* and *Xnhsbm1044*. This target glossy QTL (*QGls10*) was detected in both screening environments and also reported in previous studies (Sajjanar 2002; Folkertsma et al. 2003; Deshpande 2005; Mehtre 2006; Satish et al. 2009, 2012; Jyothi 2010; Apotikar et al. 2011, and Aruna et al. 2011). We looked into the genomic recombination events by traditional fine mapping, *Xgap001* was showing clear association with glossiness, and *glossy15* (Sb10g025053) gene is just 237 kb away from *Xgap001* within the mapped QTL region. Thus *glossy15* (Sb10g025053) could be a likely candidate gene for '*QGls10*' as it is known to control

Table 7 Summary of sorghum shoot fly resistance mapping studies detecting seedling glossiness (Gls) and trichome density (TD) QTLs on SBI-10

Reference	Trait of shoot fly resistance on SBI-10L	Marker interval	QTL size in cM	Closest marker	Physical map positions (Mb)	Size of QTL in Mb or kb	Pedigree	Population
Sajjanar 2002	Gls[a]	*Xgap001-Xtxp141*	34 cM	*Xgap001*	54.50–58.24	3.74 Mb	BTx623/IS18551	252 RIL
Sajjanar 2002	TD[a]	*Xgap001-Xtxp141*	34 cM	*Xtxp141*	54.50–58.24	3.74 Mb	BTx623/IS18551	252 RIL
Deshpande 2005	TD	*Xgap001-Xcup67*	22 cM	–	54.50–12.27	42 Mb	296B/IS18551	213 RIL
Mehtre 2006	TD	*Xgap001-Xcup67*	22 cM	–	54.50–12.27	42 Mb	296B/IS18551	213 RIL
Satish et al. 2009	Gls[a]	*Xgap001-Xnhsbm1043*	10 cM	–	54.50–56.88	2.38 Mb	296B/IS18551	168 RIL
Satish et al. 2009	TD	*Xgap001-Xnhsbm1043*	10 cM	–	54.50–56.88	2.38 Mb	296B/IS18551	168 RIL
Satish et al. 2009	TD[a]	*Xnhsbm1013-Xnhsbm1048*	11 cM	–	55.04–57.39	2.35 Mb	296B/IS18551	168 RIL
Aruna et al. 2011	Gls[c]	*Xtxp320-Xcup16*	17 cM	–	55.38–57.76	2.38 Mb	27B/IS2122	210 RIL
Aruna et al. 2011	TD[a]	*Xtxp320-Xcup16*	17 cM	–	55.38–57.76	2.38 Mb	27B/IS2122	210 RIL
Aruna et al. 2011	TD	*Xgap001-Xtxp320*	4 cM	–	54.50–55.38	880 kb	27B/IS2122	210 RIL
Satish et al. 2012	Gls[a]	*Xgap001-Xnhsbm1011*	5 cM	*Xgap001*	54.50–54.90	400 kb	296B/IS18551	168 RIL
Satish et al. 2012	Gls[b]	*SvpepcA-XnhsbmSFC4*	5.9 cM	–	47.13–46.50	630 kb	296B/IS18551	168 RIL
Satish et al. 2012	Gls[c]	*XnhsbmSFC34-Xnhsbm1039*	8 cM	–	57.83–58.24	410 kb	296B/IS18551	168 RIL
Satish et al. 2012	TD	*Xgap001-Xnhsbm1011*	5 cM	*Xgap001*	54.50–54.90	400 kb	296B/IS18551	168 RIL
Satish et al. 2012	TD[a]	*XnhsbmSFC34-Xnhsbm1039*	8 cM	–	57.83–58.24	410 kb	296B/IS18551	168 RIL
Present study	Gls[a]	*Xgap001-Xnhsbm1044*	10 cM	*Xgap001*	54.50–56.96	2.46 Mb	RSG04008-6/J2614-11	1894 F_2
Present study	TD[a]	*Xisep0630-Xtxp141*	8 cM	–	57.40–58.24	800 kb	RSG04008-6/J2614-11	1894 F_2
Present study	Gls[a]	*XIsp10263-Xgap001*	14 cM	*Xgap001*	49.67–54.50	4.83 Mb	RSG04008-6/J2614-11	369 $F_{2:3}$
Present study	TD[a]	*Xtxp141-Xisep1011*	8 cM	*Xtxp141*	58.24–60.74	2.5 Mb	RSG04008-6/J2614-11	369 $F_{2:3}$

Gls Glossiness, *TD* Trichome density, J2614-11=IS18551 introgression line in BTx623 background, [a] co-localization of QTL, [b]/[c] New QTLs

transcriptional regulation of *glossy* phenotype expression. This suggests that '*QGls10*' needs to be studied further using a fine-mapping approach with higher density markers in this region, and other possible candidate genes in the target interval.

The seedling leaf blade trichome density '*QTd10*' was better expressed in the post-rainy season (*rabi*, characterized by lower temperatures and shorter photoperiods) than in the rainy season, but in both F_2 and $F_{2:3}$ segregating populations it was detected in the same support interval. In order to see the recombination events in the support interval the '*QTd10*' QTL was highly associated with *Xtxp141* and *Xisep0630*. Trichome density is largely dependent on the environmental factors and is a complicated trait to measure. More precise microscopic field observations of trichome density may resolve the location of its controlling genomic regions – but these were not practical for the large number individuals observed in the full F_2 population. Presence of '*QTd10*' within the same support interval (*Xisep0630-Xtxp141*) across generations and seasons showed the consistency of the QTLs in sorghum molecular mapping of component traits for shoot fly resistance..

F_2 and $F_{2:3}$ QTL Mapping on Selected 369 Individuals

A consistent QTL was detected in two different seasons with two different generations, confirming the presence of a QTL region for seedling leaf blade glossiness that needs to be finely mapped in this population with a larger number of polymorphic molecular markers. We conclude that one QTL for glossiness score (with the glossy allele originating from donor parent IS18551) is present in the SBI-10L target region. QTLs for trichome density mapped differently in the post-rainy and rainy seasons, but within a support intervals sharing a common marker, *Xtxp141*. To clearly differentiate these F_2 and $F_{2:3}$ '*QTd10*' QTLs, increased marker density and more efficient phenotyping is required. Fine mapping of these QTLs will improve our understanding of the molecular basis of seedling leaf blade glossiness and trichome density traits (important morphological component traits contributing to sorghum shoot fly resistance). As the glossiness and trichome density QTLs were consistent in both the F_2 and F_3 generations but showed deviation in the F_2 population sub-set (Fig. 4b).

In F_2 sub-set rate of recombination has increased due to selected recombinants with heterozygous nature, which will increase the recombination fraction and this could affect the QTL detection power and may increase the rate of false discovery rate (FDR) QTLs. Sometimes missing marker data and segregation distortion in early generation population like F_2 may lead to disturbance in estimation of QTL position and its effects. As F_2 selected informative recombinants are highly distorted from the normal Mendelian segregation and increased heterozygosity may increase the dominance effect of the detected QTL, which may be due to over dominance effect or the pseudo over-dominance effect of the QTL. Segregating populations (F_2 and $F_{2:3}$) have heterozygous variant regions which complicate the gene action during linkage repulsion phase of two dominant alleles results in over dominance or pseudo over dominance.

When both the loci are dominant which may result in over dominance as in case of trichome density both the parents are contributing low to medium and medium to high trichome density so overall trichome density was more in the total. The statistical analysis methods, experimental designs and the phenotyping techniques variation could also affect the dominance and over dominance effects of the detected QTL (Schnable and Springer 2013). QTLs from resistant parent express dominance or over dominance; but if they segregate in the next generation they may not be detected due to less trait variation or other genomic regions might have more influence in phenotype expression. This may also be due to environment effect on trichome density levels leading to less phenotypic variation which cannot separate the genomic regions responsible for the phenotypic variation in the target QTL region detected on SBI-10L.

Major Component Traits of Shoot Fly Resistance

Glossiness

Leaf glossiness trait has multiple functions in biotic (shoot fly resistance) and abiotic stresses (drought, salinity, high temperature). Glossiness is visually observed as erect, narrow, pale green and shiny leaf appearance termed as the glossy trait but, all the characteristics may not be controlled by same gene. Cuticular waxes on leaf could be the reason for the glossy phenotype. Water sprinkling method on leaves differentiates non-glossy leaves from glossy leaves by adherence and non-adherence of water droplets, respectively (Tarumoto 1980). Scanning electronic microscopic observations show increased number of wax crystals on leaf surface of non-glossy leaves compared to glossy leaves (Tarumoto et al. 1981).

Candidate Genes for Glossiness

Seedling leaf blade glossiness variation was observed between the two mapping population parents and QTL analysis conducted on 1,894 F_2 high resolution population and 369 F_3 selected genotypes resulted in identification of very similar QTLs, which we consider to be a single entity viz., '*QGls10*'. In both phenotyping generations, this glossiness QTL was mapped near to SSR marker *Xgap001*. The mapped QTL region was searched for candidate genes and several wax synthesis-related genes were found. One of the candidate genes related to wax synthesis and deposition of wax present in the QTL region has a C2 calcium lipid-binding domain (Sb10g025040), which is involved in plant stress signal

transduction, and this C2 domain was able to bind membrane lipid ceramides (de Silva et al. 2011). These wax-deficient mutant loci in maize, brassica and sorghum are defined as '*glossy*' loci and in *Arabidopsis* and barley are named as *ceriferum* (*cer*) mutant loci (Kunst and Samuels 2003, 2009).

One of the candidate genes, *glossy15* (Sb10g025053), encodes an *APEPETAL2* (AP2) -like transcription factor involved in the transition from juvenile leaf epidermis characteristics to adult leaf epidermis characteristics, and is expressed after second leaf growth stage (Moose and Sisco 1994, 1996). AP2/ERF transcriptional factors are reported to be involved in wax biosynthesis (Tiwari et al. 2012). Recently Go et al. (2014) reported AP2/ERF (Sb10g025053) acts as a bi-functional transcriptional factor that down regulates the wax biosynthesis pathway by interacting with promoter regions of wax synthesis proteins. MYB transcription factor present in the mapped glossy QTL region (Sb10g024950) has been reported to be involved in activation of AP2/ERF transcription factors involved in wax biosynthesis (Cominelli et al. 2008)

Trichome Density

Trichomes are non-glandular, cellular appendages that protrude above the epidermis (Maiti and Gibson 1983). Trichomes act as physical barriers between the insect pests and the leaf blade epidermis that inhibit egg laying and/or larval movement, which leads to reduction in 'dead heart' formation. Trichome density is genetically controlled and negatively correlated with oviposition- and dead heart incidence-based (Maiti and Gibson 1983; Dhillon et al. 2005) measures of susceptibility sorghum shoot fly.

Candidate Genes for Trichome Density

An MYB transcription factor gene homolog (Sb10g027280) is present in the trichome density QTL region-. Liang et al. (2014) showed that in *Arabidopsis* a WD40 + HLH + MYB transcriptional factor complex regulates the trichome initiation process programmed by cell development. This complex recognizes the specific DNA motifs in gene regulatory regions to activate or repress transcription, mostly by interacting with other proteins like Armadillo repeats, Speckle-type POZ-like proteins, F-box domain proteins, WRKY proteins, MYB transcription factors, ethylene zinc finger proteins, EF-hand Ca^{2+}-binding proteins, and thumatin-like proteins. In *Arabidopsis thaliana*, *TRANSPARENT TESTA GLABRA2* (*TTG2*) encodes a WRKY transcription factor and is expressed in young leaves, trichomes, seed coats, and root cells which are not involved in root hair production. During epidermal cell differentiation, MYB transcription factors and HLH transcription factors regulate *TTG2*, which modulates *Glabra2* expression in trichomes (Eulgem et al. 2000; Johnson et al. 2002; Ishida et al. 2007). One additional WRKY transcription factor gene homolog (Sb10g025600) is present in the target trichome density QTL region; this is one of its probable candidate genes. An ethylene zinc finger protein gene homologous with Sb10g027550 has a key role in regulating trichome development in *Arabidopsis*. ZFP5 and ZFP6, the zinc finger proteins, necessary for gibberellic acid and cytokinin signalling to regulate trichome cell differentiation (Zhou et al. 2013). An Armadillo repeat protein gene that appears to be homologous to Sb10g027680 regulates both the gene expressions and cell-cell adhesion. Patra et al. (2013) demonstrated that *ubiquitin protein ligase3* (*upl3*) N-terminal domain has Armidallo repeats that interact with the C-terminal domain of *Glabra3*/*Enhanced Glabra3* for trichome development in *Arabidopsis*. An F-box domain protein homologous to Sb10g027730 also has Armadillo repeats that may act as transcriptional factors and involved in the degradation process plant developmental processes (Coates 2008). An EF-hand Ca^{2+}-binding protein gene homolog (Sb10g027680) is also one of the candidate genes underlying the putative *QTd10* trichome density QTL. Kinesin-like calmodulin (KIC) is a EF-hand Ca^{2+}-binding protein that interacts with a microtubule motor protein and regulates trichome morphogenesis. Over expression of KIC inactivates kinesin-like calmodulin binding protein (KCBP) by disrupting its interaction with microtubules and its participation in trichome morphological complex resulting in trichomes with less branches/no branches (Reddy et al. 2004). Jakoby et al. (2008) mentioned Speckle-type POZ proteins (homologous to Sb10g026780) were also expressed in trichomes.

In case of the glossiness QTL the increase in score value indicates non-glossiness and lower scores are more preferred for the trait (glossy). Glossiness is also inherited from resistant parent where the moderately large F_2 population had more dominance effect due to large population size and high scores which could influence the dominance nature of the detected QTL. In both generations, a glossiness QTL was detected within the same support interval. This confirms that a single glossiness QTL is located in the target marker interval. Further, fine mapping and focused gene expression studies can be carried out utilizing this high resolution cross. This should reveal which of the underlying candidate genes is responsible for the observed variation and its functional role. In contrast, the putative QTL for trichome density on the lower surface of seedling leaf blades, thought to have been introgressed from grandparent IS18551 into BTx623-background line J2614 by Jyothi et al. (2010), was detected in the full F_2 population and the sub-set of 369 informative recombinants selected from this under lower-temperature, short-day length post-rainy conditions, but was not clearly detected in the derived F_3 progenies when these were evaluated in the rainy season. This warrants considerable further study – starting with phenotyping of the same F_3 progenies

for lower leaf blade trichome density during the post-rainy season using available remnant seed. Expression of this QTL only under post-rainy conditions vs. rainy season conditions would warrant considerable further study to understand environmental regulation of this QTL for this trait. Based on F_2 genotyping data of 7 co-dominant SSR markers and $F_{2:3}$ phenotyping data, we have selected a further reduced sub-set of 182 most informative recombinants, and selfed their corresponding F_3 progenies to produce F_4 seeds which can go for replicated field trials and can be used for further study to restrict the genomic region that appears to contribute to the control of sorghum seedling leaf blade glossiness and lower surface trichome density (Usha Kiranmayee et al. 2015a).

Implications of This Study in Breeding Program

As both the parental lines are introgression lines, but having different genetic backgrounds, the background noise for the interested traits has not been reduced substantially. None-theless, we could identify genotypes having combinations of RSG04008-6 stay-green (drought tolerance) trait with glossines and trichome density. Selfing until homozygosity of the pyramided genotype should lead to development of a multiple resistance trait donor for use in breeding and crossing programs.

Materials and Methods

Parents

Parent J2614-11 (glossy, highly trichomed) is a single plant selection from a shoot fly resistant introgression line derived from IS18551 alleles introduced by MABC into BTx623 background having validated donor alleles for seedling leaf blade glossiness and trichome density on SBI-10 (Fig. 1b) (Jyothi 2010). RSG04008-6 (non-glossy, less trichomed) is a single-plant selection from a high yielding drought tolerant but shoot fly susceptible introgression line (IL) with E36-1 alleles for stay-green-associated drought tolerance in highly senescent R16 background (Fig. 1a) (Kassahun 2006).

Plant Material: Development of the F_2 Population and Recombinant $F_{2:3}$ Progenies

At ICRISAT-Patancheru, a manually emasculated and pollinated plant×plant cross was made between RSG04008-6 (susceptible) and J2614-11 (resistant) during post-rainy 2010 to produce F_1 seeds. Morphologically and genotypically confirmed F_1 plants were self-pollinated using selfing bags to produce F_2 seed lots. A moderately-large, high-resolution mapping population of 1,894 F_2 individuals derived from a single selfed F_1 plant (U110055), was grown in three batches in plastic pots during late post-rainy (late *rabi*) 2011 (Feb - Jun 2012) with triply-repeated parents for each F_2 sowing. The parental population were thinned to 3 plants per plot per sowing. Plants were labelled individually (with plant number starting from U120001 to U121931) for F_2 progenies, while parents were tagged with their names (Fig. 4c). DNA samples of a emerged F_2 plants and parents (9 repeats in total for each parent) were isolated and genotyped initially with 5 simple sequence repeat (SSR) markers namely *Xgap001*, *Xnhsbm1044*, *Xiabt340*, *Xisep630* and *Xtxp141* on SBI-10L where QTLs for seedling leaf blade glossiness and trichome density from donor parent IS18551 had previously been mapped, and then introgressed into BTx623 background to produce parent J2614-11 (Jyothi 2010). By careful examination of the genotyping data across the target QTL region a subset of homozygous and nearly homozygous recombinant F_2 plants was selected. All the selected sub-set of recombinant F_2 individuals were advanced by selfing to the F_3 generation (Fig. 5). All 1,894 individual F_2 plants were scored for both of the target traits, i.e., seedling leaf blade glossiness abbreviated as '*Gls*' and seedling leaf blade trichome density abbreviated as '*Td*'. The selected subset of recombinant F_3 progenies were sown in the field during rainy season (*kharif*) 2012 (July), in a single plot of 4 m per entry with 10–12 plants per plot after thinning, with two replications of parent. Phenotyping in F_3 generations was similar to F_2 generation.

Phenotyping for Traits

F_2 plants were tagged and scored individually for the two traits during *rabi*11/12, whereas in the $F_{2:3}$ generation the plants were segregating for traits within each family; so maximum group of plants with similar phenotype were scored for each genotype family during *kharif* 2012. Seedling leaf blade glossiness was scored visually at 12–15 days after emergence (DAE) as described in Sharma et al. (1992) where 1=shiny, pale green, pointed, narrow and erect leaves (glossy) and 5= dull, dark green, broad and droopy leaves (non-glossy) (Fig. 1a and b). Leaf blade trichome density was scored by visual appearance of trichomes on leaves as described in Bourland et al. (2003), but based on the trait variation, in the present population, scores were defined as follows: As trichomes are hairy leaf structures, leaf surface roughness indicated degree of trichome density and smooth leaf surfaces indicated absence of trichomes. Scores were given as 0=absent, 1=very low density, 2=low density, 3=medium density, 4=high density, 5=very high density.

DNA Isolation and Genotyping

Single plant DNA was extracted from each of the 1,894 F_2 seedlings, and each sample of the two parents, using a modified-CTAB and phenol:chloroform:isoamyl alcohol

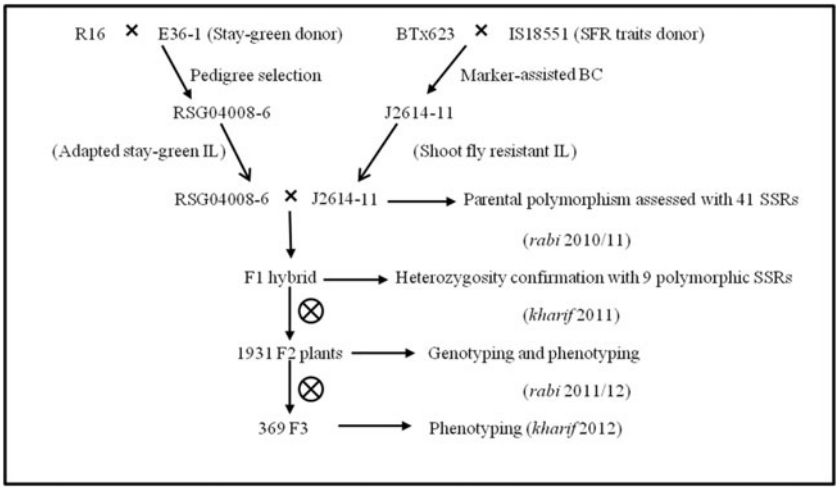

Fig. 5 Schematic representation for developing ILs and their genetic material from ILs for mapping '*QGls10*' and '*QTd10*' on SBI-10L

(25:24:1) method as described by Mace et al. (2003). DNA was quantified on 0.8 % agarose gel and normalized to 2.5–5 ng/μl using distilled water. PCR was performed in 5.0 μl reaction volumes with 1.0 μl of normalized DNA, 2.0 mM $MgCl_2$, 0.1 mM of dNTPs, 1× PCR buffer, 0.4 pM of each primer and 0.1 U of DNA polymerase enzyme using a Gene Amp® PCR system 9700 thermal cycler (Applied Biosystems®, USA).

PCR products were resolved by capillary electrophoresis on an ABI 3730 DNA sequencer (Applied Biosystems®, USA) and the data generated was analysed with Genemapper® v4.0 software (Applied Biosystems®). This analysis provides a series of automatic fragment sizing, allele scoring, bin-building and auto panelize algorithms that helped in calling A', 'B' and 'H' allele scores ('A'=homozygous for allele of RSG04008, 'B'=homozygous for allele of J2614-11, and 'H'=heterozygous) for PCR products from each SSR primer pair. A set of 41 SSR markers mapping single-copy loci in the extended target region of sorghum chromosome SBI-10L (Table S3). For parental polymorphism assessment a sub-set of nine ([1]*Xgap001*, [2]*Xnhsbm1008*, [3]*Xnhsbm1011*, [4]*Xisep0643*, [5]*Xiabtp389*, [6]*Xnhsbm1044*, [7]*Xiabtp340*, [8]*Xisep0630*, [9]*Xtxp141*) polymorphic SSRs were identified for the initial target marker interval region of *Xgap001-Xtxp141*.

Due to the large F_2 population, initially a set of five SSRs distributed across the *Xgap001-Xtxp141* interval on SBI-10L were selected based on their amplification, segregation patterns, good polymorphism between parents, and clear peak patterns for GeneMapper® analysis. SSR markers *Xgap001* (Brown et al. 1996), and *Xtxp141* (Bhattramakki et al. 2000) were previously identified as flanking markers for the target region (Deshpande 2005; Sharma et al. 2005; Dhillon et al. 2006; Mehtre 2006; Jyothi 2010; Ramu et al. 2010). Three SSR markers mapping between these, viz. *Xnhsbm1044* (Satish et al. 2009), *Xisep0630* (Ramu et al. 2009), and *Xiabt340* (Ramu et al. 2010), were selected based on their physical map positions, polymorphism and distribution across this interval. To extend the flanking regions on either side of the target interval, three additional SSR markers (*Xisep0621*, *Xisp10263* and *Xisep1011*) were later added. Due to large percentage of missing data (>50 %) in 369 selected recombinants, SSR marker *Xiabt340* was dropped from linkage analysis, and a new linkage map was developed with seven SSRs (Fig. 3a and b).

Statistical Analysis

From the F_2 and F_3 generations, observed phenotyping data was analysed using SAS software package (SAS Institute, USA). A PROC – MIXED augmented design analysis using 'entries' as random model was used to provide covariance parameter estimates and Best Linear Unbiased Predicted means (BLUPs) were derived (with *Z* estimates). Heritabilities values (h^2) were estimated from the covariance parameter values. The F_2 population was sown in 3 blocks, with each block including 600–650 individual F_2 plants along with three replicates of the parental checks in each block. Block effect was estimated from the means of repeated checks and adjusted for each F_2 phenotype value in each block in order to minimize the rate of experiment-level error. In case of the F_2-derived F_3 progenies, parents repeated twice were used as checks.

Linkage Map Construction and QTL Analysis

F_2-population

To tide over the resources and time required for genotyping the complete F_2-population, we strategically selected only five (*Xgap001*, *Xnhsbm1044*, *Xiabt340*, *Xisep0630*, *Xtxp141*) out of total nine available polymorphic SSR markers spanning the initial target region (*Xgap001-Xtxp141*). Genotyping data on 1894 F_2 population for these five SSRs was generated and

used as input for JoinMap V3.0 (Van Ooijen and Voorrips 2001). This data was used to select a sub-set of informative F_2-individuals (to develop F_3 progenies) capturing the recombination events in steps. The Kosambi map function was used to convert recombination fractions into centi-Morgans (cM) (Kosambi 1943). Marker order was assigned at minimal LOD 3 and segregation distortion and *chi*-square values were calculated using JoinMap V3.0 (Van Ooijen and Voorrips 2001). QTL mapping for the 1,894-entry F_2 population was performed using Composite Interval Mapping (CIM) implemented in QTL Cartographer Windows V2.5 (Wang et al. 2010) with default settings (window size of 10 cM, walking speed of 1 cM, control markers=5 and backward regression). Significance of each QTL interval was determined with the threshold level estimated at 1000 permutations with $P \leq 0.05$ for significant QTL detection.

$F_{2:3}$ population

Genotyping data of additional three markers (*Xisep0621*, *Xisp10263* and *Xisep1011*) on selected 369 F_2 was used for generating a new linkage map with 7 SSR markers with JoinMapV3.0 (Van Ooijen and Voorrips 2001). The phenotyping data for the 369 selected recombinant F_3 progenies along with their respective F_2 individuals was merged with the corresponding F_2 genotyping data, and QTLs were positioned and their effects estimated by CIM (Zeng 1994; Jansen 1994) implemented in PLAB QTL version 1.2 (Utz and Melchinger 1996) for both target traits. LOD 2 was set as criteria for detecting QTLs at 1000 permutations. QTL Cartographer Windows V2.5 was used for F_2-population QTL analysis and we used PLABQTL for $F_{2:3}$ progenies.

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Biotechnological Approaches to Evolve Sorghum (*Sorghum bicolor* (L.) Moench) for Drought Stress Tolerance and Shoot fly Resistance

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Abstract

Sorghum is a model tropical grass that uses C_4 photosynthetic activity.But its yield is affected by many abiotic stresses likeheat, drought, cold,salt and also biotic stresses such as shoot fly, midges, and stem borerfromseedling stages to maturity. This article summarizes the terminal drought stress tolerance mechanism with stay-green phenotype expression during post-flowering and also mechanisms of early shoot fly resistance during seedling stages of crop growth. The trait stay-green is extensively studied and its correlation to yield makes the stay-green trait more special for research and in marker assisted back cross programs. Under terminal drought stress conditions, stay-green trait is expressed with a complex mechanism involving many transcription factors, chlorophyll retention and nitrogen remobilization from leaves to maintain longer photosynthetic activity. Shoot fly resistance on the other hand, involves manyphysico-chemical, biologicaland morphological traits. Out of the many morphological traits, seedling leaf blade glossiness and trichome density are well characterized at genetic level and can assist as shoot fly resistance sources in marker-assisted breeding programs as they are highly negatively correlated with shoot fly dead heart formation. However, quantitative trait loci (QTL) mapping studies and candidate genes identified for the stay-green and shoot fly component traits need to be further validated with fine mapping, gene cloning and expression level studies. Pyramiding these two traits into a high yielding sorghum variety may lead to multiple stress resistance which could ultimately benefit the marginal farmers in India.

Keywords: Sorghum, shoot fly resistance, stay-green, drought tolerance, QTL, marker-assisted selection

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a cultivated tropical crop plant that belongs to the family Poaceae, tribe Adropoganeae and genus *Sorghum*. Sorghum is largely self-pollinated diploid crop (2n=2õ=20) with fully sequenced genome length of ~730Mb (1). It is the fifth most important cereal crop globally (2) providing food, feed, fiber, fuel, and chemical/ biofuels feed-stocks across a range of environments and production systems.USA, India, México, Nigeria, Sudan and Ethiopia are the major producers of sorghum. Other sorghum producing countries include Australia, Brazil, Argentina, China, Burkina Faso, Mali, Egypt, Niger, Tanzania, Chad and Cameroon. Grain is mostly used as food (55%), in the form of breads and porridges in Asia and Africa, and as feed (33%) in the Americas. Its stover is an increasingly important source of dry season

fodder for livestock, especially in Asia (http://www.icrisat.org/crop-sorghum.htm). Its remarkable ability to produce yields under adverse conditions like arid and semi-arid regions, where water limited conditions exists alongside heat stress. This makes sorghum an important 'fail-safe' source of food, feed, fiber, and fuel in the global agro-ecosystem. Sorghum is a representative of tropical grasses that use C_4 photosynthesis, which results from complex biochemical and morphological specializations that improve carbon assimilation at high temperatures. While the world's average annual yield for sorghum was 1.08 tonnes per hectare in the year 2012, total production from all sorghum producing countries was 57 million tonnes. FAO reported the United States of America as the top sorghum producer with a harvest of 1.22 million tonnes followed by India, Nigeria, Mexico and Sudan (3). In India, with its large population and fragile balance in the production and demand equation for food grains, sorghum plays a crucial role in national food security. Attempts to increase the production of sorghum with the introduction of new high-yielding varieties and hybrids since 1966, was largely unsuccessful because of the susceptibility of the improved cultivars to various abiotic (drought)and biotic(shoot fly) (4,5,6,7)stresses. But therate of loss due to biotic and abiotic stresses in sorghumyear by year is increasing.

Drought stress and stay-green trait : Abiotic stresses are the most harmful constraints concerning the growth and productivity of crops worldwide. After soil nutrient deficiency, drought stress is the most important abiotic constraint for sorghum production globally(8) Sorghum is well adapted to semi-arid environments and regarded as model crop for studying drought stress tolerance among grass species. So, breeders mostly have focused on improving drought stress tolerant varieties of sorghum (9). If plants withstand drought spell occurring at grain filling stage, it is defined as terminal drought tolerance. Drought stress during and after flowering typically causes premature leaf senescence which in turn lead to stalk lodging, stalk rot disease, reduced grain filling, and significant grain and stover yield losses.Plant characters best associated with post-flowering drought tolerance, may be due to delay in leaf senescence or non-senescence or "stay-green" trait(9,10,11,12,13,14,15,16). Therefore, the "stay-green" trait is more than the ability of the plant to maintain functional green leaf area (GLA), to improve quality of residues (17), to support the continuation of carbon fixation and supply of starch to the grain filling site (18), to prevent premature death and stalk lodging (19) andto sustain grain-filling under water stress to improve yield (14,20).Stay-green is of three types. Type A stay-green phenotypes have a delayed onset and a normal rate of senescence following its onset. Type B stay-green phenotypes initiate leaf senescence normally but the rate of senescence is comparatively slower. Type C stay-green phenotypes retain chlorophyll despite the normal onset and progression through senescence(21). Many crop plants other than sorghum like rice, wheat, maize, barley, cotton, tobacco have been reported till date with stay-green character.

Mechanism of drought tolerance/stay-greenand factors associated with stay-green : Molecular mechanisms underlying delay in senescence which extend the duration of active photosynthesis in sorghum have not been elucidated completely. Rosenow et al. (20) observed positive impact of delayed leaf senescence on crop performance of plants under water limited conditions during grain filling. Presence of stay-green phenotype is a result of balance between nitrogen (N) demand by grain and nitrogen captured by vegetative parts of plants like increasing the supply of water by modified root architecture which increases water extraction from soil or reducing water demand by reducing transpiration loss. Nitrogen remobilization from leaves maintain longer photosynthetic activity and supply adequate carbohydrates to developing grains (10,22,23). It appears that carbon, nitrogen ratios and ABA levels affect senescence. Besides them,

cytokinins also play a role in leaf senescence and increased production of cytokinins lead to delayed leaf senescence (24).Stay-green was influenced by genetic factors, environmental factors like high temperature, soil-water holding capacity, soil moisture content at planting, vapor pressure depict, rain fall during cropping and management factors like population size and planting time (14). Leaf chlorophyll content was also significantly correlated with stay-green scores under drought conditions as pointed out by Xu et al. (25).

Nodal root angle depends on vertical and horizontal distribution of rootsin soil. Theirprofile is relevant to drought adaptation and is co-localized with stay-green genomic regions which show that roots and their growth are related tostay-green phenotype expression (12,26,27).Stay-green is highly negatively correlated with flowering time and stover yield (9). These correlation studies indicate early flowering is associated with green leaf area. But, stay-green shows positive association with grain yield (9,11,14). Stay-green is negatively correlated with flowering time, canopy size, size of upper leaf,tillering. Under drought conditions stay-green enhances grain yield, by altering the canopy development and modifyingthe size of the leaf (leaf anatomy), root growth (nodal root angle) and water uptake mechanisms (11,12,28). Reduction in leaf size leads to transfer of photosynthetic nutrients to grains without undergoing the drought stress.

Identification of genetic factors involved in stay-green : Genomic regions responsible for stay-green trait were detected with the help of molecular markers and the phenotyping data of the stay-green lines locate the variation in the genomic regions which are important for drought tolerance breeding programs. Quantitative trait loci(QTLs) for stay-green have much importance in improving the productivity under drought stress conditions (23).Many QTL mapping studies contributing to stay-green expression under drought stress conditions have been evaluated in mapping populations (8,15,29, 30,31,32,33, 34,35,36,37,38) introgression lines (9) and near isogenic lines (29,30,31,33,34,35,15). Several stay-green sources have been field evaluated and used for crosses (39,40). Best stay-green sources are B35,E36-1,and SC56 that are involved in different marker assisted breeding programs. Cross B35 (stay-green) × R16 (senescent) was developed (9) and their introgression lines were field evaluated.B35 (stay-green) × Tx7000 (senescent) was also extensively studied and their introgression lines were used for fine mapping of different stay-green QTLs (15,33,35). B35 × Tx430 (32), SC56 × Tx7000 (36), N13× E36-1, IS9830 × E36-1 (8),M35-1× B35 (16) crosses were made and different stay-green QTLs were identified.Stay-green was extensively studied in crops other than sorghum like in maize (41), wheat (42), barley(43), rice (44),and *Arabidopsis*(45).It appears therefore that stay-green genotypes need to be utilized in sorghum breeding programs aimed at developing drought tolerant plants.

Marker-assisted breeding for stay-green : Drought stress may be alleviated by developing crops that are well adapted to dry-land environments with marker assisted breeding crop improvement programs.Increasing marker density and identifying QTLs and narrow downing the QTLs to smaller regions will improve marker assisted breeding.Different types of stay-green QTLs are influenced by different backgrounds (28) and many crossing programs introgressed stay-green into senescent breeding lines. Therefore, marker assisted breeding programs help us develop drought tolerant lines in sorghum.

Stay-green candidate genes : An alteration in the chlorophyll break down mechanism influenced by many key factors like plant hormones, transcriptional factors and genes lead to delayed degradation of chlorophyll.Cytokinins are plant hormones involved in regulating senescence process, and thecytokinin receptor (AHK3), the type-B response regulator (ARR2) and the recently identified cytokinin response factor (CRF6) are involved in senescence signal responses (46). No apical meristem (NAC/NAM)

transcriptional factor is a developmental regulator and accelerates senescence and increases nutrient remobilization from leaves to developing grains (47).In *Arabidopsis*,*At*NAP encodes NAC transcription factor which is closely associated with senescence (48).OsNAP is a NAC transcriptional activator identified in rice involved in senescence pathway. Reduced OsNAP expression lead to improved grain filling and seed setting and subsequently increased grain yield (49).Senescence associated genes (SAGs) were up- and downregulated under stress conditions (50). Chlorophyll catabolic enzymes and STAYGREEN1 (SGR1), STAYGREEN2 are regulators of chlorophyll degradation and their mutants (sgr) exhibit stay-green phenotype which is a desired phenotype for drought tolerance (45).WRKY family transcriptional factors are also involved in senescence pathwayand over expression of WRKY transcriptional factors lead to improved drought tolerance (51). Thus, the above candidate genes appear to be crucial for imparting drought stress tolerance. Their overexpression in sorghum can certainly lead to transgenic sorghum lines that can withstand water limited conditions.

Shoot fly resistance : Apart from abiotic stresses, many biotic stresses are caused by plant pathogens and insect pests.Nearly, 150 species of insect pests damage sorghum, of which sorghum shoot fly *Antherigonia soccata* (Rondani), is the major insect pest in Africa, Asia and Mediterrian Europe (6). Shoot fly belongs to the family Muscidae and is a devastating pest in sorghum. It mostly attacks tropical grass species like wheat, barley and sorghum.Female shoot fly lays white, elongated,cigar shaped eggs singly on abaxial (lower) surface of leaf, parallel to mid-rib. Eggs hatch in 1-2 days of incubation and larvae crawl into central leaf whorl and cuts the growing tip resultingin typical wilting and drying of the central whorl leaf known as 'dead heart'. As a result of dead heart formation, the young seedlings may be killed outright or they may produce axial tillers, which are rarely productive. The axial tillers serve as a mechanism of recovery resistance if they remain undamaged, but if shoot fly infestation continues, the seedling may die or present a rosette appearance and fail to produce any grain (52). Larvae feed on the decaying tissuewhich may lead to seedling mortality and the crop gets damaged within 1-4 weeks after seedling emergence.

Mechanisms of shoot fly resistance : Agronomic practices (timely sowing), natural and synthetic insecticides, natural enemies and host plant resistance (HPR), are all components of integrated pest management practices used to minimize sorghum losses due to shoot fly infestation. Early sowing during rainy season can also be one of the resistance mechanisms (53); but HPR and timely sowing remains most preferred as they are cost-effective, eco-friendly and easily adapted by farmers. Mechanism of resistance to shoot fly is complex and depends on interplay of many component characters of plant, insect and environmental factors (54).Improvement in resistance will increase ecological fitness, reduces pesticide use, and facilitates creation of a sustainable production system with increased efficiency, profitability and enhances grain quality traits. Antixenosis for oviposition is the primary mechanism of resistance for shoot fly resistance in sorghum (55,56). Antibiosis and tolerance also plays important shoot fly resistance mechanism (52,57).Of many important morphological components of sorghum HPR identified, seedling leaf blade glossiness (58), seedling leaf blade trichome density (59), seedling vigor, and leaf sheath pigmentation are all positively associated with Shoot Fly Resistance (SFR). Leaf glossiness reflects the flies from the host and increased trichome density inhibits the larval movement on leaf surface and acts as barrier between the leaf and fly to prevent egg laying (antixenosis)(60). Rapid growth of seedling due to seedling vigor inhibits the larvae movement to reach the central leaf whorland this reduces the frequency of dead hearts(60). Cytoplasmic male sterilityalso influences the expression of shoot fly resistance mechanism (61,62). Chlorophyll content and leaf

surface wetness, and waxy bloomhave been reported to be associated with shoot fly susceptibility (63). Increased secondary metabolites also take path in shoot fly resistance mechanism (64). Shoot fly resistant genotypes were used in the breeding programs as a source forresistance. Genotypes such as IS2122, IS18551, IS2146, IS1054, IS2312, SFCR151, ICSV705, SFCR125 were used in many crossing programs as resistant donors for shoot fly resistance (65,66,67). However, many of these resistance mechanismsstill need to be evaluated clearly at the molecular level. Genes associated with these mechanisms and their cloning and overexpression studies are also needed for validation.

Factors associated with shoot fly resistance : Resistance to shoot fly is mediated by many physico-chemical, morphological,biological, environmental, biochemical, cytoplasmic and genetic factors.Chemicals and pesticides were used to control shoot flies in the field. Fipronil and imidacloprid were successfully evaluated for shoot fly control (68).As the chemicals and pesticides are not affordable by poor farmers and can cause serious environmental hazards,it is necessary to develop cultivars with shoot fly resistance with the help of marker assisted back cross (MABC) methods (64). Morphological traits like seedling leaf blade glossiness, trichome density in lower and upper leaf portions,leaf sheath pigmentation, seedling vigor are negatively correlated with percent shoot fly 'dead heart' and positively associated with shoot fly resistance.Significant correlation was observed between shoot fly dead hearts and yield(53). Morphological components like glossiness and trichome density are negatively correlated to shoot fly dead heart percentage and are significantly associated to shoot fly resistance. Combined effects of trichome density on abaxial (lower), adaxial (upper) and leaf glossiness have been shown to reduce dead heart percentage and high shoot fly resistance (66).These observations point out that glossiness and trichome density are vital for shoot fly resistance in sorghum. Environment is a major factor associated with shoot fly resistance as the rainy(Rabi) season is most suitable for shoot fly infestation when compared to the post rainy (Kharif) season.Biochemical factors like p-hydroxy benzaldehyde, cinnamic acid, luteolin, apigenin, and some unidentified compounds from damaged and undamaged seedlings of sorghum were associated with expression of resistance for shoot fly as pointed out by Chamarthi et al. (69). QTLs associated with shoot fly resistance have beenidentified in many populations and different crosses responsible. However, candidate genes need to be identified and validated in sorghum. SFR component traits have been mapped and the putative QTLs identified for individual traits and subsequently validated by marker-assisted backcross (MABC)-introgression into genetic backgrounds highly susceptible to shoot fly. The cross BTx623 × IS18551(70, 71, 72, 73) mapped the shoot fly resistance (SFR) QTLs on SBI-01, SBI-05, SBI-07, and SBI-10. Similarly, using crosses 296B (susceptible) × IS18551 (resistant) (60,74) and cross 27B (susceptible) × IS2122 (resistant) (75)mapped the SFR. In a reciprocal cross IS18551 × 296B, Apotikar et al.(76) found SFR QTLs on SBI-01 and SBI-03. Five putative QTLs for SFR component traits from IS18551 were then validated by MABC-introgression into the genetic backgrounds of elite shoot fly-susceptible hybrid seed parent maintainer lines 296B and BTx623 (77). Thus, these studies point out that it is possible to transfer shoot fly resistance through classical breeding programs.

MABC for shoot fly resistance : Many crossing programs at the National and International Research Centers like Directorate of Sorghum Research and ICRISAT, Patancheru, India, resulted in the development of introgression lines for shoot fly resistance which can be used in further breeding programs. Jyothi(77) introgressed SFR QTLs into BTx623 (fully sequenced) (1) and into 296B backgrounds. 296B× IS18551 and BTx623× IS18551 (60,70,71,72,73,74,77) (crosses were

extensively studied and their introgression lines were field evaluated for the introgressed trait validation. Utilizing these introgression lines in future molecular breeding programs may help in increasing the shoot fly resistance in different genetic backgrounds and can be pyramided along with other preferred traits to attain multiple resistances to the sorghum plants.Gene pyramiding is a breeding strategy that serves to combine favorable alleles at multiple genetic loci into a single plant genotype. This process of stacking of genes/QTL into a single elite cultivar background can now be efficiently performed by marker-assisted selection (MAS), using backcrossing or pedigree approaches. This approach expedites the varietal development process by providing the opportunity to select for all desirable genes/QTLs simultaneously, as well as eliminating the time-consuming process of inoculation for different races or isolates at different time intervals (78). Pyramiding of multiple genes or common major QTLs for biotic and abiotic stresses are important approaches for genetical improvement of any sorghum genotype. Fine mapping can be achieved by large scale population with more markers showing more recombination events. In early generation populations like F_2, F_3populations many recombination events can be utilized but, heterozygosity segregation distortion, dominance and epistasis need to be overcome to fine map the interested regions. Advance molecular toolsincrease the precision of crop improvement. A genome-wide association study (GWAS) is a further advanced method to understand the marker trait associations based on linkage disequilibrium and can identify the SNP associated with the candidate genes (79).

Candidate genes responsible for shoot fly resistance : Candidate genes underlying the target QTLs like seedling leaf blade glossines and trichome density have been reported by Satish et al. (60,74) and Aruna et al. (75). Data derived fromsorghum genome database and studies on trichome density and glossiness in different crops are consistent with the identified QTLs. Identification of genes, pathways and mechanism involved in sorghum seedling leaf blade glossiness and trichome density have not yet been clearly studied nor cloned in sorghum. Majority of the studies were carried out in model crop plants like *Arabidopsis* and maize. But studies on sorghum are very few. Wax deficient mutant loci in*Zea mays* (maize), *Brassica napus* and sorghum are defined as '*glossy*' loci whereas in *Arabidopsis thaliana* and *Hordeum vulgare* (barley), they were named as '*ceriferum*' (cer) mutant loci (80). In *Arabidopsis*,shine (*shn*) mutants were reported. Ithas been found that the *shn* gene encodes for APETALA (AP2)/ethylene response element binding protein (EREBP) transcriptional factors that act in up- and downregulation of lipid biosynthesis (81). More than 30 '*glossy*' loci have been identified and a few were cloned (*gl1, gl2*, *gl3*, *gl4*, *gl8*, *gl13* and *gl15*) in maize (82) and their functional role in glossiness has been reported. Similarly,many studies reported that WRKY, MYB transcription factors play important roles (83,84,85,86)for developmental regulation of trichomes and trichome morphology can also play important roles in SFR (60). Further, *Mir*1 gene encodes for cysteine proteasewhich can reduce the growth of larvae as reported by (60). Transparent Testa Glabra1 (TTG1), Glabrous 2 (Gl2) and Glabrous 3 (Gl3) are involved in trichome initiation and TTG2 is also involved in trichomes throughout their development (83,87). Thus, these data appear that genes associated with both glossiness and trichome density have been identified and can be used in genetic engineering techniques for generating transgenics with better resistance.

Conclusions

Recent advances in genomics, molecular breeding and next generation sequencing and re-sequencing methodologies can be utilized in future to decipher stay-green and morphological traits of shoot fly resistance in sorghum. We need to further fine map the mapped QTL genomic regions and look for the maker trait associations with the help of genome wide association studies

(GWAS) in sorghum. Genes responsible for stay-green, leaf blade glossiness and trichome density need to be cloned and their introgression and expression level studies should be made in sorghum in order to enhance the genetic architecture. In future, both these studies need to be targeted with MABC and it could be possible to pyramid the stay-green trait alongside shoot fly component traits inorder to achieve a multiple resistant variety for improved sorghum productivity.

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