

**Development and exploitation of a set of contiguous
segmental substitution lines in pearl millet
[*Pennisetum glaucum* (L.) R. Br.]**

**THESIS SUBMITTED TO
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DOCTOR OF PHILOSOPHY
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Ramana Kumari Basava



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DECLARATION

I hereby declare that the research work presented in this thesis entitled “**Development and exploitation of a set of contiguous segmental substitution lines in pearl millet [*Pennisetum glaucum* (L.) R. Br.]**”, has been carried out by me at International Crops Research Institute for Semi Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India under the supervision of Dr. C.T. Hash and under the co-supervision of Prof. P.B. Kavi Kishor, Department of Genetics, Osmania University, Hyderabad.

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:

(Ramana Kumari Basava)

Place: Hyderabad

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ABBREVIATIONS

°C	:	degree Celsius
AFLP	:	Amplified Fragment Length Polymorphism
ANOVA	:	Analysis of Variance
BAC	:	Bacterial Artificial Clones
bp	:	base pair
cDNA	:	complementary DNA
CGIAR	:	Consultative Group on International Agricultural Research
CISP	:	Conserved Intron Scanning Primers
cM	:	centiMorgan
CSSL	:	Contiguous Segmental Substitution Lines
DArT	:	Diversity Array Technology
df	:	degrees of freedom
DFID	:	Department For International Development
DH	:	Double Haploid
DM	:	Downey Mildew
DNA	:	Deoxyribonucleic Acid
Env	:	Environment
EST	:	Expressed Sequence Tag
ET	:	Evapotranspiration
FAO	:	Food and Agricultural Organization
g	:	gram
G × E	:	Genotype × Environment
GCA	:	General Combining Ability
ha	:	hectare
ICRISAT	:	International Crops research Institute for the Semi-Arid Tropics
IFAD	:	International Fund for Agricultural Development
IL	:	Introgression Lines
kb	:	kilo bases
kg	:	kilogram
L	:	Line
LG	:	Linkage Group
m	:	meter
MAB	:	Marker-Assisted Breeding
MABC	:	Marker-Assisted Backcrossing
MARS	:	Marker-Assisted Recurrent Selection
MAS	:	Marker-Assisted Selection
Mb	:	Million bases
MDE	:	Mutation detection Enhancement
mM	:	milliMolar
mRNA	:	Messenger Ribonucleic Acid
ng	:	nanograms

PAGE	:	Polyacrylamide Gel
PCR	:	Polymerase Chain Reaction
pm	:	picomole
QTL	:	Quantitative Trait Loci
RAPD	:	Random Amplified Polymorphic DNA
RFLP	:	Restricted Fragment Length Polymorphism
RIL	:	Recombinant Inbred Line
RPHL	:	Recurrent Parent Homozygous Line
SFP	:	Single Feature Polymorphism
SNP	:	Single-Nucleotide Polymorphism
SSCP	:	Single Strand DNA Confirmation Polymorphism
SSR	:	Simple Sequence Repeats
STMS	:	Sequence Tagged Microsatellite
STS	:	Sequence Tagged Sites
T	:	Tester
μl	:	microliter

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ABSTRACT

Over the past three decades, the application of molecular markers and genetic linkage maps using conventional mapping populations has allowed the identification of specific regions of the genomes controlling polygenic traits, called 'Quantitative Trait Loci' (QTLs), in all major crop species including pearl millet. The resolution power of QTL identification is limited in conventional mapping populations due to the overshadowing effect of major QTLs preventing effective estimation of 1) the number of independently segregating minor QTLs and 2) the interactions between pairs of unlinked QTLs. To overcome such limitations and considering the several utilities of introgression lines, the present study aimed to develop a set of pearl millet substitution lines from an advanced backcross population derived from two agronomically elite parental lines *viz.*, ICMB 841 and 863B, which were known to differ for important traits like downy mildew resistance, drought and salinity tolerance, straw ruminant nutritional quality, grain Fe and Zn concentration, and grain and stover yield components. One thousand four hundred and ninety-two progenies (derived from BC₅F₃, BC₅F₂ and BC₆F₁ families) expected to provide coverage across most of each of the seven pearl millet linkage groups were selected for genotyping at 74 marker loci (48 SSR, 21 SSCP-SNP and 5 STS). Based on this genotyping, a total of 124 segmental introgression lines (12 for LG1, 10 for LG2, 10 for LG3, 41 for LG4, 32 for LG5, 12 for LG6 and 17 for LG7) were identified. Among them, 56 were unique introgression segments from 863B in the ICMB 841 genetic background. These contiguous segmental substitution lines (CSSL) provide a nearly ideal set of genetic stocks for fine mapping the multitude of traits for which their parents differ, and use in unraveling the genetic and physiological mechanisms of several agronomically important but complexly inherited traits.

The productivity of major crops is severely constrained by biotic stresses and drought stress is a regular occurrence in pearl millet production environments. Hence identification of quantitative trait loci (QTLs) responsible for grain and stover yield, and their maintenance under stress conditions, has become an essential component in pearl millet improvement. To understand the genetic basis of grain and stover yield-related traits of pearl millet, testcross hybrid agronomic performance of selected homozygous introgression lines (ILs), along with their donor and recurrent parents, and a set of sample of recurrent parental allele homozygotes, was evaluated using three genetically diverse, elite inbred pollinators (H 77/833-2, PPMI 301 and RIB 3135-18) under three moisture regimes (fully-irrigated control, late-onset drought stress, and early-onset drought stress conditions) during the summer and kharif seasons at ICRISAT-Patancheru. Twelve phenotypic characters related to grain and stover yield were recorded and line \times tester analysis was performed to estimate general combining ability (*gca*) of each line in each of these environments and across these environments. Several ILs had significant positive *gca* for grain yield and stover yield, with hybrids showing better performance than that of recurrent parent ICMB 841. Introgression of the 863B-P2 segment between SSR markers *Xpsmp2069* and *Xpsmp2273* on LG1 for grain yield and *Xpsmp2270* and *Xpsmp2213* on LG6 for panicle harvest index showed better *gca* effects than recurrent parent ICMB 841. The present results validate these QTLs which were detected in earlier studies using testcrosses of an F₂-derived F₄ mapping population based on the same parental lines.

CHAPTER I

INTRODUCTION

1. INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.], commonly known as bulrush or cattail millet, is the most important of a number of unrelated small-seeded grasses grown as grain crops (*i.e.*, millets) that are collectively grown on a total of 40 million ha worldwide (FAO and ICRISAT, 1996). Pearl millet is the most widely grown type of millet, and is thought to have the greatest potential. Grown in South Asia and sub-Saharan Africa since prehistoric times, it is generally accepted that pearl millet was domesticated in Africa and subsequently introduced into India. In its traditional growing areas in India and many countries in sub-Saharan Africa, pearl millet grain is the basic staple for poor households in the hottest, driest, most marginal crop production environments where dryland crop-livestock production occurs. In addition, pearl millet grain provides the staple diet for the urban poor, including laborers who value its high energy content and slow rate of digestion. Although pearl millet appears to have been domesticated originally as a food crop and is still primarily used this way in Africa and India, its grain is most likely to be used for animal feed in the U.S.A., Latin America and Australia, and its use for this purpose is expanding rapidly in India. Several studies have been conducted on its potential for use as feed for various types of animals including poultry, ducks, cows, hogs, and catfish. Pearl millet grain is consumed by people in the form of leavened or unleavened breads, boiled or steamed foods, and beverages. In the Sahel and elsewhere in northern Africa, pearl millet is an important ingredient of couscous. Pearl millet stalks are a valued building material, fuel and fodder for ruminant livestock.

Pearl millet is a highly tillering, cross-pollinated diploid ($2n=14$) tropically-adapted C4 cereal bearing grain on the surface of its erect candle-shaped terminal spikes. It has a genome size of approximately 2350 Mb (Bennett *et al.*, 2000), which is about five times as large as that of rice and three times as large as that of sorghum, but comparable to that of maize.

Pearl millet grain generally has a crude protein level (10.3%) that is higher by 1 to 2 percentage points than sorghum grown with similar cultural practices. Pearl millet protein is deficient in several essential amino acids, but averages 35% higher lysine levels than sorghum (Rooney and McDonough, 1987). Pearl millet grain has 5 to 6% oil and a lower proportion of the less digestible cross-linked prolamins and lower levels of tannins than sorghum grain (Jambunathan and Subramanian, 1988), which together result in a markedly higher nutritional value of pearl millet grain compared to sorghum grain.

Pearl millet is well adapted to production systems characterized by low rainfall, low soil fertility, and high temperature. It performs better than other tropically-adapted cereals in soils with high salinity levels and/or high levels of aluminum saturation. Because of its tolerance to difficult growing conditions, pearl millet can be successfully grown in areas where other cereal crops, such as wheat, rice or maize, would not survive.

The productivity of major crops is severely constrained by biotic and abiotic stresses. Most often, traits related to stress tolerance in crops are controlled by several genes with complex interactions among them as well as with the environment, making it difficult to unravel their genetic basis. Over the past two decades, the application of molecular markers and genetic linkage maps has allowed the identification of specific regions of genomes controlling polygenic traits, which are referred to as 'Quantitative Trait Loci' (QTL), in all of major crop species including pearl millet. However, it is often still difficult to utilize existing mapping results in marker-assisted selection (MAS) or in map-based cloning of genes. Specifically, functional genomics research for complex phenotypes such as abiotic stress tolerance has to resolve technical difficulties so as to efficiently identify QTLs with large effects on specific target phenotypes in order to efficiently fine-map target QTLs and identify candidate genes likely to contribute substantially to genetic control of these QTLs. Only once these complex steps have been accomplished can we efficiently determine and verify functions of candidate genes controlling these QTLs. The resolution power for identification of QTLs is limited in conventional bi-parental mapping populations. They are not suitable for identification of QTLs of small effect as a major QTL can overshadow the effects of minor independently segregating QTLs by increasing the total phenotypic variation that is observed in the

segregating population so that QTLs with lesser phenotypic effects typically fall below the significance threshold for QTL detection. This overshadowing effect interferes with correct estimation of the number of QTLs contributing to control of a trait and with mapping of genes contributing to QTLs of small effect. An additional restriction of standard bi-parental populations, especially those of moderate size, may result from interactions between two unlinked QTLs. Interacting loci can reduce the difference between the subgroups of the tested QTLs and therefore one or both loci may escape detection.

The use of sets of introgression lines is expected to overcome such limitations of conventional bi-parental mapping populations. Introgression lines are typically developed by systematic backcrossing to introgress small segments of chromosomes from one or more donor parents into the common genetic background of a recurrent parent (often an elite cultivar or hybrid parental line of significant commercial importance). Such introgression lines are expected to differ for only a portion of their genome in an otherwise common genetic background. Such sets of introgression lines can be exploited to unravel the genetic and physiological mechanisms associated with both qualitative and quantitative traits. They enable the phenotypic analysis of specific QTLs in a common genetic background in which direct comparison of two lines (*e.g.*, an introgression line and its near-isogenic recurrent parent) can be used to evaluate the phenotype conditioned by a single introgressed genomic segment. These introgression lines also can be used as parents to construct fine-mapping populations following one or more additional backcrosses to their recurrent parent, thereby enabling map-based cloning of QTLs as has been done in rice and tomato. In addition, diallel or partial diallel crosses among members of an introgression line set can be used to assess the importance of epistatic interactions between different genomic regions that contribute to control of a particular phenotype of interest. Finally, introgression lines fulfilling agronomic requirements can be used directly in breeding programmes either as experimental cultivars or as potential donor parents of particular target traits.

Considering these several uses of introgression lines, work was initiated at ICRISAT to develop a set of introgression lines in pearl millet. Two agronomically elite mapping population parental lines *viz.*, 863B and ICMB 841, which were known to differ for important

agronomic traits like downy mildew resistance, drought and salinity tolerance, and combining ability for many grain and stover yield and quality traits, were used to develop introgression lines having overlapping segments from 863B in the genetic background of ICMB 841 via marker-assisted backcrossing. At the start of this specific thesis research program, introgression line development had reached various backcross generations for different portions of the genome. The current study proposed to further advance these backcross progenies to identify homozygous segmental substitution lines based on genotyping of sequence-tagged microsatellite (STMS, also know as simple sequence repeat (SSR)) markers, and single-strand conformation polymorphism single-nucleotide polymorphism (SSCP-SNP) markers, and sequence-tagged site (STS) markers based on restriction fragment length polymorphism (RFLP) marker; develop as comprehensive a set of such segmental introgression lines as is practical; and evaluate these lines (as inbred lines *per se* or as testcross hybrids) for agronomic performance (e.g., grain and stover yield, drought and salinity tolerance, and/or host plant resistance to pearl millet downy mildew). With these introgression lines, we expect further research efforts to be able to study in detail different mechanisms associated with these traits, which are of substantial economic importance.

Early beneficiaries of this thesis research will be public- and private-sector pearl millet breeders, geneticist and physiologists globally, who are attempting to exploit molecular markers to improve abiotic stress resistance in pearl millet. They are expected to be benefited from the availability of more suitable genetic materials to study the mechanisms of downy mildew resistance and abiotic stress tolerance in pearl millet and for the development of fine-mapping populations to precisely locate the individual QTLs controlling these traits and to estimate their effects and the interactions between various loci contributing or a particular target trait of interest. Preliminary information generated in the course of the current thesis research will identify genomic regions as targets for marker-assisted introgression of drought tolerance, as well as grain size and other grain and stover yield and quality component traits, from donor parent 863B to a wide array of agronomically elite pearl millet hybrid parent line backgrounds. Pearl millet producers (and the agencies that provide them with improved pearl millet cultivar seed) will be the intermediate beneficiaries once improved pearl millet hybrids and varieties with better drought and salinity tolerance can be bred. Ultimate beneficiaries will include all direct and indirect consumers of pearl millet.

With the above background, the current study was proposed with following objectives:

1. Development of a set of at least 50 pearl millet contiguous segmental substitution lines (CSSLs) having segments of 863B in the background of ICMB 841
2. Evaluation of these substitution lines for agronomic performance, under drought stress environments, for grain and stover yield and its component traits

CHAPTER II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Pearl millet

2.1.1. Importance and area of cultivation

Pearl millet [*Pennisetum glaucum* (L.) R. Br.], commonly known as bulrush or cattail millet, is the most important of a number of unrelated small-seeded grasses grown as grain crops (*i.e.*, millets) that are collectively grown on a total of about 40 million ha worldwide (FAO and ICRISAT, 1996). It is the sixth most important cereal crop (following rice, wheat, maize, barley, and sorghum) and provides food security to 200 million of the poorest people living predominantly in parts of Asia and Africa (www.fao.org). These areas are characterized by low or erratic rainfall, high temperature and low soil fertility. However, pearl millet gives stable grain yields in such harsh environments as it is well adapted to these production systems (Hash *et al.*, 2003). It performs better than other tropically-adapted cereals in soils with high salinity levels and/or high levels of aluminium saturation. Because of its tolerance to difficult growing conditions, it is considered as the hardiest crop among cereals and can be successfully grown in areas where other cereal crops, such as wheat, rice or maize, would not survive.

India is the most widely cultivated cereal in India after rice and wheat. India is the largest producer of this crop, both in terms of area (9.3 m ha) and grain production (9.5 m tonnes), with an average productivity of 1044 kg ha⁻¹. Pearl millet is cultivated in the hot arid region of India that spreads across parts of Rajasthan, Haryana and Gujarat. Rajasthan alone contributes about 60% of area and 52 of production of pearl millet in the country, followed by Maharashtra with 11% area and 10% production, and Gujarat with 5% area and 5% production (project coordinator review on pearl millet research in (www.aicpmip.res.in, 2010-2011). The other major pearl millet producing countries are Senegal, Mali, Burkina Faso, Niger, Nigeria, Chad and Sudan. Pearl millet is also grown in Oceania and the Americas, predominantly as a forage and/or mulch component of minimum tillage-based cropping systems.

2.1.2. Origin, evolution and taxonomy

Pearl millet has been grown in South Asia and sub-Saharan Africa since prehistoric times. It is generally accepted that this crop was domesticated in western Africa 40,000 years ago (National Research Council, 1996) and was introduced to eastern Africa and then to Indian sub-continent some 3000 years ago (Brunken, 1977). The genus *Pennisetum* includes pearl millet (*P. glaucum*), an important crop widely cultivated in Africa and Asia, and its two wild relatives *P. violaceum* and *P. mollissimum*, which are now known as *P. glaucum* subsp. *monodii*. The wild forms of pearl millet are found only in Africa, where they have been involved in the domestication of the crop for several thousand years. These species are not reproductively isolated and are subunits of the same gene pool (Brunken, 1977; Mariac *et al.*, 2006a, 2006b; Lewis, 2010). Other species of this genus are prized for their fodder (*P. purpureum*, *P. ramosum*) or are used as ornamental plants (*P. villosum*, *P. setaceum*).

Pearl millet, *Pennisetum glaucum* (L.) R. Br., is a monocotyledon belonging to the *Poaceae* family. It is a highly tillering, cross-pollinated, tropically-adapted, diploid ($2n=14$) C4 cereal bearing grain on the surface of its erect candle-shaped terminal spikes. It has a genome size of approximately 2350 Mb (Bennett *et al.*, 2000), which is about five times as large as that of rice and three times as large as that of sorghum, but comparable to that of maize.

2.1.3. Economic importance

Pearl millet produces nutritious grain and is a major human food for people living in the semi-arid, low input, dry land agriculture regions of Africa and south Asia. It is high in oil, protein, and energy, has balanced amino acids (except low in S-containing amino acids), is high in Ca and Fe (Malhotra and Dhindsa, 1984; Serna-Saldivar and Rooney, 1995), and contains no tannins. It generally has a crude protein level (10.3%) that is higher by 1 to 2 percentage points than sorghum grown with similar cultural practices. Pearl millet protein is deficient in several essential amino acids, but averages 35% higher lysine levels than sorghum (Rooney and McDonough, 1987). The grain has 5 to 6% oil and a lower proportion of the less digestible cross-linked prolamins and lower levels of tannins than sorghum grain (Jambunathan and Subramanian, 1988), which together result in a markedly higher nutritional value, compared to sorghum. People in northern Namibia are almost entirely dependent on pearl millet for food. Four countries in the Sahel region of Africa, with a total population of 38 million, depend on pearl millet to provide over 1,000 calories per person per day (Dendy, 1995). Pearl millet is consumed in many different ways: porridges, leavened or unleavened

breads, fermented and non-fermented beverages, snacks, popped grain, *etc.* (Murty and Kumar, 1995). Although pearl millet was domesticated as a food crop and is still primarily used this way in Africa and India, its grain is most likely to be used for animal feed in the U.S.A. Several studies have been conducted on its potential for various types of animals, including poultry, ducks, cows, hogs, and catfish. In the Sahel and elsewhere in northern Africa, pearl millet is an important ingredient of couscous. Besides providing food for human and animals, millet stems are used for a wide range of purposes, including: the construction of hut walls, fences and thatches, and the production of brooms, mats, baskets, sunshades, *etc.* (IFAD, 1999).

2.1.4. Production constraints

The productivity of major crops is severely constrained by biotic and abiotic stresses. The climate of most pearl millet producing areas can typically be described as hot and dry. Pearl millet has become the primary staple food crop in these areas because nothing else will produce a crop on a reasonably consistent basis. Drought is a major production constraint, reducing pearl millet yields in arid and semi-arid regions of the world, a regular occurrence in these regions, making stress tolerance an essential attribute of new pearl millet cultivars. Breeding for drought tolerance has been slow due to the complex physiological responses to drought, various environmental factors, and their interactions. Major factors that restrict the grain and stover production potential of pearl millet are the high mean temperatures, low and erratic rainfall, and infertile sandy soils with low water-holding capacity that are typical of pearl millet production environments (Bidinger and Hash, 2005). Soil salinity, another abiotic stress, greatly hampers pearl millet productivity by delaying germination, reducing seed germination percentage and severely affecting subsequent vegetative and reproductive growth (Ashraf and Idrees, 1992). The primary biotic constraints for pearl millet production in India include downy mildew (*Sclerospora graminicola*), pyricularia leaf blight (*Magnaporthe grisea*), rust (*Puccinia substriata* var. *indica*), and root knot nematode (*Meloidogyne arenaria*) (The Syngenta Foundation for Sustainable Agriculture, 2005; World Meteorological Organization, 1996). Among the fungal diseases, downy mildew constitutes the major disease risk to its successful cultivation (Wizard Project Information, 1999), particularly in India. The crop loss can be up to 30 percent of the harvest during years of severe attack, with losses in individual fields reaching nearly 100 percent (CGIAR, 2006).

2.2. Pearl millet crop improvement

2.2.1. Conventional breeding

Breeding objectives may vary depending on whether the plant is used for grain or forage production. Traits for grain production include seed size, weight, and color; inflorescence length and diameter; floret compactness; stalk strength; pest resistance; tillering ability; days to grain maturity; and plant height. Traits for forage production include dry matter yield, dry matter digestibility, response to daylength, regrowth potential, tillering ability and pest resistance. Anand Kumar and Andrews (1993) summarized the known plant, panicle, disease, and grain characteristics considered important in pearl millet crop improvement. Greater productivity and reliability of pearl millet cultivars have been achieved by using population improvement approaches (Witcombe, 1999). These approaches will continue to be important in the improvement of pearl millet. However, utilizing hybrid vigor to improve pearl millet forage and grain cultivars is an effective approach to maximizing the potential of this crop. Use of hybrids in India has increased since the mid-1960s, even though the total area sown with pearl millet has remained static or declined marginally. Dave (1987) estimated that 40% of the pearl millet grown in India was sown to F₁ hybrids, because of which grain production had increased by 50%. The popularity and use of hybrids has evolved because of development of superior inbreds. Most elite pearl millet inbreds are vigorous and have both good general and specific combining ability to produce hybrids in a number of different combinations as well as in specific combinations. Characteristics such as good seed set and yields, plant vigour, disease resistance, standability, plant height, and earliness have enhanced the use of hybrids (Hanna and Rai, 1999). Hybrids are beginning to be used in Africa, especially Nigeria, because significant grain yield increases are being realized. The main constraint on the use of pearl millet hybrids in Africa, however, is the lack of infrastructure for their effective seed distribution. Although pearl millet hybrids often give better grain yields than local open-pollinated cultivars, the genetically uniform single-cross hybrid cultivars currently available are more vulnerable to epidemics of pearl millet downy mildew. Such epidemics constitute the major risk to cultivation of well-adapted pearl millet hybrids (Hash, 1997). Molecular marker-assisted breeding, comprising of molecular markers, genetic linkage maps and quantitative trait loci (QTL) mapping, and marker-assisted selection is a recent time saving approach that is expected to complement conventional breeding procedures in crop improvement.

2.2.2. Molecular markers and Genetic linkage maps

Molecular markers were recognised as a powerful tool in plant breeding, more than 50 years ago (Sax, 1923). Molecular markers are specific locations on a chromosome which serve as landmarks for genome analysis. Molecular markers are useful in characterization of the available germplasm, estimating its genetic diversity with the aim of using this information for selection of parents for hybridization programs (e.g. Bhat *et al.*, 1999; Srivastava *et al.*, 2001; Prashanth *et al.*, 2002; Roy *et al.*, 2002), and also in preparation of genetic maps, a fundamental organizational tool for genomic research (Gupta *et al.*, 2002b).

Isozymes, biochemical markers comprised of multiple forms of enzymes having the same catalytic activity that are separable by electrophoretic procedures (Markert and Muller, 1959; Feret and Bergmann, 1976), were used for studying genetic variation and morphological characters in many crop species in 1970s to 1990s. Several studies in pearl millet involving isozymes, conducted prior to the 1990s were reviewed by Anand Kumar and Andrews (1993) under the category of biochemical genetic markers. There were well-designed mapping studies to detect genetic linkage with isozymes (include esterases, peroxidases, and β -1,3-glucanases) that were analyzed for their correlation with disease resistance (Shetty *et al.*, 2001) and plant height (Chhabra *et al.*, 2001). However, they have not provided good genetic markers since their level of expression shows considerable quantitative and qualitative variation, varying with tissue, growth stage and environmental conditions. During the last three decades, advances in molecular biology have provided new classes of genetic markers in large numbers at the DNA level for almost all major crop species.

The discovery of restriction enzymes (Smith and Wilcox, 1970) and the polymerase chain reaction (PCR; Mullis and Faloona, 1987) paved the way for development and exploitation of DNA-based molecular markers in genomic research. Classically, DNA-based molecular markers are genetic markers that can detect DNA polymorphisms both at the level of specific loci and at the whole-genome level. These molecular markers can be categorised mainly into three groups, depending on how the polymorphism is exhibited. (1) hybridization-based markers: restriction fragment length polymorphism (RFLP), (2) PCR-based markers: random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence-tagged site (STS), and microsatellite or simple sequences repeat (SSR), and (3) sequence or chip-based markers: single nucleotide polymorphism (SNP), diversity array technology (DArT) and single feature polymorphism (SFP). However, the utility of

these different marker systems depends on the purpose of the study such as genetic diversity assessment, molecular mapping and marker-trait association studies (methodology, traits involved and mapping population), marker assisted selection (foreground, recombinant and background), and the relative costs of these marker assays versus other selection techniques such as phenotypic selection or various bioassay systems. The multitude of potential applications of DNA marker technologies to the improvement of pearl millet were comprehensively reviewed by Hash *et al.* (2002).

2.2.2.1. Restriction fragment length polymorphism (RFLP) markers

Restriction fragment length polymorphism (RFLP) markers were the first DNA-based markers used in studies of crop inheritance and improvement. The four primary advantages of RFLP markers over morphological markers and isozymes are co-dominance, substantial number of polymorphisms, absence or limited influence of the environment, and absence of pleiotropic effects (Beckman and Soller, 1983). By the mid to late 1980s, a potentially unlimited number of RFLPs had been developed for use in major crops like rice (McCouch *et al.*, 1988), maize (Helentjaris *et al.*, 1986, Burr *et al.*, 1988), wheat (Chao *et al.*, 1989), soybean (Apuya *et al.*, 1988), tomato (Bernatzky and Tanksley, 1986), potato (Bonierbale *et al.*, 1988, Gebhardt *et al.*, 1989), and brassica (Figdore *et al.*, 1988). In pearl millet, the development of RFLP markers with *Pst*I genomic clones to construct the first genetic linkage map was initially reported by Liu *et al.* (1992, 1994a, 1994b). The total length of this map was 303cM, and it consisted of some 200 loci and seven linkage groups (LGs), which most likely correspond to the seven pearl millet chromosomes. Hash (1991), Gale and Witcombe (1992), Jones *et al.* (2002), Hash *et al.* (1997, 1999), Hash and Bramel-Cox (2000) and Hash and Witcombe (2002) emphasized the opportunities for potential use of RFLPs in plant breeding with particular reference to downy mildew resistance in pearl millet. A number of recent reports on this crop also demonstrated the use of RFLPs for mapping drought tolerance (Yadav *et al.*, 1999; Hash *et al.*, 2000; Yadav *et al.*, 2002; Yadav *et al.*, 2004; Bidinger *et al.*, 2007) and for diversity analysis (Bhattacharjee *et al.*, 2002). ICRISAT and its partners have successfully demonstrated the use of RFLP markers in the marker-assisted backcross transfer of additional downy mildew resistance into a parental line of popular pearl millet hybrid HHB 67 (Hash CT *et al.*, 2006a; Khairwal and Hash, 2007). RFLP marker assays are, however, expensive, time consuming and having potential health and environmental hazards, and hence cannot be considered for large-scale applications in marker-assisted plant breeding.

2.2.2.2. Sequence tagged site (STS) markers

RFLP markers linked with downy mildew resistance QTLs, and additional low copy RFLP probes having high levels of polymorphism in initial variability studies, were converted to sequence tagged site (STS) markers (Money *et al.*, 1994), but these have proven to be disappointing because much of the polymorphism of the RFLP markers on which they were based can no longer be detected without the use of multiple restriction enzymes. In many cases, this additional enzyme cost essentially negated any advantages of moving from co-dominant RFLP markers to the polymerase chain reaction (PCR)-compatible STS marker format.

2.2.2.3. Amplified fragment length polymorphism (AFLP) markers

The initial RFLP-based pearl millet linkage map was transferred to several additional crosses (Busso *et al.*, 1995; Liu *et al.*, 1996) in studies of sex-specific recombination rates in cultivated \times cultivated and cultivated \times wild crosses, and a pearl millet world reference mapping population was developed based on the cross 81B \times ICMP 451 (Hash and Witcombe, 1994). This map has been used for high saturation marker genotyping using amplified fragment length polymorphism (AFLP) markers, additional homologous RFLP probes from pearl millet, and heterologous RFLP probes from other grasses. This latter group of markers has permitted improved understanding of the complex relationships between the pearl millet genome and those of other cultivated graminaceous species (Devos and Gale, 1997; Devos *et al.*, 1999; Devos *et al.*, 2000). This work has extended the total pearl millet genetic linkage map length to approximately 600 cM. Unfortunately, attempts to cap the pearl millet linkage groups with telomeres have not proven successful as all polymorphic telomeric sequences detected to date in pearl millet segregate independently from the seven previously detected linkage groups. This is in agreement with the observations of Liu *et al.* (1994) and Qi *et al.* (2004) that most recombination in the pearl millet genome is localized distal to the centromeric regions, so that the length of a comprehensive pearl millet linkage map is likely to exceed 1200 cM. Vom Brocke *et al.* (2003) used AFLP markers to study pearl millet genetic diversity patterns and attributed those results to farmer's local knowledge and pearl millet seed systems in Rajasthan.

2.2.2.4. Simple sequence repeats (SSR) and Expressed sequence tags (EST)

Simple sequence repeats (SSR) or microsatellite DNA sequences are abundant in eukaryotic genomes (Tautz and Renz, 1984) as tandem repetitions of short sequence motifs. These

markers are highly variable, widely distributed across the genome, reproducible and the flanking sequences are relatively conserved among haplotypes of the same species. Length polymorphism relies on a difference in the number of repeat units in different genotypes and is revealed after PCR amplification with primer pairs designed to target the SSR-flanking regions. These PCR-based SSR markers are generally co-dominant and highly polymorphic, providing an efficient marker system for genomic analysis in many species (Bryan *et al.*, 1997; Senior *et al.*, 1998). SSR markers are widely used in fingerprinting, genetic diversity analysis, mapping and marker-assisted selection. SSR sequences have generally been identified either from DNA sequence databases or following the screening and sequencing of small-insert genomic libraries. Cregan *et al.* (1999) reported the development of SSR markers by sub-cloning a limited number of bacterial artificial clones (BACs) and Qi *et al.* (2001) isolated 25 SSRs using 3' end-anchored SSR primers from 40 pearl millet BAC pools, comprising of 384 clones without sub-cloning steps. Allouis *et al.* (2001) constructed a pearl millet BAC library covering 5.8 haploid genome equivalents and demonstrated use of this library as a valuable resource in the isolation of SSR markers. Genetic maps, produced using four different crosses segregating for drought tolerance and downy mildew resistance, were integrated to develop a consensus map of 353 RFLP and 65 SSR markers. An interesting feature of this map was the extreme localization of recombination towards chromosome ends (Qi *et al.*, 2004). Budak *et al.*, (2003) developed 18 pearl millet SSR markers by screening a small insert genomic library with (CT)₁₅ oligonucleotide probe and 11 of these 18 markers were used for assessing genetic diversity of 53 lines of pearl millet. For high throughput cloning of stress-responsive genes that are differentially expressed in response to salinity, cold, and dehydration stress in pearl millet, cDNA libraries are well established (Mishra *et al.*, 2007). Microsatellite markers were used to study the phylogeny and origin of pearl millet by Oumar *et al.* (2008).

ESTs are typically short (300–500 bp), single-pass DNA sequences obtained from the 5' or 3' end of mRNAs (cDNAs). ESTs represent a snapshot of genes that are expressed at a given developmental stage or in a given tissue. SSR marker development, which conventionally is a costly and time-consuming process, has become easier and more cost-effective with the availability of genomic or EST/genic sequences in public databases and the recent advent of bioinformatics tools for data mining (Jayashree *et al.*, 2006). The exploitation of EST databases to develop microsatellite markers was first attempted in rice (Miyao *et al.*, 1996) and has subsequently been reported from many other plant species (Varshney *et al.*, 2005;

Jayashree *et al.*, 2006; Ellis and Burke, 2007). EST-SSR markers are superior in terms of cross-species transferability, as they were derived from the most conserved regions of genome, and thus are well suited for application in phylogenetic analysis and comparative genome mapping (Zhang *et al.*, 2006). EST-SSR markers have been used for diversity analysis in rice (Cho *et al.*, 2000), wheat (Leigh *et al.*, 2003, Gupta *et al.*, 2003, Zhang *et al.*, 2006), and barley (Thiel *et al.*, 2003; Varshney *et al.*, 2007, 2008); and for mapping in wheat (Gupta *et al.*, 2003, Yu *et al.*, 2004; Balyan *et al.*, 2005), barley (Varshney *et al.*, 2006), finger millet (Dida *et al.*, 2007) and sorghum (Ramu *et al.*, 2009).

Currently large public EST data bases are available for several grass species, which can be used for the development of EST-SSR markers in orphan crops like pearl millet. Mariac *et al.* (2006b) utilised this approach to develop a set of 16 polymorphic pearl millet EST-SSR markers from 2,570 publicly available sequences, and used 10 of these (along with 15 other SSR markers) for genetic diversity studies of 46 wild and 421 cultivated accessions of pearl millet in Niger. A set of 43 polymorphic EST-SSRs were developed by Senthilvel *et al.* (2004, 2008) using publicly available sequence information from 3,520 expressed sequence tags (ESTs) of pearl millet, and mapped 17 of these mostly to the distal regions of the linkage groups, where they often filled large gaps that had existed previously. They now are being used by ICRISAT in diversity assessment and marker-aided breeding programs. Yadav *et al.* (2007) reported that they had developed 19 new EST-SSR markers using this same approach and 11 of them were amplified in the test DNA. A set of 99 new EST-SSR markers have been developed from cDNA libraries, mapped using four mapping populations, and a consensus map also has been developed recently by Rajaram *et al.* (2010).

2.2.2.5. Single nucleotide polymorphism (SNP)

Single strand DNA confirmation polymorphism (SSCP) methodology allows detection of polymorphism due to differences of one or more base pairs in PCR products, which is suitable for SNP genotyping. The methodology relies on the secondary structure being different for single strands derived from PCR products that differ by one or more nucleotides at an internal site. These conformational differences affect electrophoretic mobility of the PCR products. For assays using SSCP methodology, PCR products of different genotypes carrying the SNP site are denatured and electrophoretically separated in neutral acryl amide gel. Because of occurrence of SNPs in different genotypes, the gel will show differences in the mobility of the resolving fragments. This methodology has been used in several species

like *Picea* (Germano and Klein, 1999) and cassava (Castelblanco and Fregene, 2006). This new marker system was developed in pearl millet using annotated rice genomic sequences to initially predict the intron-exon borders in pearl millet ESTs and then to design primers that would amplify across the introns (Bertin *et al.*, 2005). However, little research has been conducted on the transferability of SSR and other markers like those detected by conserved intron scanning primers (CISPs) from major cereal crops to pearl millet. Using a similar approach, Feltus *et al.* (2006) designed 384 PCR primer pairs for conserved exonic regions flanking introns, using *Sorghum* and *Pennisetum* EST alignments to the *Oryza* genome. The system is, of course, dependant on an adequate supply of pre-existing ESTs. SSCP-SNP is, however, less polymorphic when compared to SSRs tested on the same genotype panel (Bertin *et al.*, 2005).

The redundant set of ESTs for a given species can be used for *in silico* SNP discovery by employing bioinformatics scripts and tools. A large number of bioinformatics tools or pipelines are available in the public domain for identification of SNPs. Basically, all these tools/pipelines perform clustering on redundant sets of ESTs so that the ESTs representing one gene are grouped under one cluster, which can be aligned and compared for the occurrence of SNPs. The putative SNP(s) identified using this approach, however, should be verified either by checking the sequence chromatograms of ESTs or validated by *wet lab* experiments, as *in silico* SNPs may have been observed as a result of sequencing error (Varshney *et al.*, 2010). Application of SSCP-SNPs in marker-assisted breeding is very expensive and tedious as in the wet lab these markers have to be visualised by denaturing and electrophoretically separating in MDE gels which are very costly. More recently, several alternative low-cost, high throughput genotyping systems for SNPs have been developed (Varshney *et al.*, 2010), which greatly enhance the utility of these markers for breeding applications.

2.2.2.6. Diversity Array Technology (DArT) markers

DArT markers are one of the new generation of markers, and represent a low-cost, high-throughput, robust marker system with minimal DNA requirements, which is capable of providing comprehensive genome coverage even for species that do not possess any genomic information. A DArT marker is a segment of genomic DNA, the presence of which is polymorphic in a defined genomic representation. DArT provides high quality markers that can be used for diversity analyses and to construct medium-density genetic linkage maps. The

high number of DArT markers generated in a single assay not only provides a precise estimate of genetic relationships among genotypes, but also their even distribution over the genome offers real advantages for a range of molecular breeding and genomics applications. DArT was first developed in rice (Jaccoud *et al.*, 2001). Subsequently, it has been developed for additional crops and used in linkage map construction and diversity analysis. Plant species for which DArT has been developed include rice (Xie *et al.*, 2006) barley (Wenzel *et al.*, 2004, 2006), *Arabidopsis* (Wittenberg *et al.*, 2005), eucalyptus (Lezar *et al.*, 2004), wheat (Semagn *et al.*, 2006; Akbari *et al.*, 2006), cassava (Xia *et al.*, 2005), pigeon pea (Yang *et al.*, 2006), and sorghum (Mace *et al.*, 2008). Over 500 DArT markers detected variation among 90 sorghum accessions used in a diversity analysis and 596 DArT markers were mapped onto an integrated linkage map of sorghum. Senthilvel *et al.*, (2010) and Supriya *et al.* (2011) enriched the existing pearl millet linkage map with 300 DArT markers for three mapping populations and developed a well-saturated consensus linkage map of DArT and SSR markers in pearl millet.

2.2.3. QTL mapping

A quantitative trait locus (QTL) is a gene or gene block that underlies quantitative traits (Gelderman, 1975), which were referred to as polygenes (Mather, 1949) before the discovery of molecular markers. QTLs are the specific regions of the genome controlling particular polygenic traits. QTL analyses are predicted based on associations between phenotypic values for a quantitative trait and the marker alleles segregating in the mapping population. Sax (1923) first reported association of simply inherited genetic markers with a quantitative trait in plants when he observed segregation for seed size associated with segregation for seed coat color in beans.

2.2.3.1. QTL mapping for drought tolerance

Drought stress at various periods during the growing season is a common occurrence in pearl millet (van Oosterom *et al.*, 1996c) that influences crop yield and yield stability (van Oosterom *et al.*, 1996a, 1996b). Post-flowering drought stress is one of the most important environmental factors reducing pearl millet grain yield and yield stability (Mahalakshmi *et al.*, 1987). Terminal drought stress (flowering through grain filling) is more damaging to pearl millet productivity than stress at the vegetative or pre-flowering reproductive crop growth stages. This is because pearl millet's asynchronous tillering behaviour and rapid growth rate allow it to recover rapidly from intermittent drought stress during these earlier

stages of plant development, but provide no advantages under unrelieved terminal drought stress (Bidinger *et al.*, 1987; Mahalakshmi *et al.*, 1987). Therefore, pearl millet breeding programs aim at improving the adaptation of the crop to terminal drought stress environments in order to increase productivity and yield stability (Yadav *et al.*, 2002, 2004; Bidinger and Hash, 2004; Bidinger *et al.*, 2005, 2007). The large variability in the timing and severity of drought stress and the inadequate understanding of its complexity have made it difficult to characterize the physiological and/or phenotypic traits required for screening and improving crop performance under drought stress. Consequently this difficulty has limited the use of a trait-based approach in plant breeding to enhance the drought tolerance of crops. Statistical procedures have been developed to partition crop yield under stress into effects of stress escape, yield potential and stress response (Bidinger *et al.*, 1987), and emphasis has been on identification and selection for traits related to stress tolerance rather than yield. A series of empirical selection experiments using panicle harvest index as a tolerance criterion resulted in small (5%) but significant gains in yield in selected materials under terminal stress (Bidinger *et al.*, 2000). QTL mapping provides a means to dissect complex phenotypic characters such as drought tolerance into their component traits (QTLs), and allows the identification of molecular markers linked to desirable QTL alleles, so that they can be directly used in marker-assisted selection (MAS) (Tanksley, 1993; Mohan *et al.*, 1997; Prioul *et al.*, 1997). Significant progress has been made in mapping a number of QTLs for components of grain and stover yield, as well as yield maintenance, under terminal drought stress conditions in pearl millet (Yadav *et al.*, 2002, 2003, 2004; Nepolean *et al.*, 2006; Bidinger *et al.*, 2007).

Testcrosses of a set of F₂-derived F₃ mapping-population progenies, derived from a cross of two inbred pearl millet pollinators (H 77/833-23 and PRLT 2/89-33) that differ in their response to drought, were evaluated in a range of managed terminal drought-stress environments. A QTL associated with drought tolerance of grain yield was obtained on linkage group 2 (LG2) in two of the three stress environments, explaining up to 32% of the variation in drought tolerance response of grain yield (Yadav *et al.*, 1999, 2002). QTLs associated with drought tolerance of 100-seed mass (three environments), harvest index (two environments), panicle harvest index (two environments), and panicle number m⁻² (one environment) were co-mapped in this same interval on LG2. A QTL for drought tolerance of stover and biomass yield in the most severe stress environment also mapped to this interval on LG2. Using a mapping population progeny set derived from cross ICMB 841 × 863B

(Yadav *et al.*, 2004; Bidinger *et al.*, 2007), a QTL on LG2 was again observed to be associated with drought tolerance of testcross grain yield in early stress environments using both (H 77/833-2 and PPMI 301) testers. Its effect on drought tolerance of grain yield, however, was achieved differently depending on the tester used. Bidinger *et al.* (2007) also identified a major grain yield QTL on LG2 with substantial LOD scores in all three moisture environments (LOD 6.3–6.9), including the stress-free fully-irrigated control, as well as across environments (LOD 7.9), which accounted for a significant proportion of the phenotypic variance for grain yield in both stress (27–38%) and the stress-free irrigated control (28%) environments, as well as across these environments (25%). The grain yield QTL co-mapped with a QTL for harvest index across environments and with QTLs for both grain number and individual grain mass under severe terminal stress.

Besides the major QTL on LG2, a number of QTLs associated with maintenance of grain yield-determining component traits were detected on other pearl millet linkage groups. One QTL for grain yield on LG1 was detected in late-onset stress environments for H 77/833-2 × PRLT 2/89-33 (Yadav *et al.*, 2002) and for ICMB 841 × 863B (Bidinger *et al.*, 2007) in early-onset stress environments, and one strong QTL for grain number also mapped on LG1 across moisture environments, with the favourable allele from ICMB 841. In testcrosses of the (ICMB 841 × 863B)-based F2-derived F4 mapping population, a QTL associated with drought tolerance of testcross grain yield was also obtained on LG5, which explained 15% of the variation in reduction in grain yield (Yadav *et al.*, 2004). This QTL was detected only for the H 77/833-2 tester in the early-onset stress treatment, and was not expressed in the background of the PPMI 301 tester. Alleles from ICMB 841 in this interval increased the grain yield drought tolerance as well as stover and biomass yields. Similarly, a QTL associated with grain yield drought tolerance in late-onset stress environments was obtained on LG6. The effect of this QTL was again evident only in the genetic background of tester H 77/833-2. This QTL exerted its effect on increased maintenance of grain yield via its effect on maintenance of increased harvest index and panicle harvest index. Grain yield QTLs on LG3 and LG4, which accounted for smaller fractions (12-17%) of the phenotypic variance for grain yield both in the late-onset stress environments and across environments were detected using (ICMB 841 × 863B)-derived F4 testcrosses by Bidinger *et al.* (2007).

A particularly interesting stover yield QTL detected from 863B on LG3 segregated independently from flowering time QTLs. Hence it may be possible to improve the stover

yield potential of (some) hybrids of male-sterile line ICMA 841, without delaying their maturity, by marker-assisted backcross transfer of part of LG3 from 863B into the genetic background of maintainer line ICMB 841 (Hash *et al.*, 2000). At least two QTLs associated with stover yield were detected and a major QTL was found on LG6. This QTL explained up to 40% of observed phenotypic variation and the allele from PRLT 2/89-33 increased stover yield. QTLs of smaller effect were obtained on LG2 and LG4, and in each case it was the allele from H 77/833-2 that enhanced stover yield. In most cases, parental alleles that were associated with increased flowering time were also associated with increased stover yield and biomass yield (Yadav *et al.*, 2002, 2003). Nepolean *et al.* (2006) identified three QTLs for dry stover yield, distributed across LG3, LG5 and LG6, and together these controlled a significant proportion of observed phenotypic variance for dry stover yield among the testcrosses. Favourable alleles for the QTLs mapped on LG3 and LG6 were provided by 863B, whereas alleles of ICMB 841 were favourable for the QTL on LG5. With substantial grain yield variation, three grain yield QTLs were also observed on LG5 with the testcrosses of the same mapping population. The grain yield QTL at the bottom of the LG5 mapped near to those for dry stover yield and stover quality traits. Thus positive association makes this genomic region a potential target region for marker-assisted selection.

QTL mapping results are determined by several factors including recombination, the number of progeny in the mapping population and the number of markers. QTL mapping typically under-estimates the number of genes that are involved in controlling a trait, and over-estimates their genetic effects, particularly when mapping population size is small. The putative QTLs identified need to be validated by testing the reliability of markers to predict phenotype in order to indicate whether a marker in the QTL region could be used in routine screening for marker-assisted selection. Initial evaluation of the putative QTLs associated with pearl millet drought tolerance was done by comparing hybrids made with topcross pollinators based on progenies selected from near isogenic lines of H 77/833-2 (into which various putative QTL segments have been introgressed from the donor parent PRLT 2/89-33 by marker-assisted backcrossing) for presence of the tolerant allele at the target QTL versus field performance in the phenotyping environments (Bidinger *et al.*, 2005; Serraj *et al.*, 2005). The QTL-based hybrids were significantly, but modestly, higher yielding in a series of both absolute and partial terminal stress environments.

In addition to drought, the pearl millet crop also suffers when excessive salts are present in the soil or are added by poor-quality irrigation water. Improvement of adaptation to the dual stresses of drought and salinity is thus a desirable trait to incorporate in pearl millet cultivars. Sharma *et al.* (2011) reported that the testcross hybrids of near-isogenic lines introgressed with the LG2 drought tolerance QTL contributed by PRLT 2/89-33 (drought tolerant donor parent) in the background of H 77/833-2 (drought sensitive recurrent parent) showed favourable effects on growth and productivity traits under salt stress by limiting Na⁺ accumulation in leaves. It was recently shown that pearl millet genotypes carrying a terminal drought tolerance quantitative trait locus (QTL) have a lower rate of water loss per unit leaf area under well-watered conditions (Kholová *et al.*, 2010a). This water-saving mechanism operating under non-stress conditions could leave water available in the soil profile for grain filling and could be beneficial for terminal stress conditions. Kholová *et al.*, (2010b) reported that introgression of the terminal drought tolerance QTL on pearl millet LG2, previously found to correlate to a lower transpiration rate, also correlated with higher abscisic acid levels in the leaves and with increased sensitivity of transpiration to high vapor pressure deficit levels, even under well-water conditions.

2.2.3.2. QTL mapping for downey mildew resistance

Pearl millet downy mildew disease is caused by *Sclerospora graminicola* (Sacc.) Schroet., a soil- and wind-borne pathogen. It is the major disease risk to the successful cultivation of pearl millet (Hash *et al.* 1997), particularly in India. In India, up to 30 percent of the harvest can be lost during years of severe attack, with losses in individual fields reaching nearly 100 percent (CGIAR, 2006). Although pearl millet hybrids often give better grain yields than local open-pollinated cultivars, the genetically uniform single-cross hybrid cultivars currently available in India are much more vulnerable to pearl millet downy mildew epidemics. QTLs associated with pearl millet downey mildew resistance effective against pathogen populations from western and central Africa as well as populations from south Asia were described by Jones *et al.* (1995), using susceptible × resistant cross (LGD 1-B-10 × ICMP 85410). QTLs with large effects contributed a large portion of the detected variation in disease reaction to each pathogen population, and were consistently detected in repeated screens. QTLs of smaller effects and variable effects were also detected. No single QTL was effective against all four of the pathogen populations studied, indicating that pathotype-specific resistance is a major mechanism of downy mildew resistance. For most, but not all QTLs, resistance was inherited from the “resistant” parent. Similarly, resistance at most QTLs was inherited in a

dominant or over-dominant manner. Similar results were found for mapping populations from crosses PT 732B × P1449-2, 81B × ICMP 451, and ICMB 841 × 863B (Hash *et al.*, 2000). Breese *et al.* (2002) identified QTLs for downy mildew resistance and seedling heat tolerance from pearl millet mapping populations produced from crosses ICMP 451 × H 77/833-2 and H 77/833-2 × PRLT 2/89-33. QTLs for host-plant resistance effective against African and Indian populations of the downy mildew pathogen were identified in a new mapping population based on cross W 504 × P310 (Kolesnikova, 2001). Based on the all above results, a routine screening parents of all pearl millet mapping populations against specific downy mildew populations (*e.g.*, Thakur *et al.*, 1998) from regions where this disease is major constraint to pearl millet production is recommended. Where differential reactions are observed for pearl millet mapping population parent pairs, then progenies from that mapping population can be screened and the phenotypic scoring data thus obtained can be used to map downy mildew resistance QTLs currently effective against that particular pathogen population.

Forty-six male-sterile A-lines and their male-fertile maintainer counterpart B-lines were evaluated for resistance to four diverse downy mildew pathotypes from India, including the Patancheru pathotype, under field conditions in disease nurseries and under greenhouse conditions using seedling inoculation. Although selection during inbreeding and at backcross stages had been made for resistance to the Patancheru pathotype, 863B was found to be downy mildew-free under both conditions for three pathotypes (Patancheru, Durgapura and Jalna) and ICMA 841 had diseased plants against all the four pathotypes under one or both of the screening conditions (Thakur *et al.*, 2001). A subsequent study involving nine pathotypes, showed that 863B-P2 had 0–6% downy mildew incidence against the eight pathotypes, and 11% disease incidence against the Banaskantha pathotype under high disease pressure in the greenhouse (63–100% disease incidence in susceptible control, 81B) (Hash *et al.* 2006b). Line 841B-P3 had 5–96% downey mildew incidence, with 30–96% incidence against four pathotypes. Two major QTLs for resistance effective against a range of DM pathogen populations from eastern Africa and India have been detected on LG4 of 863B.

2.2.4. Marker Assisted Breeding

Once markers that are tightly linked to genes or QTLs of interest have been identified, prior to field evaluation of large number of plants, plant breeders may use specific DNA marker alleles as diagnostic tools to identify plants carrying the more desirable alleles at specific

genes or QTLs. The procedure is called ‘marker-assisted selection’ or ‘marker-aided selection’ (commonly referred as MAS) or ‘marker-assisted breeding’ (MAB). MAS has a number of important advantages in terms of transferring desirable genes over conventional breeding methods, but the main one is that the breeding process is more rapid, selection being based on the test for highly heritable marker alleles rather than on less reliable testing for phenotype in the field. MAS was originally clearly limited to a small number of target QTLs, as population sizes and costs for marker analyses increased significantly as target QTL numbers increase. Therefore it was important to select QTL targets for MAS on the basis of as much information as possible. The primary considerations in selection of target QTLs were (i) their likely direct (on the target trait) and indirect (on other traits) effects, and (ii) the expected stability of expression of the QTL across environments (Bidinger *et al.*, 2007). However, rapidly declining genotyping costs are driving development of more marker-intensive breeding strategies such as genome-wide selection (GWS) and marker-assisted recurrent selection (MARS). Hash *et al.* (1997, 1999), Witcombe and Hash (2000), Hash and Witcombe (2002) described how multiple resistance gene pyramids can be used practically to deploy resistance genes in a potentially more durable manner than has been previously practised. At CIMMYT, (Dreher *et al.*, 2003; Morris *et al.*, 2003) compared the costs involved with the use of conventional breeding methods and MAS for the quality protein trait in maize line conversion and concluded that conventional breeding is more expensive and time consuming than MAS. A recent economic study of molecular breeding of rice for tolerance to salty and low-phosphorus soils in selected Asian countries has estimated that the method saves a minimum of 2–3 years, which results in significant incremental benefits in the range of US\$300–800 million (Alpuerto *et al.*, 2009).

Varshney *et al.* (2009) have been reviewed some successful examples of product development of marker-assisted breeding programs in cereals such as release of US barley variety Tango that contains two QTLs for adult resistance to stripe rust (Toojinda *et al.*, 1998); advancement of a ‘Sloop type’ variety with cereal cyst nematode resistance for commercial release (Langridge, 2005); release of two Indonesian rice cultivars ‘Angke’ and ‘Conde’, in which marker-assisted selection (MAS) was used to introduce *xa5* into a background containing *xa4* (Toenniessen *et al.*, 2003); development of an improved version of Pusa Basmati 1 (PBI) variety of rice after introgressing the genomic segments, harbouring the bacterial blight resistance namely *xa13* and *Xa21* have been transferred to PB1 from a non-Basmati donor through MAS (Mohapatra T, NRCPB, IARI, India; personal

communication); and pyramiding of stay-green QTLs in elite but drought sensitive sorghum lines (Kassahun *et al.*, 2010). Similarly, MAS approach in cassava has already been successfully utilized to develop 14 genotypes that combine cassava mosaic disease resistance and high yields by using eight markers associated with the dominant CMD2 gene (Okogbenin *et al.*, 2007).

In case of a marker-assisted backcrossing program (MABC), use the molecular markers associated with the trait for foreground selection, while neutral markers covering the whole genome can be used for background selection. The foreground and background selection with molecular markers can be conducted in subsequent generations until the backcrossing products have the desirable chromosomal segment from the donor genotype introgressed into the recipient genotype background. Hash *et al.*, (2000) described several alternative marker-assisted backcrossing procedures that can be used for transferring QTL from a donor to elite recurrent parent when those two lines have been used in forming the base mapping population. MABC approach was used in the development of submergence tolerant rice cultivars, which has improved yields in more than 15 million hectares of rain-fed lowland rice in South and Southeast Asia (Septiningsih *et al.*, 2009). In chickpea, root traits are being used in marker-assisted backcrossing (MABC) programmes in elite cultivars of India and Africa. By using the ICC 4958 genotype as a donor genotype, and JG 11 as a recurrent genotype, MABC has led to development of BC3F3 lines at ICRISAT (Varshney *et al.*, 2010).

Howarth and Yadav (2002) reported a successful MAS approach in pearl millet in transferring of downy mildew resistance into H 77/833-2 from ICMP 451 and also a major region of the genome controlling grain yield during drought stress again into H 77/833-2 (male parent of the popular pearl millet hybrid, HHB 67), but from PRLT 2/89-33. ICRISAT and its partners have successfully demonstrated the marker-assisted backcross transfer of additional downy mildew resistance into a parental line of popular pearl millet hybrid “HHB 67” and a new version of this hybrid, “HHB 67 Improved” based on the improved parental line, was released for commercial cultivation in 2005 as the first public-bred product of marker-assisted breeding in India (Hash *et al.*, 2006a). This study has demonstrated that, while conventional backcross transfer of downy mildew resistance to improve one parental line took nearly nine years, a marker-based approach facilitated its completion for the other in just three years. Bidinger *et al.* (2007) identified three QTLs (on LG2, LG3, and LG4) were

identified as primary candidates for MAS for improved pearl millet grain yield across variable post-flowering moisture environments and those on LG2 (863B allele) and LG3 (ICMB 841 allele) explained useful proportions (25 and 13%, respectively) of phenotypic variance for grain yield across environments. They also co-mapped with other grain yield associated traits and linked to SSR markers so they should be amenable to efficient MAS. Products of breeding supplemented with MAS are just now beginning to become available in India, and work is continuing to transfer downy mildew resistance and drought tolerance QTL into other economically important hybrid parental lines at ICRISAT. The next decade will hopefully see this technology spreading to benefit pearl millet farmers worldwide.

2.2.5. Contiguous segmental substitution lines (CSSLs)

Over the last two decades, the application of molecular markers and genetic linkage maps has allowed the identification of QTLs in all of major crop species including pearl millet. However, it is still complicated to detect favourable alleles of complex traits such as disease resistance in a crop relative or wild species because of often-poor agronomic performance of such non-adaptive germplasm. Specifically, functional genomics research of complex phenotypes has to resolve the technical difficulties such as to efficiently identify QTLs with large effects on specific target phenotypes, to efficiently fine-map target QTLs and determine candidate genes of QTLs; and to efficiently determine and verify functions of candidate genes conferring these QTLs. Different types of segregating populations, like Recombinant Inbred Lines (RIL), Doubled Haploids (DH), Backcross (BC) or F₂/F₃ populations have been extensively used for QTL mapping. Nevertheless, the resolution of QTL identification is limited in these mapping populations due to overshadowing effects of major QTLs on the estimation of numbers of independently segregating minor QTLs and with their fine mapping by increasing the total phenotypic variation and also due to interactions between pairs of unlinked QTLs. Moreover, in the case of interspecific crosses, hybrid sterility often hampers developing such population types. In such cases, production of a novel population type consisting of complete set of chromosome segment substitution lines (CSSLs) [*e.g.* recombinant inbred introgression lines (ILs)] provides avenues to exploit largely the genetic and physiological mechanism associated with several agronomic traits of interest that remain in the wild species (Eshed and Zamir, 1995).

Introgression lines are typically developed by systematic backcrossing to introgress a small segment of chromosome from a donor parent into the genetic background of a recurrent

parent. Sets of such lines are expected to differ for only small portions of genome of donor parent in an otherwise common genetic background of recurrent parent. Therefore, they constitute an “exotic library” where the entire donor species genome is partitioned among several lines, each carrying a single homozygous introgressed segment. These lines are in a small set (say 25-35) homozygous lines that differ from each other by pairs of introgressed segments. These lines are useful resources for genetic studies, including detection and fine mapping of QTLs for target traits. In particular, CSSLs permit the detection of QTLs with small additive effects that are masked by QTLs with large effects in filial generation 2 (F₂) and recombinant inbred line (RIL) populations. Being much less expensive to phenotype, and probably even more effective, the nearly isogenic nature of the CSSLs provides a relative advantage over other segregating populations when rapidly implementing a pyramiding approach via crosses, marker analysis and phenotyping. The lines fulfilling agronomic requirements can directly be used in breeding programs as potential donors of respective traits. The CSSLs enable systematic assessment of donor parent allelic effects in the recurrent parent genetic background, and thus facilitate transferring genes from one parent to the other in molecular breeding.

The concept of CSSLs was first proposed by Eshed and Zamir (1994a) in case of cultivated tomato to enable the identification and fine mapping of yield-associated QTL. This strategy has been successfully demonstrated and widely utilized in introgression of favourable alleles from wild relatives in tomato, rice, wheat and barley. Introgression Lines (ILs) in tomato (Eshed and Zamir, 1994a, 1994b, 1995; Fulton *et al.*, 1997; Bernacchi *et al.*, 1998; Chetelat and Meglic, 2000; Monforte and Tanksley, 2000), Chromosome Segment Substitution Lines (CSSL) or Single Segment Substitution Lines (SSSL) in rice (Ghesquière *et al.*, 1997; Doi *et al.*, 1997; Yu *et al.*, 2005; Ando *et al.*, 2008; Zhu *et al.*, 2009; Gutiérrez *et al.*, 2010); introgression lines (ILs) in wheat (Liu *et al.*, 2006) and in barley (Matus *et al.*, 2003; Pillen and Zacharias, 2003; Korff *et al.*, 2004). Several sets of introgression lines have already been developed for various other plant species, such as lettuce (Jeuken and Lindhout, 2004), soybean (Concibido *et al.*, 2003), *Brassica oleracea* (Ramsay *et al.*, 1996), *Brassica napus* (Howell *et al.*, 1996), cotton (Saha *et al.*, 2004; Stelly *et al.*, 2005), *Arabidopsis* (Koumproglou *et al.*, 2002); and rye (Falke *et al.*, 2008; Rangel *et al.*, 2008).

In general, two approaches are followed to develop CSSL population. The first method involves crossing of two elite parents with subsequent backcrossing with recurrent parent to

generate backcross progenies. An extensive marker assisted selection (MAS) is initiated until segmental substitution lines overlapping the entire genome of donor parent are developed. This method was used by Hao *et al.*, (2006) to develop CSSLs that carried the overlapping chromosome segments of entire rice genome. The second method involves choosing a specific recombinant inbred line (RIL) and backcrossing with the donor parent, assisted by molecular markers. Kubo *et al.*, (1999) implemented this second approach to generate a series of *indica* CSSL from *japonica* background in rice. A wide population containing 128 CSSLs in rice were successfully developed using a high quality physical map of ultra high density SNPs based on whole genome re-sequencing data by Xu *et al.*, 2010.

Introgression lines have been developed for various crops such as tomato, lettuce, barley, rice and *Brassica Oleracea*. During development of CSSL, molecular MAS can accelerate the process. In most cases, MAS was conducted from early backcross generation (Eshed and Zamir 1994a, Ramsay *et al.* 1996, Howell *et al.* 1996). Liu *et al.*, 2006 delayed MAS selection until the late backcross generations, BC₄F₃ during the development of 82 introgression lines from 97 selected lines of BC₄F₃. Their results proved that marker detection at the end of the process of introgression lines development may be also efficient.

Considering the several utilities of introgression lines, attempts were made at ICRISAT to develop a partial set of contiguous segment substitution lines based on two elite dwarf hybrid parental lines, 843B as recurrent parent and ICMP 85410 as donor parent. Hybrids of the inbred substitution lines developed and their recurrent parents were evaluated for drought tolerance and downy mildew resistance. Several CSSLs showed improved resistance relative to the recurrent parent, particularly whenever a previously mapped downy mildew resistance QTL was introgressed into the recurrent parent. A remarkably large difference in terminal drought tolerance of hybrids produced with testers H 77/833-2 (more tolerant) and RIB 335/74 (more sensitive) was noted. This means that an agronomically elite drought-sensitive pollinator (RIB 335/74) could be used as a mapping population parent in any future QTL mapping of pearl millet terminal drought tolerance (Hash, technical report of DFID project). Besides this partial set of introgression lines, two agronomically elite parental lines *viz.*, 863B and ICMB 841, which were known to differ for important agronomic traits like downy mildew resistance and drought and salinity tolerance were also used to start to develop

a more complete set of introgression lines having overlapping segments from 863B in the background of ICMB 841 through marker-assisted backcrossing. At the start of this PhD thesis research program, these were in various backcross generations.

CHAPTER III

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Development of contiguous segment substitution lines (CSSLs)

3.1.1. Progenies selected from advanced backcross generation

Two agronomically elite inbred seed parents ICMB 841 and 863B were crossed to develop a segregating population for genetic linkage map construction and trait analysis. The two parents are known to produce hybrids that distinctly differ in their response to post-flowering stress. Parent 863B (Andrews and Anand Kumar, 1996; Rai *et al.*, 2008) was bred from *Iniadi* landrace material from Togo. It was selected for this study based on its combination of agronomic eliteness and superior combining ability for grain filling under terminal drought conditions. Parent ICMB 841 (Singh *et al.*, 1990) is the maintainer of the female parent of several high yielding hybrids that have been widely grown in India but lack tolerance to terminal drought stress.

Prior to the initiation of this thesis research project; development of the segmental substitution line set had progressed to the BC₅F₂/BC₆F₁ generation with support from the UK's Department for International Development (DFID). The DFID-supported work was completed at the start of 2006. A database containing marker genotype information and availability for selfed and backcrossed seed materials harvested from the BC₅F₂/BC₆F₁ generation was developed and used to identify the most promising BC₅F₃ and BC₆F₂ families for generation of the segmental introgression homozygotes.

3.1.2. Genotyping of the selected lines

In the present study, one thousand four hundred and ninety-two progenies (376 for LG1, 69 for LG2, 88 for LG3, 328 for LG4, 302 for LG5, 102 for LG6 and 227 for LG7) derived from the advanced backcross populations (from BC₅F₃, BC₅F₂ and BC₆F₂ families) involving single-plant selections from two genetically diverse, agronomically elite hybrid parental lines *viz.*, ICMB 841-P3 and 863B-P2 were selected for genotyping using seventy-four of repeatable and polymorphic PCR-compatible pearl millet SSR (Qi *et al.*, 2001, 2004; Allouis *et al.*, 2001; Budak *et al.*, 2003; Senthilvel *et al.*, 2008), STS (Money *et al.*, 1994), and SSCP-SNP (Bertin *et al.*, 2005) markers. In backcross generations through BC₅F₂ and BC₆F₁, prior to initiation of this thesis study, plants were selected for heterozygosity for donor parent

marker alleles on a single linkage group (LG), or rarely two LGs, combined with homozygosity for recurrent parent alleles across all other genomic regions for which marker data was available. The following Table 1 summarizes the numbers of progenies and markers used for genotyping to develop CSSLs for each of the seven pearl millet linkage groups.

Genomic DNA from leaf tissue samples of each of the selected plants, as well as their recurrent and donor parents were isolated using a high-throughput DNA extraction protocol described by Mace *et al.* (2003) and normalized to a working concentration of 2.5 ng/ μ l. In order to determine the quantity and quality of genomic DNA, an aliquot of 1 μ l of DNA from each sample along with 2.5 ng of molecular weight marker (λ DNA, Amersham Biosciences) were initially analyzed by electrophoresis on 0.8% agarose gels containing ethidium bromide (0.5 μ l/10 ml of gel) and run in 0.5X TBE (Tris Borate EDTA) buffer at a constant voltage (100 V) for one hour. The gel was viewed under UV illumination and recorded using an UVi Tech gel documentation system (DOL-008.XD, England).

Polymerase chain reaction (PCR) was performed in 5 μ l reaction volumes with following protocol:

Component	Stock Concentration	Volume
DNA	2.5 ng/ μ l	1.0 μ l
Primers	2 pm/ μ l	1.0 μ l
MgCl ₂	10 mM	1.0 μ l
dNTPs	2 mM	0.3 μ l
Buffer	10X	0.5 μ l
Enzyme	0.5 U/ μ l	0.2 μ l
(AmpliTaq Gold [®] , Applied Biosystems, USA)		
Water		1.0 μ l
	Total	5.0 μl

Table 1: Number of progenies selected from advanced backcross generation of ICMB 841×863B and markers used for genotyping

Linkage Group(LG)	LG1	LG2	LG3	LG4	LG5	LG6	LG7
Total no.of lines selected from advanced backcross generation	376, BC5F3 and BC6F2 progenies (derived from 30 BC5F2 and BC6F1 families)	69, BC5F3 and BC6F2 progenies (derived from 4 BC5F2 and BC6F1 families)	88, BC5F3 progenies (derived from 8 BC5F2 families)	328, BC5F3 and BC4F3 progenies (derived from 21 BC5F2, 1 BC5F1 and 4 BC4F2 families)	302, BC5F3 progenies (derived from 21 BC5F2 families)	102, BC5F3 progenies (derived from 8 BC5F2 families)	227, BC6F2 progenies (derived from 2 BC6F1 families)
Polymorphic markers selected for genotyping	<i>Xpsmp2069</i> <i>Xpsmp2273</i> <i>Xicmp3080</i> <i>Xicmp3017</i> <i>Xicmp3032</i> <i>Xctm12</i> <i>Xpsms35</i> <i>Xpsms38</i> <i>Xpsms58</i> <i>Xpsms86</i>	<i>Xpsmp2072</i> <i>Xpsmp2088</i> <i>Xpsmp2066</i> <i>Xpsmp2206</i> <i>Xpsmp2255</i> <i>Xpsmp2201</i> <i>Xpsmp2231</i> <i>Xpsmp2225</i> <i>Xpsmp2059</i> <i>Xpsmp2232</i> <i>Xctm21</i> <i>Xpsms13</i> <i>Xpsms30</i> <i>Xpsms75</i> <i>Xpsms84</i> <i>Xpsms82</i> <i>Xpsms73</i> <i>Xpsms22</i> <i>Xpsmp322</i>	<i>Xpsmp2267</i> <i>Xpsmp2070</i> <i>Xpsmp2214</i> <i>Xpsmp2227</i> <i>Xpsmp2249</i> <i>Xctm10</i> <i>Xpsms31</i> <i>Xpsms60</i> <i>Xpsms61</i> <i>Xpsmp108</i>	<i>Xpsmp2081</i> <i>Xpsmp2076</i> <i>Xpsmp2084</i> <i>Xpsms27</i> <i>Xpsms16</i> <i>Xpsmp716</i> <i>Xpsmp305</i>	<i>Xpsmp2202</i> <i>Xpsmp2078</i> <i>Xpsmp2229.2</i> <i>Xicmp3027</i> <i>Xicmp3078</i> <i>Xctm25</i> <i>Xpsms74</i> <i>Xpsms56</i> <i>Xpsms70</i> <i>Xpsmp318</i>	<i>Xpsmp2270</i> <i>Xpsmp2213</i> <i>Xicmp3002</i> <i>Xicmp3058</i> <i>Xicmp3086</i> <i>Xicmp3050</i> <i>Xicmp3038</i> <i>Xpsms41</i> <i>Xpsms59</i>	<i>Xpsmp2224</i> <i>Xpsmp2271</i> <i>Xpsmp2074</i> <i>Xpsmp2063</i> <i>Xpsmp2263</i> <i>Xpsmp2203</i> <i>Xicmp3043</i> <i>Xicmp3048</i> <i>Xctm8</i>
Total SSR loci	6	11	6	3	6	7	9
Total SSCP-SNP loci	4	7	3	2	3	2	-
Total STS loci	-	1	1	2	1	-	-
Total markers = 74	10	19	10	7	10	9	9

PCR reactions were carried out in a GeneAmp[®] PCR System 9700 thermal cycler (Applied Biosystems, USA) with a touchdown (61-51) program using the following cyclic conditions:

- Step 1:** Denaturation at 94°C for 15 min
- Step 2:** Denaturation at 94°C for 15 sec
- Step 3:** Annealing at 61°C for 20 sec
(Temperature reduced by 1°C for each cycle)
- Step 4:** Extension at 72°C for 30 sec
- Step 5:** Go to Step 2 for 10 times
- Step 6:** Denaturation at 94°C for 10 sec
- Step 7:** Annealing at 54°C for 20 sec
- Step 8:** Extension at 72°C for 30 sec
- Step 9:** Go to Step 6 for 40 times
- Step 10:** Extension of 20 min at 72°C.
- Step 11:** Store at 4°C

Amplified PCR products were resolved on 6% native polyacrylamide gels (PAGE) coupled with silver staining as described by Tegelstrom (1992) in case of un-labelled STS markers and SSR markers. The PCR amplicons of SSCP-SNP markers were denatured at 94°C for 5 min and separated on mutation detection enhancement (MDE) gels (Rockland, ME 04841 USA).

In case of some SSR markers, the forward primer was directly labelled with one of the four fluorescent dyes *viz.* *Fam*, *Ned*, *Pet* and *Vic*. Addition of a fluorescently-labelled forward primer into the PCR reaction, along with reverse primers, adds the fluorescent label to the PCR amplicon. This facilitates high-throughput genotyping and marginally reduces the cost of such genotyping.

The same PCR protocol was used with some modifications using direct labelled primers as follows:

Component	Working Conc.	Final Conc.	Volume
DNA	2.5 ng/μl	2.5 ng	1.0 μl
Primer			
Labelled forward primer	2.0 pm/μl	0.08 pm/μl	0.2 μl
Reverse primer	2.0 pm/μl	0.16 pm/μl	0.2 μl
MgCl ₂	10 mM	2 mM	1.0 μl
dNTPs	2 mM	0.12 mM	0.3 μl
Buffer	10 X	1 X	0.5 μl
Enzyme	0.5 U/μl	0.1 U	0.2 μl
(AmpliTaq Gold [®] , Applied Biosystems, USA)			
Water			1.0 μl
		Total	5.0 μl

The same PCR program described above, touchdown 61-51 was used for amplification of all polymorphic markers. After amplification, PCR products were pooled based on their amplicon size. Pooling was done using the following protocol:

Component	Volume
Hi-Di Formamide	7.0 μl
LIZ size standard	0.1 μl
PCR products	
FAM labelled	1.0 μl
VIC labelled	1.0 μl
NED labelled	1.0 μl
PET labelled	1.0 μl
Water	2.9 μl
Total	10.0 μl

Pooled PCR products of different markers labelled with different fluorescent dyes were denatured at 95°C for 5 minutes. Capillary electrophoresis of denatured pooled products was performed using ABI3130x1 DNA genetic analyzer. After completion of the electrophoresis

run, the raw data files created by the ABI machine were processed through GENESCAN 3.7 v software (Applied Biosystems) for sizing the PCR amplified fragments based on their relative mobility compared to the internal *Liz* size standards. Allele calling was done using GENOTYPER 3.7 v software (Applied Biosystems).

Based on the amplicon sizes, data were scored for all optimized primers run against template DNA from each individual sample. Data points were scored as ‘A’, ‘B’, ‘H’ or ‘-’.

‘A’ – Homozygous allele of donor parent (863B)

‘B’ – Homozygous allele of recurrent parent (ICMB 841B)

‘H’ – Heterozygous (presence of both parental alleles)

‘-’ – Missing data (failed amplification)

3.1.3. Identification of CSSLs

Based on the above genotyping results, a number of CSSLs were identified by the presence of homozygous segmental introgression of donor parent 863B in the background of recurrent parent ICMB 841. A few homozygous recurrent parent homozygous lines and heterozygous lines were also identified.

3.2. Agronomic performance of CSSLs

3.2.1. Testcross hybrid seed Development and self seed multiplication

The selected set of homozygous segmental substitution lines, a set of samples of selected recurrent parent homozygous lines (identified from the first objective of this study) along with their donor and recurrent parents (863B and ICMB 841, respectively) were evaluated for drought tolerance as testcross hybrids, rather than using their derived inbred progenies (Yadav *et al.* 2002, 2003, 2004) for several reasons:

- to restore heterotic vigor to the advanced generation backcross-derived progenies that might otherwise be too weak for effective screening under stress conditions (pearl millet is highly cross-pollinated in nature and suffers considerably from inbreeding depression); and
- to have test units that approximate the genetic structure of the F₁ hybrids grown by farmers rather than agriculturally irrelevant BC₅F₄ or BC₆F₃ inbred lines.

During the 2008/09 summer and kharif seasons at ICRISAT-Patancheru, selfed seed multiplication and testcross hybrid seed production involving morphologically and genetically diverse elite restorer line testers RIB 3135-18, PPMI 301 and H 77/833-2 was carried out for the selected CSSLs along with their parents (863B and ICMB 841) and samples of selected recurrent parent homozygous lines. Receptive stigmas on bagged panicles of each of these lines were dusted with bulk pollen from each three testers. Characters of these testers and their released hybrids helped to distinguish the reasons for their selection. The first tester RIB 3135-18 is highly sensitive to drought. It is the male parent of released full season hybrid RHB127 (ICMA 89111 × RIB 3135-18) developed at Rajasthan Agricultural University, Agricultural Research Station Durgapura. The second tester PPMI 301 is also sensitive to terminal drought stress. It is the male parent of released full-season hybrid Pusa 301 (841A × PPMI 301) developed at the Indian Agricultural Research Institute, New Delhi. The third tester used for testcross hybrid development, H 77/833-2, is the male parent of number of thermo-tolerant extra-early, high tillering and high yielding pearl millet hybrids, including HHB 67 (843A x H 77/833-2; Kapoor *et al.*, 1989) developed at Haryana Agricultural University, Hisar. HHB 67, which was widely, cultivated in Haryana and the Thar Desert margins of Rajasthan in north-western India. Besides this testcross seed production, seed multiplication was also done by selfing to produce inbred seeds. These selfed seed lots were stored for use in future research.

3.2.2. Drought nursery trials

Field trials of testcross hybrids produced with all three testers for sets of CSSLs for pearl millet linkage groups LG3, LG4 and LG5 (10 for LG3, 20 for LG4, and 13 for LG5) along with their donor (863B) and recurrent (ICMB 841) parents and a set of samples of recurrent parent homozygotes (3 for LG3, 8 for LG4, and 3 for LG5), were conducted at ICRISAT-Patancheru during the 2009 summer season under fully-irrigated non-stress conditions and early-onset terminal drought stress conditions. Field trials of testcross hybrids of CSSLs for LG1, LG2, LG3, LG6 and LG7 (12 for LG1, 10 for LG2, 10 for LG6 and 14 for LG7) along with their donor (863B) and recurrent (ICMB 841) parents and a set of samples of recurrent parent homozygotes (7 for LG1, 4 for LG2, 2 for LG6, and 11 for LG7), were conducted at ICRISAT-Patancheru during the 2010 summer season under three environments: *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions (more severe), and late-onset terminal drought stress conditions (less severe).

3.2.2.1. Statistical Design

Drought nursery trials of testcross hybrids were conducted separately for each linkage group and all trials were sown in alpha (incomplete block) designs to provide for as much adjustment capability to local variation in stress intensity as possible. Small blocks of between 6 and 9 plots were used (18-27 m²/block), with the total number of blocks variable, depending upon the numbers of entries in the trial and replicated thrice. It was generally found that the effect of blocking was statistically significant, despite the general precautions taken in managing these experimental crops. Individual plots were one row of 4.0 m length with rows 0.6 m apart and net (harvested) plot area was one row of 3.0 m by 0.6 m (1.8 m²).

3.2.2.2. Field/Crop Management

For pearl millet drought tolerance field screening at ICRISAT-Patancheru, only a single designated six ha field with a shallow and relatively uniform soil profile is used. When at field capacity, the profile of this field contains enough plant-available water for about 6 days of full evapotranspiration (ET) during April, when pan evaporation rates averages 8-10 mm day⁻¹. As a part of the development of this field for surface irrigation, the A and B horizons of the original soil (50-75 cm depth) were removed, the gravelly subsoil material graded to a uniform slope of 1.5% and the surface soil spread evenly over the graded subsoil. Thus the major source of heterogeneity in the original field—the variable depth of soil to the gravelly C-horizon, and the consequent variable amount of plant-available water—has been largely removed (Bidinger *et al.*, 1987).

Sprinkler irrigation is used to supply water to the crop before flowering, adjusting the amounts of water applied to meet increases in transpiration demand as the season progresses, as pearl millet is sensitive to low soil oxygen tensions that occur following surface (furrow) irrigation during cooler times of the year. Sprinkler lines are placed 14.4 m (24 crop rows) apart, with each sprinkler line in the center of four border rows, so that leakage from the sprinkler lines does not affect test plots.

At the time of initiation of the stress, furrow irrigation is used to be sure that the full soil profile is wetted. The furrows are filled rapidly, one twenty-row strip at a time, to have a sufficient head of water for this purpose. Water is held in the furrows for 4 hours and then drained rapidly to prevent water logging. All irrigation operations are managed carefully to assure that irrigation is done as precisely and uniformly as possible.

The time of sowing of trials in the summer drought nursery is standardized to have the crop flower and fill grain during the period of maximum evaporation demand and irrigation is managed to achieve a 50-60% reduction in yield for a severe stress and a 30-40% reduction for a moderate stress. Standard crop management procedures (described below) are followed to obtain uniform pre-flowering crop growth and initiate the stress(es) at fixed crop developmental stage(s). Irrigation in the early-onset stress treatment was terminated approximately one week before flowering of the main shoot to initiate the stress about mid-flowering to affect both seed number and seed filling. Irrigation in the late-onset stress treatment was terminated one week later to initiate the stress in early to mid-grain filling to affect primarily seed filling.

A number of ways to improve the uniformity of the pearl millet crop growth in the ICRISAT Patancheru drought nursery prior to the initiation of the stress treatments have been learned by experience. 1) The field is land planed every 2 to 3 years to remove local surface irregularities that result in collection of excess irrigation water and reduced crop growth. 2) Fertilizer is banded into ridges with a precision applicator, rather than broadcasting it, to assure that all seedlings have equal access to nutrients. 3) Light sprinkler irrigation is provided prior to sowing, to moisten the surface soil and improve control over the depth of seed placement. 4) Over sowing is done with a precision planter and seedlings thinned about 10 days after emergence to achieve uniform plant stands. 5) Sprinkler irrigation is used in the early crop growth stages, rather than furrow irrigation, to prevent excess water application and reduced crop growth. 6) Sprinkler irrigation is provided at the time of secondary root initiation to assure that these roots penetrate the soil rapidly and completely. 7) Weed management is practiced during the entire year in the screening field to prevent the build-up of weed seed and cultivation is done early and as often as necessary to remove weed seedlings in early stages before they can establish. 8) Prophylactic pest and disease control is applied whenever a problem is suspected.

3.2.2.3. Traits evaluated

The observations and measurements taken during the field trials were as follows:

1. Flowering time (FT): Time of flowering was recorded as days from seedling emergence to stigma emergence in 75% of the main shoots in a plot.

2. Plant height (PH): Plant height (cm) was measured from the base of the stem to the tip of the main culm panicle at the maturity. Data was recorded on three random plants from the middle of each row.
3. Panicle length (PL): Length of the panicle (cm) was measured for main culms of sample plants considered for plant height in each plot.
4. Panicle diameter (PD): Panicle diameter (mm) was measured using Vernier callipers on all those panicles for which panicle length was recorded.

At the time of harvest

5. Plant count (PC): Number of plants in the middle 3 m of one row of each plot was counted for all the entries.
6. Head count (HC): Panicles from the middle 3 m of one row of each plot were harvested and counted for all the entries.
7. Effective tiller (ET): Number of productive tillers per plant was calculated by dividing HC by PC.
8. Panicle yield (PY): After harvesting was completed, panicles were put in an oven for 24 hours and dried at a temperature of 60°C. The dry weight of the panicles from each plot was then recorded before threshing.
9. Grain yield (GY): Panicles were threshed and their grain cleaned. The weight of the grains from each plot was recorded.
10. Fresh stover yield (FSY): After panicles were harvested, the stems and the tillers were cut for biomass analyses from the middle 3 m length of one row per plot for all entries.
11. Subsample fresh stover weight (SWS): Samples of fresh stover were then collected from each entry and chopped and fresh weight of these samples was taken.
12. Subsample dry stover weight (SDS): The chopped samples were kept in a drier for two days at a temperature of 60°C and their dry weights were then recorded.
13. 100-grain mass (HGM): One hundred grains (g) were counted in two replicates and their weight was recorded for each entry.
14. Number of grains per panicle (PGN): Number of grains per panicle was derived from these primary data ($= (100*GY) / (PN*HGM)$).
15. Stover dry matter fraction (SDMF): Stover dry matter fraction was calculated for each plot as the ratio of SDS and SWS.
16. Dry stover yield (DSY): Dry matter yield was calculated as the product of FSY and SDMF.

17. Biomass yield (BMY): Biomass yield was calculated for each plot as the sum of PY and DSY.
18. Grain harvest index (GHI): Grain harvest index was calculated for each plot as the ratio of GY and BMY.
19. Vegetative growth index (VGI): Vegetative growth index was calculated by using the formula: $VGI = \frac{BMY}{FT+10}$.

3.2.2.4. Statistical Analysis

The statistical analysis *viz.*, Line \times Tester analysis, was performed using the software, GENSTAT 10th edition (2007). Analysis of variance was calculated and also general combining ability (*gca*) was estimated for each line and tester for each observed or calculated trait under different moisture treatments (fully irrigated non-stress control, early-onset stress, and late-onset stress) and also across treatments. Pooled environment analysis interaction was assessed in the combined analysis across the three treatments to detect significant differences, if any, in drought tolerance for grain and stover yield-component traits. Mean agronomic performance of testcrosses of each CSSL was compared with that of the recurrent parent, and significant differences were attributed to the presence of QTL(s) for the particular trait in the introgressed donor segment(s).

CHAPTER IV

RESULTS

4. RESULTS

4.1 Development of contiguous segment substitution lines (CSSLs)

One thousand four hundred and ninety-two progenies derived from the advanced backcross populations (from BC₅F₃, BC₅F₂ and BC₆F₁ families), involving two genetically diverse, agronomically elite hybrid parental lines *viz.*, ICMB 841 and 863B were genotyped using 74 polymorphic markers (Table 1) expected to provide coverage across most of each of the seven pearl millet linkage groups to identify contiguous segment substitution lines (CSSLs) for pearl millet.

4.1.1. Linkage Group 1 (LG1)

A total of 376 BC₅F₃ and BC₆F₂ progenies (derived from 30 BC₅F₂ and BC₆F₁ families) were successfully genotyped with ten polymorphic markers (6 SSRs and 4 SSCP-SNPs, *viz.*, *Xpsmp2069*, *Xpsmp2273*, *Xpsmp3080*, *Xpsmp3017*, *Xpsmp3032*, *Xctm12*, *Xpsms35*, *Xpsms38*, *Xpsms58* and *Xpsms86*) distributed across linkage group 1 (LG1) to identify contiguous segmental substitution lines for LG1. The genotyping results of all 376 plants for LG1 are presented in Table 1 of the Annexure. Based on the analysis of the 10 markers used, nine 863B alleles were represented in at least one plant. Based on this genotyping, 143 lines (selfed progeny of the genotyped plants) were identified as recurrent parent homozygotes that lack any substituted donor segment from 863B, and 220 lines exhibited 1-3 heterozygous segments substituted from 863B. Finally the remaining 13 lines showed 1-2 homozygous segments substituted from 863B in the genetic background of recurrent parent ICMB 841. Genotyping data of these 13 CSSLs of LG1 is shown in Table 2. Among these 13 CSSLs, 12 lines have only one homozygous segment and remaining one line has two homozygous segments from the donor parent. A total of 11 unique homozygous 863B introgression segments were achieved with these 13 CSSLs, which subsequently constituted our CSSLs for LG1 though few lines have overlapping segments. The distribution of the introgressed segments along this chromosome was not random. The majority of 863B introgression segments were often at one terminal position. No introgression segment was found to include the marker *Xctm12* position at another terminal of LG1.

Table 2: Genotyping data of 13 contiguous segmental introgression lines of Linkage Group1(LG1)

Locus/ Line number	<i>Xpsms86</i>	<i>Xpsmp2069</i>	<i>Xpsmp2273</i>	<i>Xpsms35</i>	<i>Xpsms38</i>	<i>Xpsms58</i>	<i>Xicmp3080</i>	<i>Xicmp3017</i>	<i>Xicmp3032</i>	<i>Xctm12</i>
71080	A	A	A	B	B	B	B	B	B	B
71085	-	A	A	B	B	-	B	B	B	B
71268	B	A	A	B	A	B	B	B	B	B
71134	-	A	A	A	-	-	B	B	B	B
71174	-	A	A	-	-	-	B	B	B	B
71178	-	A	A	-	-	-	B	-	B	B
71273	B	A	A	A	A	B	B	B	B	B
71281	B	A	A	A	A	B	B	B	B	B
71118	B	A	A	A	A	A	A	A	-	B
71074	-	-	B	B	A	-	B	B	B	B
71218	B	B	B	B	B	A	A	A	A	B
71220	B	B	B	B	B	A	A	A	A	B
71103	B	B	B	B	B	B	A	B	B	-

4.1.2. Linkage Group 2 (LG2)

Sixty-nine individuals comprised of BC₅F₃ and BC₆F₂ progenies (derived from 4 BC₅F₂ and BC₆F₁ families) and 19 polymorphic markers (11 SSRs, 7 SSCP-SNPs and one STS, viz. *Xpsmp2072*, *Xpsmp2088*, *Xpsmp2066*, *Xpsmp2206*, *Xpsmp2255*, *Xpsmp2201*, *Xpsmp2231*, *Xpsmp2225*, *Xpsmp2059*, *Xpsmp2232*, *Xctm21*, *Xpsms13*, *Xpsms30*, *Xpsms75*, *Xpsms84*, *Xpsms82*, *Xpsms73*, *Xpsms22* and *Xpsmp322*) distributed across the LG2 were used to identify CSSLs for LG2. The genotyping results of all 69 plants for LG2 are presented in Table 2 of the Annexure. Based on the analysis of the 19 markers used, eight 863B alleles were represented in at least one plant. Thirteen plants were identified lacking any substituted donor segment from 863B, 9 plants showed single homozygous segments substituted from donor parent 863B, and the remaining 47 plants contained heterozygous donor segments. Genotyping data of these nine CSSLs of LG2 is shown in Table 3. Among nine homozygous LG2 introgression lines identified, three were unique lines containing three different homozygous introgression segments of the donor parent, occupying one terminal position and part of middle portion of the LG2. There were no introgression segments of the donor parent, observed at many marker positions of LG2, as substitution line development for this portion of LG2 was the subject of an earlier research program (Sathish Kumar, 2004), and hence not targeted in the present study.

4.1.3. Linkage Group 3 (LG3)

To construct CSSLs for LG3, 88 BC₅F₃ progenies (derived from eight BC₅F₂ families) were selected and genotyped with 10 polymorphic markers (Six SSRs, three SSCP-SNPs and one STS, viz. *Xpsmp2267*, *Xpsmp2070*, *Xpsmp2214*, *Xpsmp2227*, *Xpsmp2249*, *Xctm10*, *Xpsms31*, *Xpsms60*, *Xpsms61* and *Xpsmp108*). Based on the analysis of the 10 markers used, eight 863B alleles were represented in at least one plant. The genotyping results of all 88 lines (selfed progeny of the genotyped plants) for LG3 are presented in Table 3 of the Annexure. Of the 88 lines analysed, 4 lines had no detectable alleles from donor parent 863B, and hence were recurrent parent allele homozygotes at all genotyped loci. Finally, 10 CSSLs were identified. Three CSSLs out of 10 showed a single homozygous donor parent introgression segment, five lines showed two homozygous introgression segments, and two lines showed three homozygous introgression segments from donor parent 863B in the background of recurrent parent ICMB 841. Genotyping data of these 10 CSSLs of LG3 is shown in Table 4.

Table 3: Genotyping data of 9 contiguous segmental introgression lines of Linkage Group2 (LG2)

Locus/ Line Number	Xpsmp322	Xpsmp2072	Xpsmp2088	Xpsmp2066	Xpsms13	Xpsms30	Xpsmp2059	Xpsms75	Xpsmp2232	Xpsmp2255	Xpsmp2225	Xpsms84	Xpsms82	Xpsms73	Xpsmp2206	Xpsmp2201	Xpsmp2231	Xpsms22	Xctm21
72001	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
72030	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B
72038	B	B	B	B	B	B	-	B	B	A	A	B	B	B	B	B	-	B	B
72039	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B
72060	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B
72066	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B
72067	B	B	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B
72054	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	-	B	B
72069	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B

Table 4: Genotyping data of 10 contiguous segmental introgression lines of Linkage Group3(LG3)

Locus/ Line number	<i>Xpsmp108</i>	<i>Xpsms60</i>	<i>Xpsmp2267</i>	<i>Xpsmp2070</i>	<i>Xpsmp2214</i>	<i>Xpsmp2249</i>	<i>Xpsmp2227</i>	<i>Xpsms61</i>	<i>Xpsms31</i>	<i>XCTM10</i>
73052	A	B	B	A	B	B	B	B	B	B
73079	A	A	B	B	B	B	B	B	B	B
73082	B	A	B	B	A	A	A	B	B	A
73086	B	A	B	B	A	A	A	B	B	A
73087	B	A	B	B	B	B	B	B	B	B
73048	B	A	A	A	A	A	A	B	B	A
73014	B	B	A	A	B	B	B	B	B	B
73023	B	B	A	A	A	A	A	B	B	A
73029	B	B	A	A	A	A	A	B	B	A
73046	B	B	A	A	A	A	A	B	B	A

A total of seven unique homozygous 863B introgression segments were achieved from 10 CSSLs, which subsequently constituted our CSSLs for LG3. The distribution of the introgression segments along the chromosome was not random and could not cover the portion of LG3 which include marker positions *Xpsms61* and *Xpsms31*.

4.1.4. Linkage Group 4 (LG4)

A total of 328 BC₅F₃, BC₅F₂ and BC₄F₃ progenies (derived from 21 BC₅F₂, 1 BC₅F₁ and 4 BC₄F₂ families) were successfully genotyped with seven polymorphic markers (three SSRs and two SSCP-SNPs and two STSs, viz. *Xpsmp2081*, *Xpsmp2076*, *Xpsmp2084*, *Xpsms27*, *Xpsms16*, *Xpsmp716* and *Xpsmp305*) distributed across the central portion of linkage group 4 (LG4) to identify contiguous segmental substitution lines for LG4. Based on analysis of the seven markers used, all seven alleles of 863B were represented in at least one plant. The genotyping results of all 328 lines (selfed progeny of the genotyped plants) for LG4 are presented in Table 4 of Annexure. Based on this genotyping, 62 lines were identified lacking a substituted donor segment from 863B, 225 lines exhibited 1-4 heterozygous segments substituted from 863B, and 41 lines showed 1 -2 homozygous segments substituted from 863B in the background of recurrent parent ICMB 841. Many of these lines have overlapping introgressed segments. Genotyping data and Graphical genotypes of these 41 CSSLs of LG4 is shown in Table 5. Among these 41 lines, 33 lines showed one and eight lines showed two homozygous introgression segments from the donor parent with remaining genetic background of the recurrent parent. A total of seven unique homozygous 863B introgression segments were achieved across these 41 CSSLs, which subsequently constituted our CSSLs for LG4. The distribution of the introgression segments along this chromosome was also random, overlapping in some lines and covering the majority of the central portion of LG4.

4.1.5. Linkage group 5 (LG5)

The CSSLs for LG5 were constructed by genotyping 302 BC₅F₃ progenies (derived from 21 BC₅F₂ families) with 10 polymorphic markers (six SSRs, three SSCP-SNPs and one STS viz. *Xpsmp2202*, *Xpsmp2078*, *Xpsmp2229.2*, *Xicmp3027*, *Xicmp3078*, *Xctm25*, *Xpsms74*, *Xpsms56*, *Xpsms70* and *Xpsmp318*) distributed across LG5. The genotyping results of all 302 plants for LG5 are presented in Table 5 of the Annexure. Based on the analysis of the 10

Table 5: Genotyping data of 41 contiguous segmental introgression lines of Linkage Group4 (LG4)

Locus/ Line number	<i>Xpsmp716</i>	<i>Xpsmp2076</i>	<i>Xpsmp2081</i>	<i>Xpsmp305</i>	<i>Xpsmp2084</i>	<i>Xpsms27</i>	<i>Xpsms16</i>
74875	A	B	B	B	B	B	B
74535	A	B	B	B	B	B	B
74574	A	B	B	B	B	B	B
74578	A	B	B	B	B	B	B
74585	A	B	B	B	B	B	B
74614	A	B	B	B	B	B	B
74448	A	A	-	B	B	B	B
74449	A	A	-	B	B	B	B
74382	A	A	A	B	B	B	B
74383	A	A	A	B	B	B	B
74411	A	A	A	B	B	B	B
74416	A	A	A	B	B	B	B
74685	A	A	A	B	B	B	B
74472	A	A	A	B	B	B	B
74478	A	A	A	B	B	B	B
74481	A	A	A	B	B	B	B
74482	A	A	A	B	B	B	B
74523	A	A	A	B	B	B	B
74526	A	A	A	B	B	B	B
74534	A	A	A	B	B	B	B
74537	A	A	A	B	B	B	B
74539	A	A	A	B	B	B	B
74543	A	A	A	B	B	B	B
74549	A	A	A	B	B	B	B
74779	A	A	A	B	B	B	B
74567	A	A	A	B	B	B	-
74570	A	A	A	B	B	B	A
74571	A	A	A	B	B	B	A
74605	A	A	A	B	B	B	A
74618	A	A	A	B	B	B	A
74620	A	A	A	B	B	B	A
74689	A	A	A	-	B	B	A
74698	A	A	A	B	B	B	A
74714	A	A	A	B	B	B	A
74135	B	B	B	A	A	A	B
74011	B	-	B	A	A	A	A
74014	B	B	B	A	A	A	A
74015	B	B	B	A	A	-	A
74601	B	B	B	B	B	B	A
74607	B	B	B	B	B	B	A
74633	B	B	B	-	B	B	A

markers used, all these 10 alleles of 863B were represented in at least one plant. Of the 302 lines (selfed progeny of the genotyped plants) analysed, 43 lines were identified as recurrent parent homozygotes that lack any detectable donor parent 863B specific allelic pattern, and 225 lines exhibited 1-4 heterozygous segments substituted from 863B. Finally, 23 CSSLs were identified containing 1-3 homozygous introgression segments of donor parent 863B in the background of recurrent parent ICMB 841 genome. Genotyping data and Graphical genotypes of these 23 CSSLs of LG5 is shown in Table 6. Among these 23 lines, 18 lines showed one and five lines showed two homozygous introgression segments from the donor parent with remaining genetic background of the recurrent parent. A total of 14 unique homozygous 863B introgression segments were achieved from 23 CSSLs, which subsequently constituted our CSSLs for LG5. The distribution of the introgression segments could cover the entire length of LG5 except at the *Xpsmp2229.1* marker locus.

4.1.6. Linkage Group 6 (LG6)

One hundred and two BC₅F₃ progenies (derived from 8 BC₅F₂ families) and nine polymorphic markers (seven SSRs, two SSCP-SNPs viz. *Xpsmp2270*, *Xpsmp2213*, *Xicmp3002*, *Xicmp3058*, *Xicmp3086*, *Xicmp3050*, *Xicmp3038*, *Xpsms41* and *Xpsms59*) covering across the LG6 were successfully used to identify CSSLs for LG6. The genotyping results of all 102 plants (selfed progeny of the genotyped plants) for LG6 are presented in Table 6 of Annexure. Based on the analysis of the nine markers used, all nine alleles of 863B were represented in at least one line. Eight lines were identified lacking substituted donor segment from 863B, 47 lines contained heterozygous segments and remaining 11 lines showed single homozygous segments of ICMB 863 in the genetic background of ICMB 841. Genotyping data and Graphical genotypes of these 11 CSSLs of LG6 is shown in Table 7. Six unique homozygous introgression segments of donor parent were achieved from the above set of 11 CSSLs for LG6.

4.1.7. Linkage Group 7 (LG7)

A total of 227 BC₆F₂ progenies (derived from 2 BC₆F₁ families) were successfully genotyped with nine polymorphic SSR markers viz, *Xpsmp2224*, *Xpsmp2271*, *Xpsmp2074*, *Xpsmp2063*, *Xpsmp2263*, *Xpsmp2203*, *Xicmp3043*, *Xicmp3048* and *Xctm8* distributed across LG7 to

Table6: Genotyping data of 23 contiguous segmental introgression lines of Linkage Group5 (LG5)

Locus/ Line number	<i>Xpsmp2202</i>	<i>Xicmp 3027</i>	<i>Xicmp3078</i>	<i>Xctm25</i>	<i>Xpsmp318</i>	<i>Xpsms74</i>	<i>Xpsms56</i>	<i>Xpsmp2078</i>	<i>Xpsms70</i>	<i>Xpsmp2229</i>
75173	A	A	A	A	A	A	B	B	B	B
75093	A	A	A	A	A	A	B	A	A	B
75023	-	A	A	A	A	A	A	B	B	-
75117	A	A	A	-	A	A	A	A	A	-
75075	A	A	A	A	A	A	-	A	A	B
75159	A	A	A	A	A	A	A	A	A	B
75022	A	A	A	A	A	A	A	A	A	B
75257	B	A	B	-	B	-	-	-	B	B
75218	B	B	A	B	B	B	B	B	A	B
75231	B	B	A	B	B	B	B	B	A	B
75098	B	B	B	A	B	B	B	A	A	B
75186	B	B	B	A	B	B	A	A	B	B
75289	B	B	B	A	A	A	A	B	B	B
75291	B	B	B	A	A	A	B	B	B	B
75298	B	B	B	A	A	A	B	B	B	B
75126	B	B	B	B	A	B	B	B	B	B
75297	B	B	B	-	A	A	-	B	B	B
75187	B	B	B	-	B	B	A	A	B	B
75190	B	B	B	B	B	B	A	A	B	B
75074	B	B	B	B	B	B	A	A	A	B
75209	B	B	B	B	B	B	B	B	A	B
75242	B	B	-	B	B	B	B	B	A	B
75099	B	B	B	B	B	B	B	-	A	B

Table 7: Genotyping data of 11 contiguous segmental introgression lines of Linkage Group6 (LG6)

Locus/ Line number	<i>Xpsmp2270</i>	<i>Xpsmp2213</i>	<i>Xicmp3058</i>	<i>Xicmp3086</i>	<i>Xicmp3050</i>	<i>Xpsms59</i>	<i>Xicmp3002</i>	<i>Xicmp3038</i>	<i>Xpsms41</i>
86019	A	A	B	B	B	B	B	B	B
86025	A	A	B	B	B	B	B	B	B
86027	A	A	B	B	B	B	B	B	B
86066	A	A	A	A	A	A	A	B	B
86052	A	A	A	A	A	A	A	A	B
86053	A	A	A	A	A	A	A	A	B
86064	A	A	A	A	A	A	A	A	B
86096	B	B	A	A	A	A	A	B	B
86100	B	B	A	A	A	A	A	B	B
86014	B	B	B	B	B	B	A	A	A
86042	B	B	B	B	B	B	B	A	B

identify contiguous segmental substitution lines for LG7. Based on the analysis, 863B alleles for all nine markers used, were represented in at least one line. The genotyping results of all 227 plants (selfed progeny of the genotyped plants) for LG7 are presented in Table 7 of Annexure. Based on this genotyping results, 25 lines were identified as recurrent parent homozygous lines, lacking any substituted donor segment from 863B, 185 lines exhibited 1-3 heterozygous segments substituted from 863B and the remaining 17 lines showed 1-3 homozygous segments substituted from 863B in the genetic background of recurrent parent ICMB 841. Genotyping data and Graphical genotypes of these 17 CSSLs of LG7 is shown in Table 8. Out of these 17 CSSLs, fourteen lines have one homozygous introgression segment and one line has two and the remaining two lines have three homozygous introgression segments of donor parent. A total of eight unique homozygous 863B introgression segments were achieved from this set of 17 CSSLs, which subsequently constituted our CSSLs for LG7. The distribution of the introgression segments along the chromosome was not random and covered the entire length of LG7 though there were few lines having overlapping segments.

For all seven linkage groups of pearl millet, a total 124 CSSLs were achieved which were consequently selected to construct a contiguous segmental substitution line population (CSSL population) and among these 124 lines, 56 lines have unique homozygous introgression segments. In this population, each CSSL contains 1-3 defined chromosomal introgression segments, few lines have overlapping segments and based on the analysis of the 74 markers used, 60 863B alleles were represented in at least one plant. The length of introgression segments were covered by 1-4 marker loci and some are by more than 4 marker loci. Different introgression frequencies were observed among the seven linkage groups; among 54 unique introgression segments, 14 introgression segments existed on LG5, while only 3 on LG2. However, the major portion of LG2 was not covered and also three regions on LG1, LG3 and LG5 were not covered even by overlapping introgression segments.

Table 8: Genotyping data of 17 contiguous segment introgression lines of Linkage Group7

Locus/ Line number	<i>Xicmp3048</i>	<i>Xpsmp2224</i>	<i>Xpsmp2271</i>	<i>Xpsmp2074</i>	<i>Xpsmp2063</i>	<i>Xpsmp2263</i>	<i>Xpsmp2203</i>	<i>Xicmp3043</i>	<i>Xctm8</i>
87071	A	A	B	B	B	B	B	B	B
87049	A	A	A	A	A	A	A	A	A
87070	A	A	A	A	A	A	A	A	A
87081	B	A	A	A	A	A	A	A	A
87167	B	A	A	A	A	A	A	A	A
87188	B	A	A	A	A	A	A	A	A
87005	B	A	-	A	A	A	-	-	A
87195	B	A	A	A	A	B	A	A	A
87002	B	B	A	A	A	A	A	A	A
87032	B	B	A	A	A	B	B	B	B
87130	B	B	-	A	-	-	A	-	A
87134	B	B	A	A	A	A	A	A	A
87148	B	B	B	A	A	A	A	A	A
87214	B	B	B	B	B	A	A	A	A
87217	B	B	B	B	B	A	A	A	A
87222	B	B	B	B	B	A	A	A	A
87226	B	B	B	B	B	A	A	A	A

4.2. Agronomic performance of CSSLs

4.2.1. Testcross hybrid seed Development and self seed multiplication

A set of selected contiguous segmental substitution lines (CSSLs) and samples of selected recurrent parent homozygous lines (RPHLs) (to serve as near-isogenic controls) for all seven linkage groups (identified from the first objective of this study) along with their donor and recurrent parents 863B and ICMB 841 respectively were evaluated for drought tolerance as testcross hybrids, rather than using their derived inbred progenies to avoid inbreeding depression and to bring the same genetic structure of F₁ hybrids of what farmers generally use in their fields.

During the 2008-09 summer and kharif seasons at ICRISAT-Patancheru, testcross hybrid seed production involving morphologically and genetically diverse elite restorer line testers RIB 3135-18, PPMI 301 and H 77/833-2 was carried out successfully for a set of selected CSSLs and samples of selected RPHLs for all seven linkage groups along with their donor and recurrent parents 863B and ICMB 841, respectively. Besides the testcross seed production, seed multiplication was also done by selfing to get inbred seeds. This self seed lot is stored for further research.

4.2.2. Drought nursery trials

Field trials of testcross hybrids of selected CSSLs and RPHLs for LG1, LG2, LG3, LG6 and LG7 along with their donor (863B) and recurrent (ICMB 841) parents were conducted at ICRISAT-Patancheru during the 2010 summer season under three environments *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions. Field trials of testcross hybrids of selected CSSLs and RPHLs for LG3, LG4 and LG5 along with their donor (863B) and recurrent (ICMB 841) parents were conducted at ICRISAT-Patancheru during the 2009 summer season under two moisture environments, *i.e.*, fully-irrigated control conditions and late-onset terminal drought stress conditions. The drought nursery trials were conducted for each linkage group separately. ANOVA and line × tester analysis were performed to estimate general combining ability (*gca*) of each line and tester in each environment: fully-irrigated, early on-set and late on-set drought stress and across these three environments. Genotype × environment (G×E) interaction was assessed in the combined analysis across the three treatments to detect

significant differences, if any, in drought tolerance for grain and stover yield-component traits. Mean agronomic performance of testcrosses of each CSSL was compared with that of the recurrent parent, and significant differences were attributed to the presence of QTL(s) for the particular trait in the introgressed donor segment(s). The results of the above experiments are described for each linkage group of all seven linkage groups of pearl millet here in this chapter.

4.2.2.1. Linkage Group 1 (LG1)

A total 63 testcross hybrids (involving three testers) of 12 homozygous segmental introgression lines and a sampling of 7 recurrent parent homozygotes (to serve as near-isogenic controls) of LG1, which were selected based on marker genotyping of 376 plants at 10 marker loci of LG1 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in three environments *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions during summer 2010 at ICRISAT, Patancheru. The results of analysis of variance and general combining ability (*gca*) for grain and stover yield related characters for all three moisture regimes and across these three moisture regimes are presented below.

4.2.2.1.1. Fully irrigated non- stress treatment

4.2.2.1.1(A). Analysis of variance

The analysis of variance results for LG1 under fully irrigated non- stress conditions during summer 2010 are presented in Table 9. Testcross hybrids showed highly significant differences for all characters except for biomass yield and dry stover yield. There were clear significant differences between lines for time to 75% flowering, grain yield, plant height, panicle length and panicle diameter, panicle number, 1000 grain mass and grain number per panicle. As expected, effects of testers were highly significant for all observed agronomic characters. Due to line \times tester effects, only panicle diameter and 1000 grain mass were found to be significant.

Table 9 : Analyses of variance for testcross hybrids of LG1 in fully irrigated non-stress conditions

Source of Variation	df	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Replication	2	16.94**	1.45	1.5	1.88	3.39*	0.11	41.07**	4.23*	0.21	1.57
Hybrids	62	201500.7**	4.91**	1.87**	41233844.82**	903.02**	8479.67**	129444.27**	7.99**	2.08**	14448.37**
Lines (L)	20	2.37**	2.37**	1.11	1.31	3.34**	2.45**	5.56**	1.58	1.75*	1.39
Testers (T)	2	14.84**	116.89**	12.94**	111.64**	25.7**	72.89**	34.77**	3.78*	23.08**	18.42**
L × T	40	1.32	0.58	0.67	0.84	1.43	1.29	1.45*	0.93	0.91	1.33
Error	124										

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative			Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
			Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)					
Replication	2	0.68	0.52	0.94	1.28	0.65	4.1*	2.85	1.96	1.29
Hybrids	62	0.87	754.64**	47786444.82**	59695.52**	0.97	32480849.92**	423133.35**	4.17**	2.86**
Lines (L)	20	0.61	0.67	0.9	1.04	0.65	0.69	14.69**	2.71**	1.33
Testers (T)	2	6.92**	6.45**	54.75**	14.77**	4.18*	8.18**	588.15**	71.2**	58.96**
L × T	40	0.69	0.72	0.85	1.02	0.86	0.84	2.00**	0.84	0.8
Error	124									

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

4.2.2.1.1(B). General Combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG1 in fully irrigated non- stress conditions during summer 2010 are presented in Table 10. For flowering time, the best negative combiners among lines were 71080 and donor parent 863B. The donor parent 863B exhibited significant positive *gca* effects for grain yield, panicle yield, panicle harvest index, plant height and panicle diameter as expected. For grain yield, only one CSSL 71178 showed significant positive *gca* and only one line 71181, a recurrent parent homozygote, showed significant negative *gca* effects. This later line 71181 showed significant negative *gca* effects for panicle yield and panicle harvest index. The remaining lines did not exhibit any significance for these three traits. CSSL 71134 showed significant positive *gca* effects for plant height and panicle length. CSSL 71220 exhibited significant positive *gca* effects for both panicle length and panicle diameter but CSSL 71218 showed significant negative *gca* for panicle diameter. The donor parent 863B exhibited significant negative *gca* effects for panicle number and tiller number. All 12 CSSLs tested, did not show any significant *gca* effects for these two traits. The donor parent 863B exhibited significant positive *gca* values for biomass yield, vegetative growth index, grain harvest index, fresh stover yield and dry stover yield. All 12 CSSLs did not exhibit significant *gca* effects for all these traits except only one CSSL 71085 showed significant positive *gca* effects for dry stover yield. For 1000 grain mass, two introgression lines 71080 and 71178 had significant positive *gca* but one introgression line 71268 and four recurrent parent homozygous lines (71066, 71139, 71181, 71226) showed negative *gca* results. CSSLs 71134 and 71178 exhibited significant positive *gca* effects for grain number per panicle and grain number per m² respectively. From the above results, it was clear that only one CSSL 71178 is the best of all CSSLs tested for grain yield, grain number and grain mass in this fully irrigated non- stress conditions.

Among testers, RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number and tiller number. PPMI 301 showed positive *gca* results for all observed traits except for panicle number and tiller number. Tester H 77/833-2 showed positive effects for all traits except for panicle length, panicle diameter, stover dry matter fraction and 1000-grain mass.

Table 10 : Estimates of *gca* effects of lines and testers of LG1 in fully irrigated non-stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle Yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Number ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/ m ²
71066	-0.26	-216.2	-21.3	-3.25	-5.66*	-1.09*	-0.15**	-4.98	36.75	0.31*	547.1	11.4	0.23	256.4	205.5	0.022	-0.552**	-94	-204
71074	-0.04	-91.3	-21.1	-0.03	-2.10	0.02	-0.13	4.35	-36.58	-0.28	-141.0	-3.0	0.12	-378.0	-126.4	0.032	-0.273	90	-89
71080	-0.82**	-202.3	-350.9	-3.25	-2.88	-0.65	0.09	16.00**	12.75	-0.13	-88.3	0.2	0.34	-36.9	-100.3	0.002	0.346*	-183*	-1722
71083	0.07	68.5	149.1	1.97	-3.31	0.68	-0.01	-0.53	-0.36	0.02	837.5	16.2	2.90	429.7	325.2	0.024	0.144	57	277
71085	-0.59	80.3	-55.7	-1.14	1.79	0.13	-0.05	-3.76	-24.25	-0.07	556.6	12.5	0.79	998.6	472.5*	0.002	-0.171	16	443
71088	-0.04	98.2	30.6	4.42	-2.99	0.02	-0.03	2.47	-22.25	-0.19	-219.5	-4.0	1.12	-148.0	-174.6	-0.016	0.200	109	333
71117	-0.48	-190.1	-50.7	-1.03	-3.77	-0.65	-0.13	2.47	17.53	0.04	447.9	10.0	1.34	185.3	136	0.006	-0.153	-80	-502
71118	0.30	-19.7	-129.4	3.97	2.01	-0.10	-0.12	-0.20	-3.92	-0.02	-306.5	-7.0	1.68	-549.2	-135.6	0.035	-0.006	23	61
71134	0.74*	237.7	-284.3	-0.47	9.67**	1.68**	0.06	5.02	-36.03	-0.28	-604.3	-14.0	-1.88	133.1	183.3	0.013	0.107	244**	996
71139	-0.37	-262.0	-150.9	-3.36	-4.32	-1.43**	-0.02	3.69	8.97	0.03	-1171.4	-22.4	-6.10	-1323.6*	-511	-0.026	-0.369*	-107	-878
71174	0.19	-168.0	-18.2	-1.36	-3.10	-0.43	-0.11	-0.53	40.97	0.25	-169.7	-2.8	-2.10	-234.7	-123.3	0.004	-0.008	-167	-857
71178	0.63*	567.2**	380.5	2.20	1.01	0.46	0.09	-5.53	-8.14	0.00	-75.0	-2.6	2.01	537.5	27	-0.037	0.430**	51	2347*
71181	-0.15	-527.9**	-942.2**	-10.58**	-4.10	0.35	-0.10	2.35	6.53	0.02	-956.5	-18.8	-4.55	-280.3	-141	0.007	-0.607**	-226**	-2392*
71218	-0.15	-46.9	-15.2	0.75	1.79	-0.32	-0.20**	-6.31	-14.03	-0.02	-79.8	-1.2	-1.88	-189.2	3.3	0.023	0.169	-8	-475
71220	0.19	-22.9	115.8	-0.58	4.34	1.24*	0.27**	-19.70**	-9.58	0.28	-226.4	-5.3	-0.21	-411.4	-239.5	-0.003	0.070	5	-612
71226	0.52	-310.7	-69.4	-4.25	-2.10	0.46	-0.08	5.02	29.53	0.11	-405.3	-9.4	-4.43	-771.4	-170.3	0.036	-0.502**	-112	-709
71268	0.63*	114.6	199.7	2.20	-0.77	0.57	0.03	5.69	12.75	0.03	791.7	14.1	3.57	327.5	229.2	0.007	-0.526**	96	1676
71273	-0.15	232.2	345.4	3.53	-1.88	-0.10	-0.05	1.91	21.97	0.11	-7.1	0.6	0.23	-149.2	-91.3	-0.054	-0.132	43	1558
71281	0.52	-151.9	40.5	-2.25	1.57	0.57	0.12	0.58	10.97	0.05	-57.6	-2.8	-3.77	362.0	35.3	-0.016	-0.271	-55	-96
ICMB 841	0.19	14.7	121.9	1.31	-0.21	-0.10	-0.06	5.13	38.64	0.16	-207.0	-4.4	1.12	-159.2	-249.9	-0.028	-0.129	-76	375
863B	-0.93**	796.3**	725.6*	11.19**	14.56**	-1.32**	0.58**	-13.00*	-82.20**	-0.40**	1534.4*	32.8*	9.45**	1400.8*	445.9*	-0.033	2.232**	374**	470
H 77/833-2	0.42**	321.7**	81.9	6.83**	2.21*	-1.27**	-0.16**	4.80*	22.80**	0.08	145.2	1.6	2.75*	403.4*	132.1	-0.017	-0.300**	130**	2455**
PPM1 301	0.04	568.7**	363.3**	10.16**	3.59**	1.81**	0.16**	-1.58	-46.90**	-0.28**	701.5*	13.9*	8.24**	564.0**	139.7	-0.021	1.538**	185**	543
RIB 3135-18	-0.47**	-890.5**	-445.2**	-16.99**	-5.79**	-0.54**	0.00	-3.31	24.10**	0.20**	-846.8**	-15.5**	-11.00**	-967.4**	-271.8*	0.038**	-1.238**	-314**	-2998**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

4.2.2.1.2. Early-onset drought stress treatment

4.2.2.1.2(A). Analysis of variance

The analysis of variance results for LG1 under early-onset drought stress condition during summer 2010 are presented in Table 11. Hybrids were highly significant for all characters except for biomass yield and dry stover yield as the same observed in the results of fully irrigated control treatment. There were clear significant differences between lines for grain yield and also for other grain yield related traits but there were no significant differences for biomass yield, vegetative growth index and stover yield. As expected, effects of testers were highly significant for all observed agronomic characters except for biomass yield and vegetative growth index. Specific significant differences were not exhibited for any trait due to line x tester effects in this early-onset drought stress conditions.

4.2.2.1.2(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG1 in early-onset drought stress treatment, during summer 2010 are presented in Table 12. For flowering time, the better negative combiners among lines were 71080, 71083, 71117, 71174, 71268 and donor parent 863B when compared with that of recurrent parent ICMB 841. This implies that these lines showed early flowering when compared to recurrent parent 841B. Among testers, RIB 3135-18 followed by PPMI 301 showed negative *gca* effects for flowering time and H 77/833-2 expressed significant positive *gca*. CSSLs 71080 and 71178 and donor parent 863B expressed significant positive *gca* values for grain yield. Four CSSLs (71080, 71134, 71178 and 71281) and one recurrent parent homozygote 71083 showed better *gca* results than that of recurrent parent ICMB 841B for this trait. CSSL 71178 and 863B exhibited significant positive *gca* values for both panicle yield and panicle harvest index also. Out of 21 lines tested, 13 lines (8 CSSLs, 4 RPHLs and donor parent) showed better *gca* results for panicle yield than that of recurrent parent. Three CSSLs 71080, 71134 and 71178 expressed better *gca* values than that of ICMB 841 for panicle harvest index. For plant height, CSSLs 71178 and 71218 along with donor parent showed significant positive *gca* values. Eight CSSLs and three RPHLs exhibited better *gca* effects for panicle length than that of recurrent parent ICMB 841B. For panicle diameter, three CSSLs (71174, 71178 and 71268) and 863B expressed better *gca* effects. One CSSL 71268 had significant positive *gca* values for panicle number and tiller number whereas CSSL 71220 showed significant negative *gca* effects for these two traits. Significant *gca* effects were not observed in case of biomass yield and

Table 11 : Analyses of variance for testcross hybrids of LG1 in early-onset terminal drought stress conditions

Source of Variation	df	Time to Flowering (d)	Grain Yield (kg/ha)	Panicke yield (kg/ha)	Panicke Harvest Index (%)	Plant Height (cm)	Panicke Length (cm)	Panicke Diameter (cm)	Plant Population ('000/ha)	Panicke Number ('000/ha)	Tillers per Plant (Number)
Replication	2	4.06*	25.33**	24.5**	18.64**	10.11**	3.37*	4.93**	1.48	0.4	1.14
Hybrids	62	75208.4**	4.43**	2.56**	14953674.7**	246.98**	3077.27**	54514.86**	2.48**	5.12**	36342.11**
Lines (L)	20	3.38**	4.5**	1.96*	4.13**	5.45**	3.4**	8.52**	1.2	2.95**	2.02*
Testers (T)	2	5.77**	69.77**	6.42**	94.41**	27.06**	157.4**	84.85**	0.04	96.15**	71.69**
L × T	40	0.82	1.12	1.07	1.22	0.85	1.11	0.85	0.76	1.46	1.04
Error	124										

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicke	Grain Number/m ²
Replication	2	5.32**	5.36**	24.19**	31.83**	0.44	88.53**	66.65**	11.95**	13.08**
Hybrids	62	0.82	689.27**	40566533.15**	37899.97**	1.09	1981611.22**	259231.89**	5.21**	2.48**
Lines (L)	20	0.74	0.85	3.04**	1.04	0.89	1.48	11.43**	4.21**	2.15**
Testers (T)	2	1.34	1.14	43.11**	7.05**	4.63*	22.82**	242.68**	76.56**	32.79**
L × T	40	0.83	0.81	0.54	1.24	0.96	1.19	1.31	1.33	1.11
Error	124									

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 12 : Estimates of *gca* effects of lines and testers of LG1 in early-onset terminal drought stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
71066	0.10	-7.3	38.1	-2.53	-2.53	-0.41	-0.11	7.39	26.17	0.08	-174.7	-3.6	-2.41	238.7	-11.5	-0.023	-0.464**	-58	134
71074	0.21	-149.8	-203.1	-2.75	-2.20	0.03	-0.05	2.95	-4.05	-0.06	-272.7	-5.9	-0.63	-277.9	-199.6	-0.008	-0.409**	-45	-512
71080	-0.46	220.5*	225.9	5.25	-4.86*	-0.52	0.02	-1.94	18.28	0.17	362.0	8.1	4.48*	414.3	6.0	-0.053	0.398*	66	1239
71083	-0.13	76.0	-0.9	2.58	-0.86	0.70	-0.01	-4.50	-2.28	0.03	-269.7	-5.1	-0.19	-52.4	-161.9	-0.001	-0.031	91	609
71085	-0.02	32.7	63.2	1.58	0.03	-0.41	-0.08	-6.39	2.61	0.10	510.1	10.1	0.48	285.4	316.4	0.041	0.028	-6	139
71088	0.32	-88.1	-93.3	-2.09	-5.08*	-0.52	-0.07	11.39	2.06	-0.11	160.7	2.4	-0.52	-37.9	123.8	0.045	-0.278	-21	-422
71117	-0.24	-201.6	-128.4	-5.31	-1.86	0.25	-0.03	0.50	-19.05	-0.12	208.6	4.5	-2.74	89.8	206.8	0.050	-0.089	-101	-1080
71118	0.10	-150.5	-131.0	-5.86*	-10.64**	-0.41	-0.08	1.84	0.72	-0.03	-842.0	-16.9	-3.96	-1109**	-474.5*	0.032	-0.323*	-116	-959
71134	0.43	63.7	-45.9	4.14	4.25	1.14**	-0.05	-1.28	-12.05	-0.03	-471.7	-10.1	0.59	-396.8	-256.6	-0.035	0.163	88	318
71139	0.21	-150.0	-132.9	-3.42	-4.09	-0.96*	-0.12*	12.80*	16.28	-0.04	-23.3	-1.0	-1.07	16.5	-20.5	0.002	-0.149	-107	-743
71174	-0.35	-197.4	-97.5	-5.53	-3.75	-0.96*	0.08	0.39	17.39	0.08	297.1	6.5	-2.96	286.5	264.6	0.023	-0.108	-146*	-1082
71178	0.43	336.5**	282*	8.24**	10.02**	1.47**	0.10*	-4.39	-26.94	-0.12	140.0	1.9	3.37	704.3	29.0	-0.049	0.181	259**	2228**
71181	-0.02	-51.1	-61.3	-1.42	-2.09	0.14	-0.12*	8.28	21.17	0.02	-277.7	-5.4	-1.41	-399.0	-81.2	0.055	-0.042	-75	-287
71218	0.10	-33.3	-69.8	0.03	5.02*	0.37	-0.01	-2.05	-26.94	-0.14	144.2	2.6	0.59	154.3	83.8	-0.008	-0.114	26	122
71220	0.32	-156.2	85.7	-6.52*	1.14	1.03**	0.03	-2.05	-37.60*	-0.21*	-494.8	-10.1	-1.96	-380.2	-330.5	-0.040	0.146	-54	-1563*
71226	-0.02	-97.4	64.0	-4.42	-1.97	-0.63	-0.10	-0.28	31.80**	0.19	489.2	9.7	-1.74	307.6	295.0	0.027	-0.483**	-61	-237
71268	-0.24	-10.8	98.1	-0.97	-1.09	0.37	0.08	-2.05	33.70*	0.24*	-18.7	-0.1	-2.19	-259.0	63.1	-0.021	-0.199	-41	260
71273	0.10	-122.7	-108.6	-2.75	3.36	0.70	0.03	-10.50	14.50	0.21*	370.3	7.0	-1.74	219.8	349.1	0.055	-0.202	-85	-540
71281	0.32	108.0	81.0	3.92	0.80	0.37	-0.06	5.50	17.39	0.04	-99.0	-2.6	0.82	-37.9	-69.2	-0.013	-0.108	65	1022
ICMB 841	0.43	-159.7	-314.9*	-1.75	-0.09	-0.85*	-0.07	-4.50	-2.94	0.02	-357.5	-8.1	-0.19	-290.2	-172.6	-0.007	-0.052	-89	-893
863B	-1.57**	738.4**	449.2**	19.58**	16.47**	-0.85*	0.63**	-11.16	-70.20**	-0.33*8	619.8	16.1	13.37**	523.2	40.4	-0.073*	2.133**	409**	2246**
H 77/833-2	0.23**	47.4	66.8	1.50	3.88**	-1.27**	-0.17**	0.49	40.60**	0.22**	192.9	3.4	0.24	392.9**	183.4*	-0.011	-0.048	-28	614*
PPM1 301	-0.05	300.2**	69.9	10.01**	1.47	2.17**	0.20**	-0.24	-62.70**	-0.37**	-195.7	-3.7	5.08**	10.3	-192.7*	-0.050**	0.956**	226**	1115**
RIB 3135-18	-0.19*	-347.7**	-136.61**	-11.51**	-5.35**	-0.90**	-0.02*	-0.24	22.10**	0.14**	2.8	0.4	-5.32**	-403.2**	9.3	0.061**	-0.908**	-198**	-1729**

** Significant at 0.01 level of probability. * Significant at 0.05 level of probability

vegetative growth index. For grain harvest index, one CSSL 71080 and 863B expressed significant positive *gca* effects. For fresh and dry stover yield also, significant *gca* effects were not observed except one CSSL 71118, which was negatively significant. For 1000 grain mass, CSSL 71080 and 863B exhibited significant positive *gca* effects and for grain number per panicle and grain number per m², CSSL 71178 and 863B showed significant positive *gca* effects. It was observed that three CSSLs (71080, 71178, and 71281) exhibited better *gca* effects for both grain number per panicle and grain number per m². These *gca* results under early-onset drought stress treatment demonstrated that four CSSLs (71080, 71134, 71178 and 71281) found be better lines out of 12 CSSLs tested, for grain yield, panicle yield and grain number. It was also noticed that RPHL 71083 also exhibited better results for these traits.

Among testers RIB 3135-18 showed significant negative *gca* effects for all grain yield related traits except for panicle number, tiller number and stover dry matter fraction PPMI 301 showed positive *gca* results for all observed traits except for panicle number, tiller number, vegetative growth index, dry stover yield and stover dry matter fraction. Tester H 77/833-2 showed positive effects for many traits except for panicle length, panicle diameter, stover dry matter fraction, 1000-grain mass and grain number per panicle.

4.2.2.1.3. Late-onset drought stress treatment

4.2.2.1.3(A). Analysis of variance

The analysis of variance results for LG1 under late-onset terminal drought stress conditions during summer 2010 are presented in Table 13. Hybrids showed highly significant differences for all characters except for biomass yield and dry stover yield. There were clear significant differences between lines for grain yield and also for other grain yield related traits but there were no significant differences for biomass yield, vegetative growth index and stover yield. As expected, effects of testers were highly significant for all observed agronomic characters. Due to line x tester effects, plant height, 1000 grain mass and grain number per panicle were found to be significant.

Table 13 : Analyses of variance for testcross hybrids of LG1 in late-onset terminal drought stress conditions

Source of Variation	df	Time to									
		75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Replication	2	0.11	5.02**	14.09**	0.25	23.23**	20.99**	0.14	16.6**	2.18	7.89**
Hybrids	62	239940.05**	11.78**	5.41**	56837213.28**	807.04**	8016.76**	121933.67**	3.85**	3.86**	25665.84**
Lines (L)	20	4.66**	5.95**	2.63**	6**	9.84**	4.57**	11.2**	0.97	2.82**	1.8*
Testers (T)	2	11.56**	287.53**	35.99**	356.25**	61.09**	239.24**	108.82**	1.83	67.78**	54.59**
L × T	40	1.21	0.91	0.66	1.22	1.82**	1.29	1.22	0.8	0.84	0.85
Error	124										

Source of Variation	df	Biomass		Vegetative		Dry		Stover		Grain	
		Yield (kg/ha)	Yield (kg/ha)	Growth Index (kg/ha/d)	Harvest Index (%)	Fresh Stover Yield (kg/ha)	Stover Yield (kg/ha)	Matter Fraction	1000-Grain Mass (g)	Number/Panicle	Number/m ²
Replication	2	1.31	1.33	1.33	0.95	6.98**	2.15	5.17**	1.81	0.58	3.06
Hybrids	62	1.2	1010.02**	41950751.4**	76366.21**	14315154.08**	1.33	287749.03**	10.5**	5.33**	
Lines (L)	20	1.36	1.42	2.68**	1.46	18.07**	1.37	41.65**	5.93**	2.07**	
Testers (T)	2	8.9**	7.75**	97.76**	45.87**	10.31**	419.83**	1.1	210.6**	118.76**	
L × T	40	0.74	0.73	0.68	0.69	1.63*	0.77	1.54*	1.24		
Error	124										

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

4.2.2.1.3(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG1 under late-onset terminal drought stress conditions, during summer 2010 are presented in Table 14. For flowering time, one CSSL (71080), two RPHLs (71139 and 71226) and donor parent 863B were the best negative combiners. Among testers, RIB 3135-18 followed by PPMI 301 showed negative *gca* effects for flowering time as in early-onset stress treatment. Two CSSLs 71080 and 71178 and donor parent 863B expressed significant positive *gca* effects for grain yield where as only one CSSL 71074 and two recurrent parent homozygous lines 71066 and 71139 showed significant negative *gca* effects for this same trait. Out of 12 CSSLs tested, 6 CSSLs (71080, 71134, 71178, 71220, 71273 and 71281) showed better *gca* effects for grain yield than that of recurrent parent. For panicle yield, introgression line 71080 and 863B showed significant positive effects while a recurrent parent homozygote 71181 and recurrent parent ICMB 841B showed significant negative effects. It was observed that 14 lines out of 21 tested (10 CSSLs: (71074, 71080, 71085, 71134, 71174, 71178, 71218, 71220, 71273 and 71281), 3 RPHLs and donor parent) exhibited better *gca* results for panicle yield when compared with that of recurrent parent. None of the lines except donor parent 863B showed significant positive *gca* effects for panicle harvest index. For plant height, CSSL 71220 and donor parent expressed significant positive *gca* effects whereas four RPHLs (71066, 71139, 71181 and 71226) and two CSSLs (71074 and 71080) showed significant negative *gca*. Anyhow, 7 CSSLs (71085, 71134, 71178, 71218, 71220, 71268 and 71281) had better *gca* values for plant height than that of ICMB 841. For panicle length, four CSSLs (71178, 71220, 71268 and 71281) expressed significant positive results whereas 863B, one CSSL 71174 and one RPHL 71139 had significant negative *gca* values. Only one introgression line 71268 and donor parent 863B expressed significantly positive *gca* for panicle diameter. It was noticed that 7 CSSLs (71080, 71118, 71178, 71218, 71268, 71273 and 71281) out of 12 exhibited better *gca* effects than that of ICMB 841 for panicle diameter. Donor parent 863B was negatively significant for both panicle number and tiller number and none of the lines showed significant positive *gca* effects for these traits. There were no lines observed with significant positive *gca* for biomass yield and vegetative growth index except that donor parent, showed significant positive *gca* for vegetative growth index. One CSSL 71080 and 863B expressed significant positive *gca* for grain harvest index. For fresh stover yield, one CSSL 71134 and 863B exhibited significant positive *gca* effects. One RPHL 71083 showed significant positive *gca* for dry stover yield and stover dry matter fraction. CSSL 71281 was negatively significant for biomass yield, vegetative growth index, fresh stover yield and dry stover yield. For 1000

Table 14 : Estimates of *gca* effects of lines and testers of LG1 in late-onset terminal drought stress con

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/ m ²
71066	0.46*	-387.4**	-68.8	-10.2**	-6.43**	-0.69	-0.06	5.01	21.10	0.06	-580.6	-12.5	-8.59**	-524.0	-239.0	0.040	-0.625**	-207**	-1480*
71074	0.35	-281.9**	91.4	-9.42**	-3.77*	-0.69	-0.11*	15.40*	-7.35	-0.21*	139.6	1.8	-3.70	-126.2	-74.2	-0.002	-0.333*	-79	-1123
71080	-0.64**	258.7*	241.5*	3.91	-5.21**	-0.47	0.04	1.78	19.87	0.11	374.2	9.0	4.85*	577.1	10.2	-0.084*	0.102	55	1461*
71083	0.13	-35.0	74.7	-1.65	-2.00	0.53	-0.02	-1.22	-10.57	-0.07	810.0	15.9	-1.70	239.4	612.6**	0.083*	0.156	3	-358
71085	-0.09	56.2	24.3	1.80	1.78	-0.47	-0.03	-2.55	2.43	0.05	242.6	4.9	1.96	121.6	95.8	0.015	-0.118	33	598
71088	0.02	-95.7	-139.3	-0.98	-2.00	-0.13	-0.09	9.23	17.54	-0.01	-69.3	-1.4	0.74	28.3	-52.4	-0.011	-0.267	-73	-134
71117	0.24	-149.3	-80.0	-2.76	-1.22	0.09	-0.07	-1.44	23.43	0.16	-583.2	-11.8	-4.81*	-521.7	-236.2	0.037	-0.386*	-72	-235
71118	0.13	-68.4	-134.3	-0.09	-0.55	0.20	0.05	2.45	3.10	-0.01	-134.7	-3.0	1.41	101.6	-122.7	-0.048	-0.273	-18	78
71134	0.46*	139.0	54.0	3.13	3.56	0.42	-0.08	-3.77	-18.57	-0.08	490.4	8.4	1.85	653.8*	314.1	-0.013	-0.094	118*	975
71139	-0.42*	-208.7*	-178.3	-3.31	-4.32*	-1.35**	-0.12*	-5.66	2.43	0.09	-74.2	-0.5	-1.04	-151.7	-18.3	0.012	-0.166	-101	-793
71174	-0.20	-191.8	-47.3	-4.53*	-1.66	-0.91**	-0.14*	1.01	28.32	0.16	152.6	3.5	-1.70	16.0	77.4	0.017	-0.020	-176**	-1139
71178	0.13	262.4*	189.6	3.91	3.23	1.31**	0.07	2.01	-23.35	-0.17	-378.9	-7.7	-1.37	-101.7	-153.9	-0.032	0.332*	139**	809
71181	0.02	-75.9	-296.3*	1.47	-3.99*	0.09	-0.10	-0.11	4.43	0.02	-348.8	-7.1	2.07	-198.4	-175.3	0.020	-0.249	-35	-202
71218	0.02	-201.0	131.1	-6.53**	1.67	0.42	0.02	3.12	3.76	-0.02	597.9	11.8	-3.37	221.6	344.4	0.047	0.081	-115*	-1536*
71220	0.24	127.2	32.7	3.35	5.45**	1.31**	-0.04	-3.11	-20.90	-0.08	249.3	4.2	2.85	312.7	93.9	-0.016	0.181	92	411
71226	-0.42*	-117.2	-212.2	0.02	-5.66**	-0.69	-0.13**	1.78	19.87	0.13	-208.3	-3.2	0.96	-265.1	-118.4	0.013	-0.631**	-5	335
71268	-0.20	44.0	-125.7	3.24	1.12	0.31	0.17**	-10.00	-0.13	0.13	-645.1	-12.5	-0.15	-642.9	-233.3	0.039	-0.201	47	714
71273	0.57**	95.4	107.0	1.91	-1.22	0.75*	0.04	1.23	27.76	0.14	470.9	7.8	1.74	314.9	241.4	0.008	0.004	-2	754
71281	0.24	99.0	127.5	0.91	2.67	0.97**	0.05	-2.55	-0.68	0.03	-1107.6*	-22.3**	-4.26	-710.6*	-515.4*	-0.070	-0.128	50	807
ICMB 841	0.13	-114.2	-323.9**	1.35	-3.44	-0.02	-0.11*	0.56	-8.57	-0.02	-210.8	-4.5	1.19	-54.0	-9.6	0.003	0.035	-53	-604
863B	-1.20**	844.7**	532.2**	14.46**	22.00**	-1.02**	0.66**	-13.20*	-83.90**	-0.40**	813.9	19.2*	11.07**	709.4*	159.1	-0.058	2.602**	399**	661
H 77/833-2	0.25**	167.6**	106.5*	3.64**	1.51*	-1.27**	-0.16**	2.16	31.90**	0.16**	525.2**	9.7**	2.39*	740**	296.2**	-0.037**	-0.188**	14	1508**
PPM1 301	-0.02	577.9**	206.3**	13.29**	4.76**	2.37**	0.23**	1.42	-52.60**	-0.33**	-79.9	-1.4	7.74**	188.9	-151.2	-0.065**	1.258**	281**	1345**
RIB 3135-18	-0.23**	-745.5**	-312.8**	-16.93**	-6.28**	-1.10**	-0.06**	-3.58	20.70**	0.17**	-445.3**	-8.3*	-10.13**	-928.9**	-145.0	0.103**	-1.069**	-295**	-2852**

** Significant at 0.01 level of probability. * Significant at 0.05 level of probability

grain mass, CSSL 71178 and 863B showed significant positive *gca* whereas CSSL 71074 showed significant negative *gca*. In case of grain number per panicle, four CSSLs (71080, 71134, 71178, and 71220) and 863B and in case of grain number per m², 7 CSSLs (71080, 71085, 71134, 71178, 71268, 71273 and 71281) and 863B exhibited better *gca* values when compared with that of recurrent parent ICMB 841. These *gca* results under late-onset drought stress treatment demonstrated that six CSSLs (71080, 71134, 71178, 71220, 71273 and 71281) found be better lines out of 12 CSSLs tested for grain yield, panicle yield and grain number.

Among testers, RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction. PPMI 301 showed positive *gca* results for all observed traits except for panicle number, tiller number, vegetative growth index, dry stover yield and stover dry matter fraction. Tester H 77/833-2 showed positive effects for many traits except for panicle length, panicle diameter, stover dry matter fraction and 1000-grain mass.

4.2.2.1.4. Genotype x Environment interaction analysis

Line x tester analysis was performed for across above three environments. The results of analyses of variance and general combining ability for the grain yield and stover yield and its related traits across these three environments (Genotype x Environment interaction) are explained below.

4.2.2.1.4(A). Analyses of variance

The analysis of variance results for LG1 across three moisture regimes are presented in Table 15. Anova results demonstrated significant differences between moisture treatments for grain yield, panicle yield, panicle harvest index, tiller number, biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield, grain mass and grain number. But for flowering time, plant height, panicle length, panicle diameter, panicle number and stover dry matter fraction, the Anova results were non-significant between moisture treatments. Hybrids were highly significant for all observed characters. In case of

Table 15 : Analyses of variance for testcross hybrids of LG1 over three moisture regimes

Source of Variation	df	Time to		Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Grain Yield (kg/ha)							
Environments(Env)	2	2.18	96.29**	49.31**	1.58	1.26	3.95	1.92	7.67*	7.27*
Hybrids	62	4.18**	31.25**	5.65**	8.41**	16.82**	13.74**	1.36*	8.79**	6.42**
Lines (L)	20	6.9**	17.96**	6.65**	14.17**	8.05**	20.42**	1.92*	6.64**	3.95**
Testers (T)	2	30.42**	746.81**	77.4**	96.47**	404.5**	194.52**	3.57*	174.83**	129.57**
L × T	40	1.51*	2.11**	1.56*	1.13	1.82**	1.36	0.98	1.57*	1.5*
Env × Lines	40	1.25	2.06**	1.24	1.22	0.82	1.39	0.9	0.89	1.05
Env × Testers	4	1.92	40.65**	7.22**	2.84*	3.23*	2.19	1.05	2.83*	3.16*
Env × Hybrids	124	1.09	2.87**	1.31*	1.39**	0.98	1.27*	0.81	1	1.14
Env × L × T	80	0.96	1.38*	1.04	1.4*	0.94	1.17	0.76	0.97	1.08
Error df		372	349	366	372	372	372	372	370	370

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
Hybrids	62	4.11**	3.82**	42.39**	8.18**	2.43**	5.29**	53.99**	19.61**	14.53**
Lines (L)	20	4.53**	4.95**	17.21**	4.74**	2.08**	2.9**	41.93**	13.61**	6.68**
Testers (T)	2	56.79**	44.11**	1101.08**	174.86**	33.11**	111.53**	1203.11**	428.42**	328.46**
L × T	40	1.27	1.23	2.05**	1.56*	1.07	1.17	2.57**	2.16**	2.76**
Env × Lines	40	1.05	0.98	2.36**	1.21	0.95	1.08	0.99	1.09	1.85**
Env × Testers	4	4.48**	4.06**	29.56**	3.2*	0.59	10.67**	18.64**	14.82**	18.28**
Env × Hybrids	124	1.16	1.08	2.72**	1.58**	0.9	1.35*	1.68**	1.5**	2.05**
Env × L × T	80	1.05	0.99	1.56**	1.68**	0.88	1.01	1.17	1.03	1.35*
Error df		337	337	320	342	341	367	372	349	349

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

environment x hybrids effects, there were clear significant differences for grain yield and also for other grain yield related traits but not for flowering time, panicle length, tiller number, panicle number, biomass yield, vegetative growth index and dry stover yield. Both lines and testers showed significant differences for all the observed agronomic characters. The environment x line interactions were significant for grain yield, panicle harvest index, grain harvest index and grain number per m². The environment x tester effects had significant differences except for flowering time, panicle diameter and dry stover yield. Due to line x tester effects, significant differences were observed for grain yield, panicle harvest index, flowering time, panicle length, panicle diameter, biomass yield, vegetative growth index, grain harvest index, dry stover yield, grain mass and grain number. The effects due to interaction among environment x line x tester, were significant for grain yield, panicle harvest index, plant height, grain harvest index, fresh stover yield and grain number per m².

4.2.2.1.4(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG1 across three moisture regimes are presented in Table 16. CSSL 71080 and donor parent 863B expressed significant negative *gca* for flowering time and among testers, RIB 3135-18 was significant negative combiner. For grain yield, three CSSLs (71080, 71134 and 71178) exhibited significant positive *gca* effects along with donor parent where as two CSSLs (71074 and 71174) and four RPHLs (71066, 71117, 71139 and 71226) showed significant negative *gca* effects along with recurrent parent. It was observed that 7 CSSLs (71080, 71085, 71134, 71178, 71220, 71268 and 71273) out of 12 CSSLs and one RPHL 71083 exhibited better *gca* effects for grain yield than that of recurrent parent ICMB 841. Only one introgression line 71178 and 863B had significant positive *gca* for panicle yield. Nine CSSLs (71080, 71085, 71134, 71178, 71218, 71220, 71268 71273 and 71281) out of 12 CSSLs and two RPHLs (71066 and 71083) expressed better panicle yield than that of recurrent parent. Three CSSLs (71080, 71134 and 71178) and donor parent expressed significant positive *gca* values for panicle harvest index where as three CSSLs (71074, 71174 and 71218) and four RPHLs (71066, 71117, 71139 and 71226) showed significant negative *gca* effects for the same trait. Four introgression lines 71134, 71178, 71218 and 71220 along with 863B expressed significant positive *gca* effects for plant height and four CSSLs (71074, 71080, 71118 and 71174) and five RPHLs (71066, 71088, 71139, 71181 and 71226) showed significant negative *gca* for this trait. For panicle

Table 16 : Estimates of *gca* effects of lines and testers of LG1 over three moisture regimes

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
71066	0.10	-170.4**	-62.6	-4.48**	-4.87**	-0.73**	-0.10**	2.47	26.60**	0.14*	2.5	-0.1	-2.45**	79.9	42.7	0.008	-0.547**	-107**	-272
71074	0.17	-185.6**	-89.6	-4.47**	-2.69**	-0.21	-0.09**	7.50*	-17.33	-0.19**	-255.0	-5.4*	-2.20**	-341*	-173*	0.002	-0.338**	-18	-645.6*
71080	-0.64**	154.8**	146.5	3.51**	-4.31**	-0.54*	0.05	5.29	15.63	0.04	88.6	3.3	2.80**	271.7*	-59.1	-0.051**	0.282**	14	784.9**
71083	0.03	1	29	0.11	-1.91	0.63**	-0.01	-2.08	-5.74	-0.02	274.3*	5.3*	-0.71	115.6	217.3*	0.030	0.090	35	-16
71085	-0.23	83.8	111.7	1.42	1.20	-0.25	-0.05	-4.23	5.66	0.10	298*	6.5*	0.12	207.0	148.8	0.013	-0.087	32	620.2*
71088	0.10	-73.9	-112.6	-0.94	-3.35*	-0.21	-0.06	7.60*	-2.22	-0.11	-177.7	-3.7	-0.38	-84.4	-69.2	0.000	-0.115	-19	-378
71117	-0.16	-186.3**	-131.7	-3.00**	-2.28	-0.10	-0.07*	0.51	5.96	0.02	-126.2	-2.0	-2.01**	-158.0	-21.0	0.025	-0.209*	-80*	-654.4*
71118	0.17	-91.4	-176.9*	-0.57	-3.06*	-0.10	-0.05	1.36	-1.37	-0.03	-388.4**	-8.1**	-0.17	-410.2**	-188.9*	0.001	-0.200*	-28	-269
71134	0.54*8	117.4*	-5.1	4.09**	5.82**	1.08**	-0.02	-0.01	-23.50*	-0.13*	77.9	-0.2	1.29	242.2	123.1	-0.017	0.059	137**	646.6*
71139	-0.20	-252.3**	-199.3**	-4.75**	-4.24**	-1.25**	-0.08*	3.62	7.89	0.02	-355.4**	-6.6*	-3.13**	-403.6**	-128.6	0.013	-0.228*	-129**	-1108**
71174	-0.12	-180.4**	-99.6	-3.53**	-2.83**	-0.76**	-0.06	0.29	27.50**	0.15*	-32.6	0.0	-2.01**	-7.4	19.0	0.009	-0.045	-159**	-1017**
71178	0.39**	521.7**	481**	6.09**	4.75**	1.08**	0.08*	-2.64	-6.12	-0.02	549.6**	10.0**	4.56**	701.5**	110.6	-0.045**	0.314**	176**	2207.7**
71181	-0.05	-101.1	-200.7**	-0.28	-3.39**	0.19	-0.11*	3.51	9.37	0.01	-238.6	-4.8	-0.16	-142.4	-65.9	0.022	-0.299**	-52	-296
71218	-0.01	-108.6	-29.9	-2.45**	2.82*	0.16	-0.06	-1.75	-13.74	-0.07	106.7	2.2	-1.63**	46.0	106.1	0.015	0.045	-41	-726.6*
71220	0.25	13.7	32.8	-0.58	3.64**	1.19**	0.087*	-8.30*	-24.00**	-0.02	-151.3	-3.6	0.15	-104.8	-140.7	-0.025	0.132	32	-385
71226	0.03	-194.6**	-117.8	-3.35**	-3.24*	-0.29	-0.10**	2.18	25.70**	0.13*	-196.7	-4.2	-2.26**	-294.2*	-41.6	0.020	-0.538**	-65*	-224
71268	0.06	16.1	12.1	0.66	-0.25	0.42	0.09**	-2.12	14.11	0.13	62.9	0.9	-0.13	-202.1	64.5	0.029	-0.308**	24	788.9*
71273	0.17	47.2	69.2	0.44	0.09	0.45	0.01	-2.45	20.00*	0.14*	157.0	2.7	-0.25	79.1	131.8	0.017	-0.110	-21	522
71281	0.35*	-27	37.7	-0.53	1.68	0.63**	0.04	1.18	7.89	0.03	131.6	1.5	-0.02	26.4	59.0	0.010	-0.169	-4	275
ICMB 841	0.25	-131.8*	-217.6**	-1.08	-1.25	-0.32	-0.08*	0.40	7.70	0.05	-420.9**	-8.7*	-0.59	-237.3	-204*	-0.016	-0.048	-96**	-677.6*
863B	-1.23**	747.7**	523.7**	13.69**	17.68**	-1.06**	0.62*	-12.40**	-80.10**	-0.38**	593.8**	14.9**	9.17**	616.2**	69.0	-0.059**	2.322**	370**	822.3**
H 77/833-2	0.30**	170.7**	82.0**	3.98**	2.53**	-1.27**	-0.16**	2.52	30.40**	0.14**	267.9**	4.3**	1.59**	498.3**	207.7**	-0.022**	-0.179**	32**	1467**
PPM1 301	-0.01	484.0**	202.4**	10.93**	3.27**	2.12**	0.19**	-0.14	-53.30**	-0.32**	161.3**	3.3**	7.43**	280.1**	-64.3*	-0.047**	1.250**	229**	944**
RIB 3135-18	-0.29**	-654.7**	-284.4**	-14.91**	-5.81**	-0.84**	-0.03**	-2.38	22.90**	0.17**	-429.2**	-7.7**	-9.03**	-778.4**	-143.4**	0.069**	-1.071**	-261**	-2411**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

length, four CSSLs (71134, 71178, 71220, and 71281) expressed significant positive results whereas 863B, two CSSLs (71080 and 71174) and two RPHL (71066 and 71139) had significant negative *gca* values. It was noticed that 7 CSSLs (71134, 71178, 71218, 71220, 71268, 71273 and 71281) out of 12 and two RPHLs (71083 and 71181) exhibited better *gca* effects than that of ICMB 841 for panicle length. Three introgression lines (71178, 71220 and 71268) and donor parent 863B expressed significantly positive *gca* for panicle diameter. It was noticed that 6 CSSLs (71080, 71178, 71220, 71268, 71273 and 71281) out of 12 exhibited better *gca* effects than that of ICMB 841 for panicle diameter. In case of panicle number and tiller number, two introgression lines 71174 and 71273 and two recurrent parent homozygotes 71066 and 71226 had significant positive *gca* effects whereas donor parent 863B and three CSSLs (71074, 71134 and 71220) and one showed significant negative effects.

GCA results of across three moisture regimes revealed that out of 21 lines tested, 14 lines including 10 CSSLs, 3 RPHLs and donor parent showed better *gca* effects for both biomass yield and vegetative growth index when compared with that of recurrent parent. Donor parent 863B and two introgression lines 71080 and 71178 exhibited significant positive *gca* effects for grain harvest index and fresh stover yield. None of the introgression lines showed significant positive *gca* effects for dry stover yield and stover dry matter fraction. But when compared with recurrent parent, six CSSLs and two RPHLs in case of fresh stover yield and eight CSSLs and three RPHLs in case of dry stover yield showed better *gca* effects. Two CSSLs 71080 and 71178 and 863B expressed significant positive *gca* effects for 1000 grain mass but two CSSLs 7118 and 71268 and five RPHLs (71066, 71117, 71139, 71181, 71226) showed negative *gca* results. Two CSSLs (71134 and 71178) and 863B showed significant positive *gca* effects for grain number per panicle. It was observed that 10 CSSLs out of 12 and two RPHLs expressed better grain number per panicle *gca* values when compared with ICMB 841. Five CSSLs (71080, 71085, 71134, 71178, and 71268) and 863B showed significant positive *gca* effects for grain number per panicle whereas three CSSLs (71074, 71174 and 71218) and ICMB 841 exhibited significant negative *gca* for this trait. It was also notices that 7 CSSLs and one RPHL showed better grain number per m² effects than that of recurrent parent. *GCA* results across three moisture regimes demonstrated that seven CSSLs (71080, 71085, 71134, 71178, 71220, 71268 and 71273) were found to be better lines out of

12 CSSSLs tested for grain yield, panicle yield and grain number. Interestingly, one recurrent parent homozygous line 71083 also observed as better line for these traits.

Among testers, RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction. PPMI 301 showed positive *gca* results for all observed traits except for panicle number, tiller number, dry stover yield and stover dry matter fraction. Tester H 77/833-2 showed positive effects for all traits except for panicle length, panicle diameter, stover dry matter fraction and 1000-grain mass.

4.2.2.2. Linkage Group 2 (LG2)

A total 48 testcross hybrids (involving three testers) of 10 homozygous segmental introgression lines and a sampling of 4 recurrent parent homozygotes (to serve as near-isogenic controls) of LG2, which were selected based on marker genotyping of 69 plants at 19 marker loci of LG2 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in three environments *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions during summer 2010 at ICRISAT, Patancheru. Line x tester analysis was performed and analysis of variance and general combining ability (*gca*) effects were calculated. The results of analysis of variance and general combining ability (*gca*) for grain and stover yield related characters for each of three moisture regimes and across these three moisture regimes are explained below.

4.2.2.2.1. Fully irrigated control treatment

4.2.2.2.1(A). Analysis of variance

The analysis of variance for LG2 under fully irrigated non- stress conditions during summer 2010 are presented in Table 17. Hybrids showed highly significant differences for all the observed characters. There were also significant differences between lines except for panicle yield, fresh stover yield, dry stover yield, stover dry matter fraction and grain number per m².

Table 17 : Analyses of variance for testcross hybrids of LG2 in fully irrigated non-stress conditions

Source of Variation	df	Time to										
		75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	
Replication	2	3.6*	2.91	0.51	5.34**	4.74*	2.14	15**	0.25	0.57	0.47	
Hybrids	47	156928.79**	7.49**	3.28**	60288719.18**	904.94**	11194.11**	157004.35**	4.09**	3.13**	18383.28**	
Lines (L)	15	3.81**	3.7**	1.57	3.09**	5.21**	4.31**	9.2**	0.95	2.37**	2.45**	
Testers (T)	2	10.93**	128.14**	11.92**	141.44**	47.17**	95.86**	64.03**	1.05	33.18**	26.35**	
L × T	30	1.79*	1.34	1.08	1.6*	1.58*	2.33**	2.11**	0.69	1.22	1	
Error	94											

Source of Variation	df	Vegetative									
		Biomass Yield (kg/ha)	Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²	
Replication	2	0.24	0.23	2.64	9.66**	1.26	11.28**	1.07	0.43	2.01	
Hybrids	47	1.6*	1400.5**	73663582.33**	80422.33**	1.8**	43727446.34**	494070.19**	4.38**	4.65**	
Lines (L)	15	1.8*	1.93*	2.77**	1.49	1.56	1.51	9.6**	2.7**	1.75	
Testers (T)	2	5.63**	4.62*	53.96**	15.4**	7.6**	8.82**	423.5**	52.12**	76.42**	
L × T	30	1.23	1.25	1.15	1.25	1.36	1.39	2.65**	1.23	1.3	
Error	94										

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

As expected, big significant differences were observed for all the recorded characters between testers. There were some significant specific combining ability differences due to line x tester effects for panicle harvest index, flowering time, plant height, panicle length, panicle diameter and 1000 grain mass.

4.2.2.2.1(B). General Combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG2 in fully irrigated non- stress conditions, during summer 2010 are presented in Table 18. For flowering time, the best negative combiners among lines were RPHL 72044 and donor parent 863B whereas CSSLs 72054 and 72067 showed significant positive *gca* effects. However, in this experiment recurrent parent ICMB 841 exhibited significant negative *gca* effect but showed late flowering when compared with 863B. Similarly among testers, RIB 3135-18 showed negative *gca* effects and H 77/833-2 had significant positive *gca* for flowering time. Only one CSSL 72028 and donor parent 863B showed significant positive *gca* whereas a recurrent parent homozygote 72031, CSSL 72039 and recurrent parent ICMB 841 showed significant negative *gca* effects for grain yield and panicle harvest index. Significant negative *gca* was also noticed in CSSL 72039 in case of panicle yield and significant positive *gca* effects were not observed in any line for this trait. It was clear from the *gca* results of LG2 under fully irrigated non-stress conditions that 9 CSSLs (72001, 72028, 72030, 72038, 72054, 72060, 72066, 72067 and 72069) out of 10 and 3 RPHLs (72002, 72044 and 72062) out of 4 exhibited better *gca* effects for both grain yield and panicle harvest index than that of recurrent parent ICMB 841. Five CSSLs and two RPHLs expressed better *gca* for panicle yield. Two CSSLs 72028 and 72039 expressed significant positive *gca* effects along with donor parent for plant height. For panicle length, three RPHLs (72001, 72031 and 72044), both donor parent and recurrent parent exhibited significant negative *gca* effects, anyhow recurrent parent was more negatively significant than that of donor parent. Two CSSLs 72060 and 72066 showed significant positive *gca* for this same trait panicle length. This indicated that for panicle length, these two lines were better lines than that of both the parents. In case of plant height, six CSSLs and two RPHLs and in case of panicle length, nine CSSLs and two RPHLs exhibited better *gca* values when compared with ICMB 841. None of the lines showed significant positive effects except donor parent 863B for panicle diameter. For panicle number and tiller number, only one CSSL 72062 and ICMB 841 had significant positive *gca*

Table 18 : Estimates of *gca* effects of lines and testers of LG2 in fully irrigated non-stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle Yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
72001	0.44	101.6	209.2	-0.35	0.29	-0.17	0.08	11.80	-0.08	-0.15	569.0	9.7	1.15	661.3	201.3	-0.014	0.208	1	130
72002	0.22	127.6	244.5	-0.24	-0.38	-1.06**	-0.13*	-3.56	36.14	0.25	-261.5	-5.6	-4.18	-323.2	-24.6	0.023	-0.183	16	803
72028	0.22	383.6*	313.7	5.10	4.39*	0.16	-0.03	6.33	-6.86	-0.14	676.1	12.4	4.82	922.4*	204.1	-0.037	0.253	124	1499
72030	0.22	-147.1	-127.0	-0.90	-1.83	0.27	-0.09	-3.12	22.03	0.17	-138.5	-3.2	1.71	-377.6	-169.8	0.006	-0.386*	-87	-51
72031	0.00	-401.3**	-229.9	-5.35*	-6.16**	-0.95**	-0.15**	-8.45	5.36	0.17	-59.8	-1.3	-2.29	-278.7	12	0.030	-0.712*	-114	-887
72038	0.11	153.5	94.0	2.43	1.62	0.27	0.00	6.33	7.25	-0.03	572.8	10.8	1.82	574.6	320.4	0.007	0.103	25	435
72039	0.44	-338.5*	-552.9*	-6.24*	5.06*	0.60	-0.12*	-6.12	-27.31	-0.13	-2234.4**	-44.6**	-11.29**	-1331.0**	-876.9**	-0.047	0.018	-90	-1660*
72044	-0.78*	21.4	0.2	0.88	-3.27	-0.73*	-0.08	3.77	17.81	0.04	174.9	5.6	2.38	-8.7	16.3	0.016	0.086	-68	131
72054	0.67*	73.3	99.4	1.10	2.62	0.60	0.00	-0.01	-0.75	-0.03	-328.9	-7.9	-0.85	-29.9	-137.5	-0.004	0.134	5	233
72060	0.44	60.7	-44.5	2.99	-2.83	0.94**	-0.08	3.77	-21.86	-0.19	153.6	1.4	3.15	360.1	39.8	-0.029	0.341**	43	118
72062	-0.44	4.7	99.4	-1.35	-5.05*	0.60	-0.01	-7.23	56.00**	0.46**	199.3	5.1	1.82	-98.7	-58.7	0.004	-0.478*	-59	785
72066	-0.11	88.2	-2.5	3.32	-0.49	0.94**	0.00	-1.90	-24.75	-0.14	-514.3	-9.8	-0.85	-457.6	-188.8	-0.052	0.141	112	332
72067	0.78**	104.1	38.3	2.54	0.40	0.16	0.01	11.88	-50.80*	-0.44*	245.5	2.7	3.15	487.9	49	-0.029	-0.205	249**	908
72069	-0.22	-234.0	-119.6	-3.68	-3.16	0.60	0.06	1.33	-5.64	-0.06	242.4	5.3	-1.74	-92.1	203.5	0.055*	-0.305*	-59	-843
ICMB 841	-0.67*	-600.3**	-360.9	-9.24**	-5.60*	-1.40**	-0.05	-3.01	38.00*	0.33*	-136.4	-0.9	-5.07	-379.9	66.2	0.052	-0.278	-306**	-2525**
863B	-1.33*	602.3**	338.4	8.99**	14.39**	-0.84*	0.59**	-11.79	-44.53	-0.11	840.1	20.3*	6.26**	371.3	343.4	0.018	1.264**	209*	594
H 77/833-2	0.47**	435.5**	204.8*	6.51**	4.60**	-0.96**	-0.16**	1.42	29.40**	0.14*	554.8*	9.7*	3.56**	713.4**	311.7**	-0.008	-0.430**	132**	3027**
PPM1 301	-0.12	430.7**	123.1	8.81**	3.00**	1.79**	0.21**	2.19	-55.60**	-0.37**	-26.5	-0.4	5.86**	102.4	-127.5	-0.030*	1.448**	163**	-206
RIB 3135-18	-0.35**	-866.2**	-327.9**	-15.32**	-7.60**	-0.83**	-0.05**	-3.62	26.10**	0.22**	-528.3**	-9.4*	-9.43**	-815.8**	-184.2	0.038**	-1.018**	-295**	-2821**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

whereas only one CSSL 72067 had significant negative *gca* values. It was noticed that nine CSSLs out of 10 showed worse tiller number and panicle number when compared with ICMB 841. It was observed that there was only one CSSL 72039 with significant negative *gca* for the traits biomass yield, vegetative growth index, grain harvest index, fresh and dry stover yield. Remaining all CSSLs did not show any significant differences for these traits except one deviation in CSSL 72028, exhibited significant positive *gca* for fresh stover yield. It was observed that 6 CSSLs and two RPHLs expressed better *gca* effects for grain harvest index than that of recurrent parent. Only one CSSL 72069 showed significant positive *gca* effects for stover dry matter fraction. For 1000 grain mass, significant positive effect was exhibited by only one CSSL 72060 like donor parent whereas two CSSLs 72030 and 72069 and two RPHLs 72031 and 72062 had significant negative *gca*. In case of grain number, CSSLs 72067 and 72039 showed significant positive and negative *gca* effects respectively. The *gca* results also revealed that 7 CSSLs and one RPHL in case of grain mass and all 14 lines tested (including 10 CSSLs and 4 RPHLs) in case of grain number exhibited better *gca* effects when compared with ICMB 841.

The present experiment also revealed that tester RIB 3135-18 exhibited negative *gca* effects for all observed characters except for panicle number, tiller number and stover dry matter fraction whereas these results were exactly in contrast to that of PPMI 301. PPMI 301 showed significant positive values for many traits except for panicle number, tiller number and stover dry matter fraction. Tester H 77/833-2 showed positive effects for all traits except for panicle length, panicle diameter and 1000 grain mass.

4.2.2.2.2. Early drought stress treatment

4.2.2.2.2(A). Analysis of variance

The analysis of variance for LG2 under early-onset drought stress conditions during summer 2010 are presented in Table 19. Hybrids showed highly significant differences for all characters except for dry stover yield. There were clear significant differences between lines for the grain yield and also for its related traits except for tiller number and grain number. There were no significant differences due to lines for biomass yield, vegetative growth index, fresh and dry stover yield and stover dry matter fraction. As expected, effects of testers were

Table 19 : Analyses of variance for testcross hybrids of LG2 in early-onset terminal drought stress conditions

Source of Variation	df	Time to									
		75% Flowering (d)	Grain Yield (kg/ha)	Panicke yield (kg/ha)	Panicke Harvest Index (%)	Plant Height (cm)	Panicke Length (cm)	Panicke Diameter (cm)	Plant Population ('000/ha)	Panicke Number ('000/ha)	Tillers per Plant (Number)
Replication	2	1.32	4.12*	4.94**	2.51	1.34	0.66	14.11**	1.57	2.74	0.35
Hybrids	47	121731.7**	5.51**	5.08**	24057051.83**	546.04**	6689.15**	120303.28**	2.96**	4.83**	30428.85**
Lines (L)	15	3.39**	3.07**	2.76**	2.79**	7.24**	2.93**	8.37**	1.09	3.73**	1.72
Testers (T)	2	8.6**	79.73**	24.86**	95.08**	20.13**	139.18**	110.56**	1.26	58.22**	30.63**
L × T	30	1.81*	1.78*	1.99**	1.63*	1.54	1.95**	1.42	0.84	1.48	1.53
Error	94										

Source of Variation	df	Biomass			Vegetative			Dry			Grain		
		Yield (kg/ha)	Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicke	Grain Number/m ²			
Replication	2	0.14	0.18	5.18**	12.82**	1.5	46.92**	13.73**	3.48*	2.84			
Hybrids	47	1.54*	1276.24**	50815177.41**	54933.8**	1.48	19267602.8**	152996.91**	4.47**	3.28**			
Lines (L)	15	1.64	1.72	2.59**	1.13	1.24	0.89	10.32**	1.98*	1.54			
Testers (T)	2	3.65*	2.92	84.82**	20.62**	4.01*	36.76**	114.87**	61.84**	42.47**			
L × T	30	1.34	1.2	1.5	1.3	1.34	1.13	1.96**	1.37	1.51			
Error	94												

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

highly significant for all observed agronomic characters except for vegetative growth index. Significant differences were observed due to line x tester effects for grain yield, panicle yield, and panicle harvest index, time to flowering, panicle length and 1000 grain mass.

4.2.2.2(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG2 in early drought stress treatment, during summer 2010 are presented in Table 20. For flowering time, lines did not show any significant *gca* effects except donor parent 863B which had significant negative *gca* and CSSL 72054 showed significant positive *gca*. Among testers, RIB 3135-18 followed by PPMI 301 showed negative *gca* effects for flowering time and H 77/833-2 expressed significant positive *gca*. In case of grain yield, no line was found to have any significant *gca* effects except CSSL 72054 showed significant negative *gca* effect. However, five CSSLs (72001, 72038, 72039, 72060 and 72066) showed better grain yield than that of recurrent parent ICMB 841B. For panicle yield and panicle harvest index, *gca* results were not significant except CSSL 72054, which showed significant negative *gca* effects. Seven CSSLs (72038, 71039, 72054, 72060, 72066, 72067 and 72069) and three RPHLs expressed better *gca* for panicle yield when compare with ICMB 841. Only one CSSL 72067 showed significant positive *gca* results for plant height and panicle length but this line was negatively significant for panicle number. Anyhow, it was observed that 13 lines out of 14 (including 10 CSSLs and 3 RPHLs) expressed better plant height *gca* values than that of recurrent parent. Two RPHLs (72002 and 72031) showed significant positive *gca* for panicle number and only one RPHL 72062 showed the same result for tiller number. None of the lines (except 863B) found to have significant positive *gca* values for biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and stover dry matter fraction. CSSL 72030 exhibited significant negative *gca* effects for biomass yield, vegetative growth index, grain harvest index and dry stover yield and CSSL 72054 was also significantly negative for grain harvest index. Remaining eight CSSLs did not show any significant *gca* effects for these characters. For 1000 grain mass, two CSSLs 72039, 72060 and donor parent 863B showed significant positive *gca* effects whereas three CSSLs 72030, 72038 and 72069 showed significant negative results. Only one CSSL 72038 was significantly positive for grain number per panicle and grain number per m². Only one CSSL 72054 showed significant negative *gca* effect for grain number per m².

Table 20 : Estimates of *gca* effects of lines and testers of LG2 in early-onset terminal drought stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population (000/ha)	Panicle Number (000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
72001	0.40	35.7	-173.0	2.88	3.56	0.33	-0.02	-4.60	-4.24	0.00	-241.3	-5.5	2.00	-408.9	-116.9	0.029	-0.092	30	189
72002	0.29	-100.0	-141.5	-1.57	-2.33	-0.22	-0.13**	-2.38	29.50*	0.21	25.4	0.0	-1.33	-127.8	118.5	0.038	-0.368	-82	-406
72028	0.29	20.2	-158.1	4.21	0.22	0.44	0.00	-7.93	-14.69	0.00	-543.9	-11.4	-0.11	85.6	-171.4	-0.024	-0.254	63	724
72030	0.07	-217.2	-219.2	-5.46	-2.78	0.00	-0.07	-4.93	6.31	0.12	-901.9**	-18.0**	-4.44*	-645.6	-418.5*	0.000	-0.438*	-124	-1249
72031	0.07	-82.1	54.8	-2.90	-4.77*	-0.44	-0.04	16.70*	64.80**	0.17	27.9	0.4	-0.78	158.9	-75.4	-0.044	-0.384	-108	-149
72038	0.40	74.7	59.8	1.76	-0.33	-0.44	-0.02	2.63	6.20	-0.01	344.9	5.9	0.67	217.8	236.5	0.039	-0.688	186**	1741*
72039	-0.15	144.4	151.8	2.76	-0.78	0.11	0.02	6.29	12.42	-0.01	403.2	8.2	1.56	493.3	203.2	-0.012	0.418*	-8	421
72044	-0.15	-84.0	-114.3	-1.01	-2.33	-1.00**	-0.12*	4.40	-1.13	-0.08	-223.6	-4.1	-0.11	-374.4	-157.6	0.002	0.288	-48	-657
72054	0.62*	-288.8*	27.1	-10.45**	-2.89	-0.33	-0.02	5.07	-9.58	-0.11	370.1	5.9	-6.00**	138.9	294.4	0.033	-0.344	-136	-1622*
72060	-0.04	141.4	193.0	3.76	1.11	0.44	-0.04	3.74	14.87	0.04	533.9	10.6	2.22	326.7	292.2	0.006	0.441*	0	548
72062	-0.26	-67.3	-4.3	-1.35	1.00	0.44	0.02	-12.38	3.20	0.21*	97.1	2.4	-0.89	138.9	53.2	-0.021	-0.023	-34	-246
72066	0.29	134.4	85.7	2.43	-0.67	0.33	-0.02	-1.82	-10.47	-0.02	-355.7	-7.4	1.33	-156.7	-290.4	-0.022	-0.129	69	833
72067	0.07	-81.0	76.5	-3.79	4.44*	1.33**	0.09	-8.60	-29.50*	-0.08	79.9	1.4	-0.56	190.0	-44.9	-0.033	0.334	-18	-828
72069	-0.04	-40.8	62.3	-1.13	-3.22	0.56	0.00	3.74	14.87	0.02	200.8	4.0	-0.22	163.3	90.1	-0.017	-0.487*	18	498
ICMB 841	-0.49	-198.9	-363.6**	-3.13	-8.77**	-0.67	-0.11*	4.40	-12.35	-0.13	-500.8	-9.0	-1.89	-693.3*	-185.8	0.049	-0.313	-84	-957
863B	-1.37**	609.4**	462.9**	12.98**	18.55**	-0.88*	0.48**	-4.38	-70.20**	-0.34**	684.1	16.6*	8.55**	493.3	172.5	-0.021	2.039**	276**	1160
H 77/833-2	0.36**	21.2	8.8	1.43	2.37*	-1.06**	-0.19**	1.42	33.90**	0.18**	209.2	3.4	0.11	522.2**	189.4*	-0.029*	-0.067	-37	416
PPMI 301	-0.15	446.2**	248.4**	12.34**	2.52**	2.12**	0.24**	-3.83	-55.80**	-0.29**	109.0	2.4	7.77**	244.4	-129.2	-0.063**	0.961**	284**	1861**
RIB 3135-18	-0.21*	-467.4**	-257.1**	-13.77**	-4.89**	-1.06**	-0.05*	2.42	21.90**	0.10*	-318.2*	-5.7*	-7.88**	-766.6**	-60.2	0.092**	-0.894**	-247.5**	-2277**

** Significant at 0.01 level of probability, * Significant at 0.05 level of probability

Among testers, RIB 3135-18 showed significant negative *gca* effects for all grain yield related traits except for panicle number, tiller number and stover dry matter fraction whereas these results were exactly opposite to that of PPMI 301. PPMI 301 showed significant positive values for many traits except for panicle number, tiller number and stover dry matter fraction. Tester H 77/833-2 showed positive effects for all traits except for panicle length, panicle diameter and stover dry matter fraction.

4.2.2.2.3. Late drought stress treatment

4.2.2.2.3(A). Analysis of variance

The analysis of variance for LG2 under late-onset drought stress conditions during summer 2010 is presented in Table 21. Hybrids showed highly significant differences for all traits except for biomass yield and dry stover yield. There were clear significant difference between lines for grain yield and also its related component traits but there were no significant differences for tiller number, biomass yield, vegetative growth index, fresh and dry stover yields. As expected, effects of testers were highly significant for all observed agronomic characters but there were no significant differences for flowering time. Lines x tester effects were significant for panicle harvest index, plant height, panicle length, panicle number, and grain mass and grain number.

4.2.2.2.3(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG2 in late-onset drought stress conditions, during summer 2010 are presented in Table 22. None of the lines showed negative *gca* effects for flowering except donor parent 863B, which has early flowering nature. CSSLs 72038 and 72066 expressed significant positive *gca* effects. None of the testers found to have significant *gca* effects, however RIB 3135-18 followed by PPMI 301 showed negative *gca* effects for flowering time. There were no significant positive *gca* effects noticed in lines except donor parent for grain yield and panicle yield. RPHL 72031 and CSSL 72038 had significant negative *gca* values for grain yield and panicle yield respectively. Only one CSSL 72039 had significant positive *gca* effect like donor parent for panicle harvest index. Two recurrent parent homozygotes and recurrent parent showed significant negative *gca* effects for this trait. There were no significant positive *gca* effects obtained in case of all 10 CSSLs for most of the other observed characters, plant height, panicle length, panicle diameter, panicle

Table 21 : Analyses of variance for testcross hybrids of LG2 in late-onset terminal drought stress conditions

Source of Variation	df	Time to		Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population n ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Grain Yield (kg/ha)							
Replication	2	11.66**	19.47**	17.72**	7.05**	1.64	38.51**	4.23*	1.51	2.95
Hybrids	47	86033.36**	14.1**	4.92**	585.74**	6665.93**	129117.01**	1.68*	4.58**	9418.33**
Lines (L)	15	2.88**	7.15**	2.72**	3.35**	1.85*	7.2**	0.82	2.65**	0.75
Testers (T)	2	2.75	258.09**	15.48**	33.45**	93.68**	82.67**	0.61	52.9**	5.6**
L × T	30	1.56	1.31	1.53	1.77*	3.47**	0.99	0.97	1.79*	1.07
Error	94									

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number / m ²
Hybrids	47	1.24	1037.54**	40826829.78**	83201.78**	1.49	22825855.47**	344745.28**	9.57**	7.46**
Lines (L)	15	0.98	1.12	1.95*	1.09	0.64	2.04*	16.17**	5.91**	2.32**
Testers (T)	2	6.77**	5.96**	46.98**	25.47**	15.02**	53.61**	390.5**	123.15**	129.89**
L × T	30	1	1	0.83	0.82	0.88	0.82	2.18**	2.49**	1.8*
Error	94									

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 22 : Estimates of *gca* effects of lines and testers of LG2 in late-onset terminal drought stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle Yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
72001	-0.22	-126.2	-71.7	-1.98	2.90	0.41	0.01	2.33	-9.10	0.06	-423.9	-7.6	-4.75	-400.5	-137.4	0.037	-0.174	-216**	-529
72002	0.44	74.3	141.3	0.47	-1.32	-0.37	-0.15**	-13.67	32.10*	0.08	690.0	12.4	0.69	219.5	413.4**	0.045	-0.487**	12	1337**
72028	0.44	68.1	64.9	1.58	2.68	0.41	-0.03	-7.56	-17.77	-0.31	-178.9	-4.6	-2.64	-53.8	22.2	0.002	0.119	74	219
72030	0.33	-153.3	-118.0	-2.31	-1.21	0.19	-0.09	9.11	-4.66	0.04	-572.1	-12.3	-3.86	-578.3	-190.2	0.047	-0.030	-48	-612
72031	0.00	-344.1**	-225.3	-6.09**	-4.54*	-0.26	-0.04	13.22	14.45	0.10	-201.4	-3.9	-1.97	-324.9	-111.1	0.018	-0.552**	-131**	-1022*
72038	1.00**	-122.5	-259.1*	1.58	-0.21	0.08	0.00	-18.11	5.12	-0.02	-250.5	-6.9	2.25	-63.8	-126.5	-0.018	0.049	-53	-572
72039	-0.11	148.6	69.8	3.46**	2.57	-0.26	0.09	13.44	1.01	0.02	200.3	4.2	3.69	258.4	-4.6	-0.048	0.370**	15	164
72044	-0.33	-119.0	-116.6	-1.42	-3.43	-0.81*	-0.12*	-15.78	8.23	-0.03	68.0	2.1	0.58	-70.5	49.4	0.020	-0.279*	-15	-36
72054	0.22	-91.8	-195.1	0.47	3.01	0.08	-0.04	12.67	-3.66	-0.01	-357.3	-7.6	-3.42	-147.2	-11.0	0.018	0.168	-28	-743
72060	-0.22	-32.4	-177.6	1.80	-0.54	0.41	-0.03	5.22	-25.77	-0.05	-520.1	-10.2	0.03	-127.2	-186.2	-0.034	-0.057	51	-42
72062	-0.22	-75.5	222.7	-4.75**	-4.21	0.85*	-0.03	-16.89	42.70**	0.12	450.4	9.4	-0.31	90.6	92.4	-0.005	-0.239	-49	-88
72066	0.66*	-73.0	-8.6	-1.31	0.90	0.30	-0.04	4.11	0.78	0.15	170.9	1.9	0.81	92.8	44.5	-0.005	-0.065	-14	-191
72067	0.11	71.3	111.7	-0.42	-1.76	0.30	0.07	-1.33	8.90	-0.23	246.8	4.5	2.36	336.2	-0.4	-0.041	-0.053	29	319
72069	-0.11	81.1	-14.3	1.58	-4.43	0.41	-0.01	6.67	-11.55	0.01	-476.6	-9.2	-2.31	-127.2	-162.4	-0.010	-0.210	103*	789
ICMB 841	-0.56	-126.2	-44.0	-3.87**	-2.76	-0.48	-0.05	0.56	26.90	0.38	277.3	6.7	-1.31	-39.4	185.8	0.068*	-0.416**	-84	-121
863B	-1.44**	820.6**	619.7**	11.24**	12.34**	-1.25**	0.46**	6.00	-67.70**	-0.30	877*	21.1*	10.13**	935.1**	122.0	-0.095**	1.856**	354**	1128**
H 77/833-2	0.27	154.6**	77.3	3.55**	2.61**	-0.95**	-0.18**	-3.88	34.60**	0.09	577.5**	10.8**	2.41*	679.9**	365**	-0.017	-0.266**	21	1507**
PPMI 301	-0.10	560.4**	173.9**	12.17**	4.02**	1.91**	0.22**	4.38	-56.50**	-0.23**	-328.0	-6.3	6.18**	79.1	-286.1**	-0.083**	1.284**	230**	933**
RIB 3135-18	-0.17	-715**	-251.2**	-15.72**	-6.63**	-0.95**	-0.04	-0.50	21.90**	0.15	-249.5	-4.5	-8.59**	-759**	-78.8	0.100**	-1.017**	-252**	-2440**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

number, tiller number, biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and stover dry matter fraction. CSSL 72039 had significant positive *gca* effects for 1000 grain mass and CSSL 72069 was positively significant for grain number per panicle like donor parent. For this trait, CSSL 72001 showed significant negative *gca* effect which was found to be worse than that of recurrent parent ICMB 841. The *gca* effects of all recurrent parent homozygote for all traits was found to be similar to that of recurrent parent except line 72002 which had significant positive *gca* like donor parent for grain number per m². It was observed that 8 CSSLs out of 10 exhibited better *gca* effects than that of ICMB 841 for grain mass.

Among three testers, RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number and stover dry matter fraction as same as observed in other two treatments. Tester H 77/833-2 showed positive results for most of the observed traits except for panicle length, panicle diameter and 1000 grain mass whereas PPMI 301 also showed positive *gca* effects for most of the characters but not for flowering time, panicle number, tiller number, biomass yield, and vegetative growth index, dry stover yield and stover dry matter fraction.

4.2.2.2.4. Genotype x Environment interaction analysis

The testcross hybrids of lines were evaluated under three moisture regimes namely fully irrigated control, early-onset terminal drought stress and late-onset terminal drought stress conditions. The results of analyses of variance and general combining ability for the grain yield and stover yield and its related traits across these three environments (Genotype x Environment interaction) are explained below.

4.2.2.2.4(A). Analyses of variance

The analysis of variance results for LG2 across three moisture regimes presented in Table 23. There were no significant differences between moisture treatments for flowering time, plant height, panicle length, panicle diameter and stover dry matter fraction. For the remaining all traits, significant differences were noticed. Hybrids were highly significant for all observed characters. In case of environment x hybrids effects, there were clear significant differences

Table 23 : Analyses of variance for testcross hybrids of LG2 over three moisture regimes

Source of Variation	df	Time to		Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Flowering (d)									
Environments(Env)	2	0.24	97.11**	89.11**	76.44**	7.94*	1.29	4.14	9.29*	5.52*	48.75**	
Hybrids	47	5.79**	29.89**	5.12**	52.18**	10.38**	19.37**	19.78**	0.98	9.69**	5.7**	
Lines (L)	15	8.19**	13.66**	4.45**	15.97**	13.57**	7**	23.39**	0.89	6.41**	4.76**	
Testers (T)	2	19.56**	569.48**	60.82**	1064.61**	97.1**	323.5**	249.94**	1.09	140.32**	78.11**	
L × T	30	3.67**	2.04**	1.74*	2.79**	3**	5.28**	2.63**	1.01	2.62**	1.34	
Env × Lines	30	0.87	1.41	1.38	1.59*	1	0.95	0.76	1.07	1.4	1.07	
Env × Testers	4	0.58	25.17**	4.56**	15.86**	2.11	0.86	0.42	1.21	0.19	1.02	
Env × Hybrids	94	0.76	2.48**	1.55**	2.34**	1.02	1.18	0.91	0.89	1.01	0.99	
Env × L × T	60	0.71	1.5*	1.44*	1.82**	0.95	1.31	1.01	0.78	0.88	0.95	
Error df		282	274	281	274	282	282	282	276	281	275	

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number / m ²
Hybrids	47	4.99**	4.36**	47.02**	8.77**	3.93**	6.06**	43.67**	16.44**	13.3**
Lines (L)	15	3.75**	4.16**	15.06**	4.04**	2.2**	2.47**	29.4**	8.49**	2.96**
Testers (T)	2	54.31**	41.32**	958.75**	135.05**	46.52**	105.75**	761.9**	283.58**	263.36**
L × T	30	2.31**	2**	2.22**	2.72**	1.96**	1.21	2.92**	2.61**	1.8**
Env × Lines	30	1.13	1.13	1.64*	1.13	0.88	1.01	2.57**	1.39	1.31
Env × Testers	4	3.12*	2.41*	13.89**	2.47*	1.41	6.94**	9.97**	11.76**	30.29**
Env × Hybrids	94	1.35*	1.25	2.43**	1.41*	1.13	1.28	2.41**	1.92**	2.73**
Env × L × T	60	1.35	1.24	2.05**	1.48*	1.24	1.05	1.83**	1.53*	1.61**
Error df		267	267	260	268	267	280	282	273	274

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

for grain yield but non- significant for other grain yield related traits such as time to 75% flowering, plant height, panicle length, panicle diameter, panicle number, tiller number, vegetative growth index, dry stover yield and stover dry matter fraction. Both lines and testers were significant for all observed traits of grain and stover yield related traits. The environments x line interactions were significant for panicle harvest index, grain harvest index and 1000 grain number. The environment x tester effects had significant differences for many traits but non- significant for flowering time, plant height, panicle length, panicle diameter, panicle number, tiller number and dry stover yield. Due to line x tester, the effects were significant for grain yield, panicle harvest index, time to flowering, panicle length, panicle diameter, biomass yield, vegetative growth index, grain harvest index, dry stover yield, grain mass and grain number. The effects due to interaction among environment x line x tester, were significant for grain yield, panicle yield, panicle harvest index, grain harvest index, fresh stover yield, grain mass and grain number.

4.2.2.2.4(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG2 across three moisture regimes are presented in Table 24. None of the lines showed negative *gca* effects for flowering except donor parent 863B and a recurrent parent homozygous line 72044, which had early flowering nature. CSSLs 72038 and 72054 expressed significant positive *gca* effects and it was observed that all CSSLs (except 72044 and 72062) flowered later than recurrent parent ICMB 841. Among testers, RIB 3135-18 was best negative combining ability followed by PPMI 301 for flowering time whereas H 77/833-2 had significant positive *gca* effect. For grain yield, CSSL 72028 showed significant positive *gca* effects like donor parent 863B and none of the lines found to be positively significant for panicle yield except 863B. In case of panicle harvest index, two CSSLs 72028 and 72060 had significant positive *gca* effects. It was also observed that 9 CSSLs out of 10 and 3 CSSLs out of 4 exhibited better *gca* values for grain yield, panicle yield and panicle harvest index than that of recurrent parent ICMB 841. The remaining two lines (CSSL 72030 and RPHL 72031) were negatively significant like recurrent parent for these traits. Only donor parent exhibited significant positive effects for plant height and recurrent parent and three RPHLs along with one CSSL 72069 were negatively significant for this character. Both recurrent parent and donor parent exhibited negatively significant *gca* effects for panicle length but the later had more negative effect and

Table 24 : Estimates of *gca* effects of lines and testers of LG2 over three moisture regimes

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
72001	0.21	-21.6	-22.9	-0.51	2.25	0.19	0.02	0.84	5.66	0.01	-38.6	-1.3	-0.62	-60.4	-18.4	0.015	-0.020	-43	-213
72002	0.32	8.7	70.4	-1.14	-1.35	-0.55*	-0.14**	-3.44	31.90**	0.24**	217.4	3.5	-1.67**	-18.9	193.2**	0.033*	-0.345**	-34	436
72028	0.32	132.0*	62.4	2.93**	2.43	0.34	-0.02	1.08	-13.78	-0.11	210.4	3.3	1.19	522.1**	125.6	-0.022	0.039	71*	671.2*
72030	0.21	-182.3**	-165.8*	-3.05**	-1.94	0.15	-0.08**	-1.98	7.22	0.07	-349.6**	-7.4**	-0.93	-385.3**	-175.8*	0.016	-0.285**	-91**	-670*
72031	0.02	-301.1**	-144.5	-5.47**	-5.16	-0.55*	-0.07*	4.84	27.50**	0.11	-309.6*	-6.1*	-3.12**	-306.9*	-154.3*	-0.001	-0.549**	-133**	-828.7**
72038	0.50**	9.9	-46.1	1.23	0.36	-0.03	-0.01	0.66	5.51	0.02	-9.4	-1.3	0.13	84.2	47.3	0.007	-0.178	37	392
72039	0.06	79.1	55.4	1.81	2.29	0.15	0.00	2.21	-5.30	-0.07	88.7	1.6	1.15	81.6	16.3	-0.021	0.268**	10	85
72044	-0.42*	-85.8	-88	-1.22	-3.01*	-0.84**	-0.10**	0.66	7.63	0.02	-225.3	-3.4	-0.50	-309.9*	-126.8	0.011	0.032	-60	-330
72054	0.50**	-33.2	-33.9	-0.89	0.91	0.12	-0.02	3.58	-5.34	-0.09	153.6	1.9	-0.68	180.6	146.4	0.014	-0.014	-12	-260
72060	0.06	31.3	-20.7	2.15**	-0.75	0.59**	-0.05	1.91	-11.60	-0.10	43.7	0.4	1.20	170.5	50.6	-0.021	0.242*	15	65
72062	-0.31	-11.7	94.9	-1.33	-2.75*	0.63**	-0.01	-7.8*	33.30**	0.32**	17.1	1.1	-0.29	-115.0	-67.2	-0.010	-0.246**	-23	318
72066	0.28	24.6	13.8	0.78	-0.09	0.52*	-0.02	-2.20	-12.15	-0.04	29.6	0.0	0.70	73.6	-27.7	-0.011	-0.018	40	182
72067	0.32	39.7	64.4	-0.11	1.03	0.59**	0.05*	5.51	-24.50**	-0.21**	-41.0	-1.7	0.84	179.4	-94.9	-0.037*	0.025	90**	185
72069	-0.13	-89.8	-34.9	-1.77	-3.60**	0.52*	0.02	1.58	-1.45	-0.05	-3.8	0.2	-1.37*	-7.3	43.4	0.007	-0.334**	5	6
ICMB 841	-0.56**	-284.9**	-267.2**	-4.83**	-5.71**	-0.84**	-0.07*	-1.68	16.85	0.15*	-351.7**	-5.6*	-3.5**	-529.5**	-74.1	0.054**	-0.335**	-155*	-1065.2**
863B	-1.38**	685.1**	462.6**	11.41**	15.1**	-0.99**	0.51**	-5.72	-61.50**	-0.28**	568.6**	14.8**	7.49**	441.2**	116.5	-0.035*	1.719**	283**	1026.2**
H 77/833-2	0.36**	212.9**	119.1**	4.02**	3.197**	-0.99**	-0.18**	1.94	32.00**	0.17**	366.6**	6.3**	1.44**	597.8**	256.1**	-0.020**	-0.254**	40**	1692**
PPMI 301	-0.12	477**	170.8**	11.09**	3.18**	1.94**	0.22**	-1.42	-54.60**	-0.32**	45.1	1.1	7.65**	190.9**	-133.2**	-0.057**	1.231**	231**	851**
RIB 3135-18	-0.24**	-689.9**	-289.8**	-15.1**	-6.38**	-0.95**	-0.04**	-0.52	22.64**	0.15*8	-411.6**	-7.4**	-9.10**	-788.7**	-123.3*	0.077**	-0.976**	-271**	-2543**

** Significant at 0.01 level of probability, * Significant at 0.05 level of probability

it was also noticed that 11 lines (nine CSSLs and two RPHLs) out of 14, showed better *gca* effects for plant height and panicle length. For panicle diameter, only one CSSL 72067 showed significant positive *gca* like donor parent and three RPHLs and one CSSL 72030 expressed significant negative *gca* effects similar to recurrent parent. In case of panicle number and tiller number, three RPHLs had positive *gca* effects, resembling ICMB 841 and one CSSL 72067 was negatively significant like 863B. None of the lines showed significant positive *gca* effects for biomass yield, vegetative growth index and grain harvest index, fresh stover yield, dry stover yield and stover dry matter fraction except line 72028 was positively significant for fresh stover yield and line 72002, a recurrent parent homozygote was positively significant for dry matter yield and stover dry matter fraction. It was noted that CSSL 72028 had significant positive *gca* effects whereas CSSL 72030 and two RPHLs (72031 and 72044) had significant negative *gca* effects for the fresh stover yield. It was observed that minimum 9 CSSLs out of 10 and 2-3 RPHLs out of 4 exhibited better *gca* effects for biomass yield, grain harvest index and fresh stover yield. For grain mass, two CSSLs (72039 and 72060) showed significant positive *gca* results and for grain number, two CSSLs (72028 and 72067) were positively significant like donor parent 863B. In this analysis, it was observed that nine CSSLs and three RPHLs exhibited better *gca* effects for grain number when compared with recurrent parent ICMB 841.

Among three testers, RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction as same as observed in other three treatments. Tester H 77/833-2 showed positive results for all observed traits except for panicle length, panicle diameter, stover dry matter fraction and 1000 grain mass whereas PPMI 301 also showed positive *gca* effects for most of the characters but not for flowering time, panicle number, tiller number, dry stover yield and stover dry matter fraction.

4.2.2.3. Linkage Group 3 (LG3)

A total 45 testcross hybrids (involving three testers) of 10 homozygous segmental introgression lines and a sampling of 3 recurrent parent homozygotes (to serve as near-isogenic controls) of LG3, which were selected based on marker genotyping of 88 plants at

10 marker loci of LG3 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in three environments *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions during summer 2010 at ICRISAT, Patancheru. Line x tester analysis was performed and analysis of variance and general combining ability (*gca*) effects were calculated. The results of analysis of variance and general combining ability (*gca*) for grain and stover yield related characters for each of three moisture regimes and across these three moisture regimes are presented below.

4.2.2.3.1. Fully irrigated control treatment

4.2.2.3.1(A). Analysis of variance

The analysis of variance for LG3 under fully irrigated non-stress treatment during summer 2010 is presented in Table 25. Hybrids showed highly significant differences for all characters except for biomass yield. There were clear significant differences between lines for grain yield, stover yield and also its related component traits but there were no significant differences for panicle yield, panicle harvest index, biomass yield, vegetative growth index and grain harvest index and grain number. As expected, effects of testers were highly significant for all observed agronomic characters except for vegetative growth index. Line x tester effects were significant only for flowering time but not for remaining traits.

4.2.2.3.1(B). General Combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG3 in fully irrigated non-stress conditions, during summer 2010 are presented in Table 26. For flowering time, the best negative combiners among lines were CSSL 73052 and donor parent 863B and five CSSLs (73023, 73046, 73048, 73082 and 73086) showed significant positive *gca*. Among testers, RIB 3135-18 and PPMI 301 showed negative *gca* effects for flowering time and H 77/833-2 had significant positive *gca*. Only one CSSL 73082 expressed significant positive *gca* effects like donor parent 863B for grain yield and panicle yield whereas CSSL 73046 showed significant negative *gca* for panicle yield. None of the lines except donor parent (had significant positive *gca*) exhibited any significant *gca* effects for panicle harvest index. CSSL 73082 had

Table 25 : Analyses of variance for testcross hybrids of LG3 in fully irrigated non-stress conditions

Source of Variation	df	Time to		Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Grain Yield (kg/ha)								
Replication	2	25.29**	2	4.15*	1.7	8.12**	0.56	52.94**	11.49**	2.25	7.59**
Hybrids	44	110377.67**	3.43**	1.99*	23137669.77**	882.82**	10411.07**	176018.17**	6.26**	3.49**	16298.41**
Lines (L)	14	9.42**	1.84*	1.75	1.3	3.68**	2.99**	8.29**	1.98*	2.71**	1.85*
Testers (T)	2	13.99**	48.53**	4.41*	48.67**	16.09**	121.48**	94.69**	1.6	42.11**	23.49**
L × T	28	1.77*	1	0.76	0.75	1.32	1	1.28	0.8	0.7	0.81
Error	88										

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
Hybrids	44	1.1	973.72**	52749513.97**	84774.69**	1.52*	30733291.8**	571092.72**	3.52**	2.49**
Lines (L)	14	1.38	1.34	1.2	1.97*	1.82*	2.12*	18.12**	1.62	0.8
Testers (T)	2	3.58*	2.84	27.17**	12.47**	6.92**	9.78**	292.74**	39.45**	36.84**
L × T	28	0.79	0.81	0.71	1.18	0.86	1.14	1.22	0.95	0.86
Error	88									

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 26 : Estimates of *gca* effects of lines and testers of LG3 in fully irrigated non-stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle Yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
73014	-0.04	-364.3	-134.0	-6.47	-2.62	0.26	-0.04	-4.10	19.84	0.23	516.2	10.1	-2.58	13.7	310.8	0.062	-0.329*	-116	-1292
73023	1.40**	15.3	-67.5	2.87	3.27	0.70	-0.06	-1.65	-12.72	-0.07	655.4	9.3	2.31	184.8	383.4	0.060	-0.097	36	218
73029	-0.27	162.4	160.2	1.42	2.27	0.48	0.00	-3.32	-26.83	-0.12	757.8	15.2	2.98	433.7	258.3	0.030	-0.148	129	755
73046	0.73*	-289.7	-559.0*	-6.58	1.05	1.25**	0.01	5.79	11.39	-0.01	-875.6	-18.6	-3.47	-209.6	7	0.067	-0.901**	-106	-617
73048	1.29**	-279.3	-402.3	-5.91	2.16	0.37	-0.14**	-7.76	3.61	0.10	57.7	-1.3	-2.36	617.0	429.6	0.018	-0.763**	-7	135
73052	-1.16*	15.0	115.8	0.20	-2.62	0.15	-0.07	-9.54	22.39	0.29*	-817.7	-13.8	-3.13	-493.0	-401	-0.082*	-0.190	-31	308
73074	-0.38	-67.3	-179.5	2.53	-1.62	0.48	-0.01	-18.30**	-25.16	0.11	-1343.5	-24.7	-1.58	-803.0	-702.0*	-0.111**	-0.227	60	-8
73078	0.40	40.7	412.1	-1.91	0.61	0.04	-0.02	10.79	9.50	-0.11	291.0	3.8	-0.24	501.5	-75.2	-0.042	0.171	-29	415
73079	-0.27	219.7	195.3	4.53	-2.73	0.26	0.10	7.13	24.50	0.03	10.4	0.8	3.42	-244.1	-208	-0.010	0.146	15	903
73082	1.40**	520.8**	696.0**	5.31	4.83*	-0.85*	0.01	12.60*	-5.83	-0.22	1760.9*	30.3**	4.64	1822.6**	725.4*	-0.036	0.266	178*	2297*
73083	-0.27	-166.2	21.3	-2.47	-4.62*	-0.52	-0.10	2.02	18.17	0.13	-880.6	-16.5	-5.13	-650.7	-370.1	0.002	-0.029	-120	-850
73086	0.73*	-143.4	-173.9	-0.13	1.05	-0.30	0.00	-0.32	-7.83	-0.09	-483.2	-10.3	-2.36	-850.7	-209.7	0.049	-0.177	-24	-479
73087	-0.27	0.6	2.1	0.98	-3.62	-0.96*	-0.06	6.68	2.84	-0.09	544.6	11.4	2.53	570.4	202.9	-0.013	0.100	-28	-318
ICMB 841	-0.49	-232.9	-405.8	-4.91	-7.84*	-0.19	-0.14*	3.90	38.70*	0.23	-1006.2	-19.0	-3.91	-843.0	-505.3	-0.046	-0.010	-188*	-1128
863B	-2.82*	568.4**	319.4	10.53**	10.39**	-1.18**	0.51**	-3.99	-72.60*	-0.42*	812.8	23.3	8.86*	-49.6	153.9	0.051	2.186**	232**	-341
H 77/833-2	0.69**	358.5**	93.0	8.60**	1.01	-1.32**	-0.24**	3.84	31.20**	0.14*	678.1*	11.3	5.33**	903.0**	400.3**	-0.010	-0.320**	116**	2720**
PPM1 301	-0.36*	375.0**	176.0	6.73**	3.54**	2.19**	0.23**	-1.96	-55.40**	-0.34**	-116.1	-0.9	4.28**	-223.6	-222.6	-0.048**	1.359**	174**	73
RIB 3135-18	-0.33*	-733.5**	-269.0*	-15.33**	-4.55**	-0.87**	0.00	-1.87	24.10**	0.20**	-562.0	-10.4	-9.62**	-679.4**	-177.7	0.057**	-1.039**	-291**	-2793**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

significant positive *gca* for plant height and negative *gca* for panicle length, similar to donor parent. For panicle length, CSSL 73046 was positively significant and another CSSL 73087 was negatively significant. It was observed that nine CSSLs out of 10 and 2 RPHLs of 3 exhibited better plant heights than that of recurrent parent. There were no lines observed with significant positive *gca* for panicle diameter except donor parent. There was only one line 73052 that expressed significant positive effects for tiller number. Donor parent 863B showed significant negative *gca* effects for panicle number and tiller number while recurrent parent ICMB 841 showed significant positive effects for panicle number. Five CSSLs showed worse *gca* effects for panicle number and tiller number when compared with ICMB 841. Only one introgression line 73082 was found to better line as it exhibited significant positive *gca* effects for grain yield, panicle yield, plant height, biomass yield, vegetative growth index, fresh stover yield, dry stover yield, grain number per panicle and grain number per m². It was clear from these *gca* results that six CSSLs (73014, 73023, 73029, 73048, 73082 and 73086) out of 10 showed better *gca* effects for dry stover yield when compared with recurrent parent. For stover dry matter fraction, CSSL 73052 and recurrent parent homozygote 73074 and for 1000 grain mass, three CSSLs (73014, 73046 and 73048) showed negatively significant *gca* effects. It was observed that five CSSLs (73023, 73029, 73048, 73079 and 73082) and one RPHL 73074 expressed better grain number per panicle than that of ICMB 841.

Among testers, RIB 3135-18 showed significant negative *gca* effects for all observed traits except for panicle number, tiller number and stover dry matter fraction whereas these results were exactly opposite to that expressed by PPMI 301 except for flowering time. PPMI 301 was also had negative *gca* effect for flowering time next to RIB 3135-18. Tester H 77/833-2 showed positive effects for all traits except for panicle length, panicle diameter, stover dry matter fraction and 1000 grain mass.

4.2.2.3.2. Early drought stress treatment

4.2.2.3.2(A). Analysis of variance

The analysis of variance for LG3 under early-onset drought stress conditions are presented in Table 27. Hybrids showed highly significant differences for all characters except for biomass

Table 27 : Analyses of variance for testcross hybrids of LG3 in early-onset terminal drought stress conditions

Source of Variation	df	Time to Flowering (d)									
		75% Flowering	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Replication	2	12.37**	3	0.64	2.99	1.59	0.96	7.42**	0.86	0.53	1.66
Hybrids	44	47521.99**	5.58**	2.14**	20960936.95**	366.88**	3657.71**	73263.52**	2.63**	2.97**	16148.63**
Lines (L)	14	10.53**	7.57**	2.28*	5.98**	4.62**	2.2*	8.61**	1.53	2.39**	2.27**
Testers (T)	2	24.59**	38.07**	5.66**	37.89**	9.11**	124.86**	109.88**	0.14	25.3**	18.87**
L × T	28	1.18	2.27**	0.98	1.99**	1.52	1.76*	1.65*	1.61*	1.44	1.55
Error	88										

Source of Variation	df	Vegetative									
		Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number / m ²	
Replication	2	0.45	0.14	3.41*	7.38**	2.57	39.59**	6.87**	2.17	4.1*	
Hybrids	44	1.16	984.63**	65257358.8**	52515.73**	1.33	8368047.94**	158101.95**	3.69**	3.26**	
Lines (L)	14	1.05	1.38	6.86**	1.05	0.74	0.96	14.85**	2.82**	3.22**	
Testers (T)	2	2.74	1.51	34.41**	12.96**	7.73**	6.02**	96.14**	20.95**	21.31**	
L × T	28	1.09	1.06	2.08**	1.01	1.09	0.63	2.43**	2.31**	1.96**	
Error	88										

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

yield, grain harvest index and dry stover yield. There were clear significant differences between lines for grain yield and its related traits but non-significant for fresh and dry stover yields, stover dry matter fraction, biomass yield and vegetative growth index. As expected, effects of testers were highly significant for all observed agronomic characters except for biomass yield and vegetative growth index. Significant differences were observed due to line x tester effects for grain yield, panicle harvest index, panicle length, panicle diameter, grain harvest index, 1000 grain mass and grain number.

4.2.2.3.2(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG3 in early-onset drought stress treatment, during summer 2010 are presented in Table 28. For flowering time, CSSL 73052 and RPHL 73074 showed significant negative *gca* effects whereas five CSSLs 73023, 73029, 73048, 73078 and 73082 exhibited significant positive *gca* effects. In this treatment, both donor and recurrent parents showed significant negative *gca* effects, however donor parent had more negative significance than that of recurrent parent. Among testers, RIB 3135-18 followed by PPMI 301 showed negative *gca* and H 77/833-2 expressed significant positive *gca* effects for flowering time. Only one introgression line 73052 and donor parent 863B showed significant positive *gca* for grain yield, panicle yield and panicle harvest index. It was noticed that 8 CSSLs out of 10 showed worse grain yield than that of recurrent parent. CSSLs 73048 and 73086 had negatively significant results for panicle yield and CSSLs 73023, 73046 and 73048 were negatively significant for panicle harvest index. For plant height, lines CSSLs 73046 and 73082 showed significant positive *gca* effects like donor parent and CSSLs 73083, 73086 and 73087 had significant negative *gca* effects like recurrent parent. One CSSL 73048 and one RPHL 73074 expressed significant positive results unlike donor parent 863B for panicle length. For panicle number, CSSLs 73052, 73079 and ICMB 841 had significant positive *gca* and CSSL 73087 had significant negative *gca*. For tiller number, none of the CSSLs were significantly positive and CSSLs 73086 and 73087 were significantly negative. There were no CSSL lines with significantly positive *gca* effects, identified for biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and stover dry matter fraction. Many of the lines had worse *gca* effects than of recurrent parent for these traits. There was only one CSSL 73052 possessing significant positive *gca* for 1000 grain mass and grain number per m² like donor parent. Three CSSLs 73046, 73048 and

Table 28 : Estimates of *gca* effects of lines and testers of LG3 in early-onset terminal drought stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle Yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
73014	0.21	33.2	-49.3	2.04	-2.51	-0.06	-0.07	11.20	2.39	-0.15	168.1	2.9	1.42	190.9	131.9	-0.020	-0.064	29	206
73023	0.66*	-200.6*	-209.9	-6.85*	0.71	0.50	0.03	-15.60*	-19.84	0.10	-239.7	-5.8	-3.13*	-311.3	-115.2	0.019	-0.305	-130	-1510*
73029	0.77*	97.3	266.1	1.93	4.27	0.27	-0.01	-4.91	15.83	0.23	523.7	8.6	1.53	347.6	172.2	-0.007	-0.224	97	1108
73046	-0.23	-209.8**	117.9	-7.96**	6.82**	0.61	-0.01	-8.02	-9.50	0.03	-56.2	-0.6	-4.91**	197.6	-41.1	0.015	-0.532**	-109	-994
73048	0.88**	-196.9*	-297.5*	-5.74*	4.27	0.94*	0.00	-7.13	-11.28	-0.01	-140.7	-4.3	-3.24*	110.9	71.1	0.010	-0.891**	-76	-788
73052	-0.78*	217.7**	332.9*	5.92*	-0.96	0.05	-0.05	1.20	38.60*	0.26	-6.7	0.7	2.87	344.2	-173.1	-0.111**	0.508**	86	1490*
73074	-0.67*	120.4	18.5	4.48	-4.18	0.94*	0.02	-18.00*	12.83	0.41**	159.5	4.3	2.64	53.1	55.7	0.035	0.234	55	923
73078	1.21**	-114.9	-102.5	-3.63	1.38	0.05	-0.08	-0.69	-0.28	0.01	222.1	2.0	-2.02	242.0	239.1	0.009	-0.288	-73	-553
73079	-0.12	-72.4	-2.3	-3.96	-0.84	-0.50	-0.02	13.98	45.80*	0.07	-596.7	-11.6	-3.68*	-594.7	-283.5	0.003	-0.045	-155*	-923
73082	1.77**	-195*	-96.8	-5.30	5.37*	0.05	-0.01	1.64	-33.39	-0.27	-629.9	-15.2*	-3.24*	-418.0	-203.0	0.002	-0.067	-76	-1160
73083	-0.23	-14.8	-48.9	-0.07	-6.95**	-0.61	-0.11*	5.98	12.27	0.00	-19.1	0.0	0.31	-53.6	-55.8	-0.025	0.179	-39	-297
73086	0.33	-134.0	-310.5*	-2.19	-5.06*	-0.73	-0.04	10.31	-28.17	-0.32*	-217.9	-4.8	-1.80	-402.4	7.0	0.071	-0.368*	-3	-604
73087	-0.23	-93.3	-246.8	-1.07	-4.62*	-0.73	-0.11*	4.20	-38.50*	-0.31*	-194.8	-3.5	-0.69	55.3	-33.4	-0.038	-0.239	51	-385
ICMB 841	-0.67*	143.2	233.4	3.37	-5.17*	0.16	-0.06	9.20	49.80**	0.19	576.1	12.6	2.42	488.7	257.1	-0.010	-0.072	59	1468*
863B	-2.89**	619.9**	395.5**	19.03**	7.48**	-0.94*	0.53**	-3.24	-36.61	-0.22	452.2	14.7*	11.53*	-250.2	-29.1	0.047	2.173**	283**	2019**
H 77R33-2	0.85**	52.3	8.1	2.77*	1.29	-1.10**	-0.25**	-0.36	39.90**	0.26**	299.5	4.4	0.87	589.8**	249.6**	-0.035	-0.086	16	792**
PPM1301	-0.47**	190.7**	155.8*	5.85**	2.13*	2.34**	0.23**	1.44	-44.70**	-0.30**	-120.5	-1.4	3.6**	-252.2	-199.6*	-0.018	0.819**	129**	695*
RIB 3135-18	-0.38*	-243.1**	-163.9*	-8.63**	-3.42**	-1.23**	0.02	-1.09	4.80	0.04	-179.0	-2.9	-4.46**	-337.6*	-50.1	0.053**	-0.733**	-145**	-1488**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

73086 showed negatively significant *gca* effects for 1000 grain mass. Two CSSLs 73023 and 73079 had negatively significant *gca* for grain number per panicle and grain number per m² respectively. In this treatment both the parent showed significant positive *gca* effects for grain number per m² but donor parent had better effects than that of recurrent parent. Ten lines out of 13 lines tested showed worse *gca* effects for grain number per m².

Among testers, RIB 3135-18 showed negative *gca* effects for all traits except for panicle number, tiller number and stover dry matter fraction whereas these results were in contrast to that of PPMI 301 except for flowering time, for which both the testers were negative combiners. Tester H 77/833-2 showed positive effects for all traits except for panicle length, panicle diameter, stover dry matter fraction and 1000 grain mass.

4.2.2.3.3. Late drought stress treatment

4.2.2.3.3(A). Analysis of variance

The analysis of variance for LG3 under late-onset drought stress conditions is presented in Table 29. Hybrids showed highly significant differences for all characters except for biomass yield. There were clear significant differences between lines for grain yield and also its related component traits but non-significant for tiller number, fresh stover yield and stover dry matter fraction. As expected, effects of testers were highly significant for all observed agronomic characters. Lines x tester effects were significant for flowering time, plant height and 1000 grain mass.

4.2.2.3.3(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG3 in late-onset terminal drought stress conditions, during summer 2010 are presented in Table 30. For flowering time, two recurrent parent homozygous lines 73074 and 73083 showed significant negative *gca* effects whereas three CSSLs (73046, 73048 and 73082) exhibited significant positive *gca* effects. In this treatment, both donor and recurrent parents showed significant negative *gca* effects, however donor parent had more negative significance than that of recurrent parent. It was also noticed

Table 29 : Analyses of variance for testcross hybrids of LG3 in late-onset terminal drought stress conditions

Source of Variation	df	Time to		Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Flowering (d)									
Replication	2	2.19	1.34	0.31	3.39*	0.64	10.54**	3.3*	6.64**	2.92	1.43	
Hybrids	44	154383.41**	6.55**	3.04**	35272107.46**	839.38**	10615.79**	124929.78**	5.85**	3.39**	15873.23**	
Lines (L)	14	13.22**	5.7**	2.26*	3.3**	4.45**	5.32**	4.75**	1.94*	1.95*	1.16	
Testers (T)	2	14.1**	89.95**	9**	94.48**	28.19**	180.43**	90.82**	1.52	41.42**	17.68**	
L × T	28	2.3**	1.03	1.05	0.94	1	2.3**	0.98	2.09**	0.99	1.08	
Error	88											

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
Replication	2	0.18	0.26	1.64	6**	0.24	7.38**	6.9**	7.46**	3.24*
Hybrids	44	1.44	1207.76**	46820046.39**	130881.82**	2.01**	17900387.43**	263252.94**	5.66**	3.26**
Lines (L)	14	2.02*	2.22*	2.63**	1.57	2.06*	3.09**	18.39**	3.74**	2.78**
Testers (T)	2	4.13*	3.11*	62.72**	34.08**	13.13**	50.22**	235.51**	74.12**	37.14**
L × T	28	0.96	0.93	0.64	0.95	1.06	1.33	2.87**	0.76	1.06
Error	88									

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 30 : Estimates of *gca* effects of lines and testers of LG3 in late-onset terminal drought stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
73014	-0.39	100.1	307.4	-0.33	-1.16	0.16	-0.03	0.45	41.60**	0.25	554.3	11.8	0.93	483.4	172.5	-0.049	-0.224	-18	947
73023	0.50	-15.9	0.7	0.45	1.62	0.71*	-0.07	0.12	6.28	0.06	413.0	7.0	-0.07	-276.6	338.0	0.125**	-0.177	17	357
73029	0.39	161.1	89.0	3.56	1.18	-0.28	0.03	1.79	27.39	0.14	277.6	4.5	2.82	161.2	114.2	0.021	-0.679**	138*	2392**
73046	0.83**	-250.7	-76.6	-5.55	3.29	1.05**	-0.06	-2.88	3.84	0.08	36.6	-1.2	-3.19	-347.7	38.7	0.067	-0.549**	-88	-588
73048	1.72**	-198.4	-112.8	-3.99	4.18*	1.05**	-0.05	-9.88	-18.83	0.00	49.4	-2.8	-1.96	-164.4	88.1	0.037	-0.716**	56	-105
73052	-0.50	-24.7	-43.1	0.23	-5.04*	-0.50	-0.04	-2.66	2.17	0.04	76.3	2.6	0.48	114.5	45.2	-0.018	-0.211	15	93
73074	-0.60*	-71.0	-84.5	0.56	-2.16	0.94**	-0.03	-12.30*	6.50	0.24	11.1	1.5	0.59	-227.7	21.2	0.045	-0.226	-16	66
73078	-0.05	-345.7**	-218.9	-7.1*	-0.93	0.50	-0.05	0.01	-5.39	-0.07	-838.8*	-16.7*	-7.29**	-93.3	-286.8	-0.067	0.225	-215**	-2902**
73079	-0.39	52.5	75.4	1.23	-3.27	0.05	0.01	8.01	-6.72	-0.13	211.1	5.0	1.26	-13.3	61.5	0.004	-0.009	48	497
73082	1.39**	-62.9	-92.5	-0.88	4.62*	-0.50	-0.06	-3.77	2.73	0.04	288.5	2.4	-1.07	167.9	306.7	0.035	-0.187	-33	-172
73083	-0.60*	-188.1	-301.8	-5.88	-4.27*	-0.06	0.00	16.60**	-6.39	-0.25	-644.8	-11.6	-1.63	-407.7	-417.6*	-0.016	0.166	-135*	-1294
73086	0.50	-171.6	-197.3	-0.99	-4.16*	-1.39**	-0.01	1.90	-20.05	-0.13	-232.3	-5.7	-0.41	-282.1	-109.2	0.023	-0.132	-10	-631
73087	-0.05	-131.6	-183.0	-0.99	-2.60	-0.94**	-0.10	9.90	20.17	-0.03	-193.8	-3.8	-0.19	-17.7	-85.2	-0.019	-0.046	-105	-667
ICMB 841	-0.60*	125.9	45.2	3.90	-2.16	0.16	0.02	5.67	3.39	0.00	-1179.1**	-22.0**	-1.41	102.3	-591.2*	-0.138**	0.371*	22	307
863B	-2.16**	102.1**	792.8**	15.78**	10.84**	-0.94**	0.42**	-12.90*	-56.70**	-0.22	1170.9**	29.0**	11.14**	801.2**	303.8	-0.049	2.395**	323**	1699*
H 77R33-2	0.50**	84.3	55.4	2.65**	2.22*	-1.34**	-0.21**	2.70	30.60**	0.14*	391.4*	6.6	0.10	590.5**	333.6**	-0.030	-0.255**	6	1189**
PPM1 301	-0.29*	517.1**	204.2**	11.47**	3.35**	2.29**	0.25**	-3.57	-53.30**	-0.29**	-49.5	-0.3	8.37**	149.9	-246.6**	-0.095**	1.275**	244**	1140**
RIB 3135-18	-0.20	-601.5**	-259.6**	-14.12**	-5.57**	-0.94**	-0.04	0.87	22.70**	0.14*	-341.9	-6.2	-8.47**	-740.4**	-87.0	0.125**	-1.019**	-250**	-2329**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

that eight of 10 CSSLs flowered later than recurrent parent ICMB 841. Among testers, PPMI 301 followed by RIB 3135-18 showed negative *gca* and H 77/833-2 expressed significant positive *gca* effects for flowering time. There were no significant positive *gca* effects noticed in lines except donor parent for grain yield, panicle yield and panicle harvest index. There was only one line 73078 (a recurrent parent homozygote) with significant negative *gca* results for these traits. For plant height, two CSSLs 73048 and 73082 had significant positive effects as donor parent 863B and lines 73052, 73083 and 73086 had significant negative *gca*. Three CSSLs (73023, 73046 and 73048) and one RPHL 73074 showed significant positive *gca* and lines 73086, 73087 and 863B expressed significant negative *gca* for panicle length. There were no significant results observed among lines for plant diameter but donor parent showed significant positive *gca* effect as usual. Only one line 73014 showed significant positive *gca* for panicle number unlike donor parent 863B which was significantly negative. None of the CSSLs showed significant *gca* effects for the traits, tiller number, biomass yield, vegetative growth index, grain harvest index, fresh stover yield and dry stover yield. Only one CSSL 73023 was significantly positive for stover dry matter fraction. Though *gca* values for biomass yield and vegetative growth index, dry stover yield and stover dry matter fraction were not significant, it was clear that all 10 CSSLs and one RPHL exhibited better *gca* effects than that of recurrent parent for these traits. In this treatment, both 863B and ICMB 841 showed significant positive *gca* for 1000 grain mass but donor parent 863B was more significant. It was observed that all 10 CSSLs and one RPHL expressed worse *gca* effects for grain mass when compared with ICMB 841. There were no other lines found to be positively significant but CSSLs 73029, 73046 and 73048 exhibited significant negative effects for this trait. Donor parent 863B and one CSSL 73029 were identified as significantly positive for grain number.

Among three testers, RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction as same as observed in other two treatments. Tester H 77/833-2 showed positive results for most of the observed traits except for panicle length, panicle diameter, stover dry matter fraction and 1000 grain mass whereas PPMI 301 also showed positive *gca* effects for most of the characters but not for flowering time, panicle number, tiller number, biomass yield, and vegetative growth index, dry stover yield and stover dry matter fraction.

4.2.2.3.4. Genotype x Environment interaction analysis

The results of analyses of variance and general combining ability for the grain yield and stover yield and its related traits across the above three environments (Genotype x Environment interaction) are explained below.

4.2.2.3.4(A). Analyses of variance

Analyses of variance for LG3 across three moisture regimes are presented in Table 31. All the observed traits were highly significant due to effects of environment, hybrids, environment x hybrid, lines, testers, location x tester and line x tester. In case of location x line effects, significant differences were noticed for all traits except for panicle diameter. There were also highly significant differences for all observed characters due to interaction among environment x line x tester except for panicle diameter and stover dry matter fraction.

4.2.2.3.4(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG3 across three moisture regimes are presented in Table 32. For flowering time, one CSSL 73052 and two recurrent parent homozygous lines 73074 and 73083 showed significant negative *gca* effects whereas CSSLs 73023, 73029, 73046, 73048, 73082 and 73086 exhibited significant positive *gca* effects. The general combining ability effect across three moisture regimes for flowering time was observed as significantly negative in case of both donor and recurrent parents, however donor parent had more negative significance than that of recurrent parent. It was also noticed that all CSSLs flowered later than recurrent parent ICMB 841 except 73052. Among testers, both RIB 3135-18 and PPMI 301 were found to be negative combiners and H 77/833-2 expressed significant positive *gca* effects for flowering time. None of the lines (except donor parent) showed significant positive *gca* for grain yield and panicle yield. Three CSSLs (73046, 73048 and 73086) and one RPHL 73083 showed significant negative *gca* for grain yield and three CSSLs (73014, 73048 and 73086) were with significant negative *gca* for panicle yield. It was observed that only two CSSLs (73079 and 73082) exhibited better *gca* effects for grain yield than that of recurrent parent. There was only one CSSL 73023 having significant positive *gca* for panicle harvest index and 4 CSSLs 73046, 73048, 73086 and 73087 and one

Table 31 : Analyses of variance for testcross hybrids of LG3 over three moisture regimes

Source of Variation	df	Time to		Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Grain Yield (kg/ha)							
Environments(Env)	8	54.25**	343.78**	204.1**	270.03**	9.77**	5.25**	79.19**	77.3**	8.93**
Hybrids	44	26.08**	38.13**	62.47**	14.06**	48.47**	73.01**	3.32**	17.95**	11.42**
Lines (L)	14	62.86**	31.68**	33.59**	29.68**	10.88**	92.68**	3.93**	9.61**	5.65**
Testers (T)	2	71.58**	547.69**	1040.82**	73.19**	944.34**	913.74**	11.95**	301.39**	172.01**
L × T	28	4.43**	4.82**	6.98**	2.02**	3.27**	3.13**	2.4**	1.86**	2.71**
Env × Lines	112	1.8**	2.74**	3.06**	1.8**	1.38**	1.02	1.14	1.49**	1.66**
Env × Testers	16	24.51**	15.87**	22.55**	13.96**	2.7**	9.93**	2.45**	1.84*	4.23**
Env × Hybrids	351	2.76**	3.01**	4.12**	2.03**	1.43**	1.46**	1.26**	1.42**	1.64**
Env × L × T	223	1.69**	2.23**	3.34**	1.29**	1.36**	1.07	1.23*	1.36**	1.46**
Error df		792	703	711	784	792	785	783	749	742

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
Hybrids	44	7.06**	7.95**	58.57**	10.03**	7.81**	7.18**	125.5**	17.03**	13**
Lines (L)	14	6.45**	13.28**	38.12**	5.45**	3.26**	3.62**	125.04**	12.01**	3.02**
Testers (T)	2	65.25**	41.9**	917.58**	138.91**	125.57**	106.47**	1688.67**	250.78**	231.14**
L × T	28	3.12**	2.8**	7.42**	3.11**	1.68*	1.87**	14.07**	2.82**	2.42**
Env × Lines	112	2.79**	2.68**	2.9**	2.85**	1.7**	1.32*	2.86**	2.65**	3.17**
Env × Testers	16	8.48**	3.41**	23.41**	13.27**	9.85**	8.35**	21.17**	16.32**	33.7**
Env × Hybrids	351	3.09**	2.85**	3.85**	2.57**	2.02**	1.49**	3.35**	2.82**	4.15**
Env × L × T	223	2.86**	2.9**	2.92**	1.66**	1.61**	1.08	2.32**	1.93**	2.52**
Error df		674	674	624	728	711	765	787	669	701

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 32 : Estimates of *gca* effects of lines and testers of LG3 over three moisture regimes

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
73014	0.17	-39.8	-117.3*	-0.51	-0.12	0.17	-0.05**	-1.07	0.31	0.02	-87.7	-1.9	-0.63	145.5	56.5	-0.006	-0.172**	14	-588
73023	0.85**	35.8	-13.6	1.42*	1.88*	0.51**	-0.01	-1.04	-0.78	0.01	-35.5	-2.3	0.40	-187.7*	34.6	0.025**	0.143*	-25	222
73029	0.63**	-42.8	-36.6	-1.19	1.92*	0.12	-0.02	-1.05	-7.43	-0.02	8.1	-1.4	-1.01*	-77.3	47.4	0.020*	-0.438**	81**	531
73046	0.51**	-255.2**	-78.5	-4.81**	1.49	0.86**	-0.01	-2.28	-5.95	-0.03	-118.7	-3.8*	-3.65**	-184.3*	-42.6	0.022*	-0.653**	-13	-216
73048	0.87**	-187.3**	-207.2**	-2.89**	3.12**	0.15	-0.10**	-3.98	5.23	0.09	-177.3*	-4.5**	-2.40**	-47.8	41.7	0.014	-0.714**	4	353
73052	-0.78**	7.6	22.1	-0.12	-2.38**	0.15	-0.03	-0.23	10.30*	0.05	-99.2	-0.6	0.71	-144.9	-129.8**	-0.018*	0.174**	-22	-715*
73074	-0.63**	-15.4	-72.3	0.67	-3.88**	0.30*	-0.05**	-7.60**	13.50**	0.20**	-200.5*	-2.4	0.98*	-198.6*	-103.8*	0.003	0.040	-53*	-56
73078	0.28	-4.2	47.0	-0.37	0.49	0.30**	-0.04*	2.89	6.34	-0.01	24.4	-0.5	0.37	151.4	-11.6	-0.023*	-0.011	-26	211
73079	-0.27	59.5	10.7	0.79	-3.32**	-0.21	-0.01	4.40*	8.60	0.00	-35.2	-0.4	0.83	33.6	-48.2	-0.002	0.039	-20	290
73082	1.73**	72.0	33.9	0.69	4.07**	-0.54**	-0.03	3.15	-13.70**	-0.15**	165.9**	-0.5	-0.58	458.2**	149.38**	-0.017	-0.196**	84**	1115**
73083	-0.31*	-119.9**	-15.4	-2.26**	-5.00**	-0.22	-0.07**	6.10**	12.70*	0.03	-94.4	-0.8	-0.99*	-180.3*	-104.9*	-0.006	0.026	-111**	-1080**
73086	0.78**	-184.1**	-203.7**	-2.21**	-1.30	-0.72**	-0.02	1.01	-11.10*	-0.09	-203.2**	-5.6**	-1.86**	-148.7	-56.2	0.023*	-0.439**	-2	-199
73087	0.15	-4.1	97.4	-1.43*	-3.18**	-0.25	-0.08**	3.06	2.91	-0.02	205.2**	3.5*	-1.08*	279.2**	45.3	-0.023**	-0.035	-29	117
ICMB 841	-0.82**	-20.7	70.6	-0.31	-4.83**	-0.01	-0.06**	3.59	23.90**	0.16**	88.4	3.5*	0.32	-142.0	11.3	-0.003	0.003	-95**	-379
863B	-3.16**	698.8**	462.9**	12.54*	11.05**	-0.64**	0.60**	-6.90**	-44.70**	-0.24**	559.7**	18.2**	8.60**	243.7**	110.9*	-0.007	2.235**	214**	394
H 77R33-2	0.62**	136.3**	42.5	3.6**	1.53**	-1.16**	-0.22**	3.60**	29.30**	0.15**	283.9**	4.3**	1.10**	491.5**	252.8**	-0.019**	-0.334**	34**	2378**
PPM1 301	-0.33**	315.1**	174.9**	7.23**	1.88**	2.10**	0.25**	-1.70	-45.60**	-0.32**	-5.1	0.3	5.32**	-47.9	-174.4**	-0.028**	1.244**	142**	-549**
RIB 3135-18	-0.28**	-451.4**	-217.3**	-10.84**	-3.42**	-0.94**	-0.03**	-1.90*	16.20**	0.17**	-278.7**	-4.6**	-6.42**	-443.6**	-78.4**	0.047**	-0.909**	-175**	-1829**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

RPHL 73083 were negatively significant for the same trait. There were five lines 73023, 73029, 73048, 73082 and 863B showed significant positive *gca* for plant height. Even though lines 73052, 73974, 73079, 73083 and 73087 had significant negative *gca*, eight CSSLs and two RPHLs were found to have better plant height *gca* effects than that of recurrent parent ICMB 841. Four CSSLs 73023, 73046, 73074 and 73078 showed significant positive *gca* and lines 73082, 73086 and 863B expressed significant negative *gca* for panicle length. There were no significant positive results observed among lines for plant diameter but donor parent showed significant positive *gca* effect as usual. Three CSSLs 73014, 73048 and 73087 and all three homozygous recurrent parents (73074, 73078 and 73083) showed significant negative *gca* for panicle diameter similar to that of recurrent parent. For panicle number, four lines 73052, 73074, 73083 and ICMB 841 exhibited significant positive *gca* and three lines 73082, 73086 and 863B showed significant negative *gca* effects. A recurrent parent homozygote 73074 and recurrent parent expressed significant positive *gca* and CSSL 73082 and donor parent had significant negative *gca* in case of tiller number. It was observed that all lines including donor parent obtained worse *gca* effects for panicle yield and tiller number when compared with recurrent parent. There were only two CSSLs 73082 and 73087 showed significant positive effects like donor parent for Biomass yield. The general combining ability effect across three moisture regimes for vegetative growth index was observed as significantly positive in case of both donor and recurrent parents; however donor parent had better positive significance than that of recurrent parent. It was also noticed that all lines showed worse *gca* effects for this trait than that of recurrent parent ICMB 841 except 73087 which had significant positive *gca*. There were two lines 73082 and 73087 exhibited significant positive *gca* effects like donor parent for fresh stover yield. In case of dry stover yield, only one CSSL 73082 and donor parent 863B showed significant positive *gca* effects. For stover dry matter fraction, four CSSLs 73023, 73029, 73046 and 73086 obtained significant positive *gca* whereas three lines 73052, 73078 and 73087 had significant negative *gca*. There were three lines 73023 and 73052 including donor parent showed significant positive *gca* whereas 6 CSSLs out of 10 had significant negative *gca* effects for 1000 grain mass. For grain number per panicle, it was observed that all 10 CSSLs and one RPHL showed better significant *gca* effects when compared to recurrent parent ICMB 841. There was only one CSSL 73082 had significant positive *gca* and only one CSSL 73052 had significant negative *gca* for grain number per m². These above results revealed that two CSSLs 73079 and 73082 better lines for both grain yield and grain number.

Among three testers, RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction. Tester H 77/833-2 showed positive results for most of the observed traits except for panicle length, panicle diameter, stover dry matter fraction and 1000 grain mass whereas PPMI 301 also showed positive *gca* effects for most of the characters but not for flowering time, panicle number, tiller number, dry stover yield, stover dry matter fraction and grain number per m².

4.2.2.4. Linkage Group 4 (LG4)

A total 90 testcross hybrids (involving three testers) of 20 homozygous segmental introgression lines and a sampling of 8 recurrent parent homozygotes (to serve as near-isogenic controls) of LG4, which were selected based on marker genotyping of 328 plants at 7 marker loci of LG4 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in two environments *i.e.*, fully-irrigated control conditions and terminal drought stress conditions during summer 2009 at ICRISAT, Patancheru. Line x tester analysis was performed and analysis of variance and general combining ability (*gca*) effects were calculated. The results of analysis of variance and general combining ability (*gca*) for grain and stover yield and its related characters for two environments and across environment are presented below.

4.2.2.4.1. Fully irrigated control treatment

4.2.2.4.1(A). Analysis of variance

The analysis of variance for LG4 under fully irrigated control treatment during summer 2009 is presented in Table 33. Hybrids showed highly significant differences for all characters except for biomass yield and grain number per panicle. There were clear significant differences between lines for the grain yield, panicle yield, flowering time, panicle length and 1000 grain mass and for remaining traits, there were no significant differences. As expected, effects of testers were highly significant for all observed agronomic characters. There were no significant differences observed for any traits due to line x tester.

Table 33 : Analyses of variance for testcross hybrids of LG4 in fully irrigated non-stress conditions

Source of Variation	df	Time to									
		75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Replication	2	6.77**	0.99	2.96	3.35**	0.74	2.56	0.27	6.47**	7.36**	8.21**
Hybrids	89	9545.45**	1.92**	1.63**	14867079.02**	152.07**	9233.65**	44040.97**	4.94**	1.48*	3465.69**
Lines (L)	29	2.4**	1.54*	1.67*	1.26	1.49	2.47**	1.39	0.94	1.07	0.86
Testers (T)	2	30.08**	41.96**	5.45**	29.05**	30.08**	423.09**	53.71**	0.66	19.9**	13.57**
L × T	58	1.37	0.73	0.9	0.78	1.55**	1.31	1.18	0.84	0.77	0.78
Error	178										

Source of Variation	df	Vegetative									
		Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²	
Replication	2	0.86	0.53	3.44*	0.32	0.71	0.95	6.63**	3.85*	0.31	
Hybrids	89	1.06	1033.64**	29469146.81**	158779.61**	1.8**	55003387.8**	167900.74**	1.3	1.54**	
Lines (L)	29	0.77	0.77	0.76	0.71	0.72	1.07	3.91**	1.12	0.98	
Testers (T)	2	8.92**	4.92**	15.71**	28.94**	38.25**	28.52**	371.19**	13.62**	32.31**	
L × T	58	0.93	0.85	0.79	1.06	0.91	1.27	1.17	0.66	0.76	
Error	178										

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

4.2.2.4.1(B). General Combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG4 in fully irrigated non- stress conditions, during summer 2009 are presented in Table 34. For flowering time, the best negative combiners among lines were 74692 (a recurrent parent homozygote) and donor parent 863B. It was observed that 18 lines flowered later than recurrent parent and there were no CSSLs having significant earlier flowering behaviour. Among testers RIB 3135-18 and PPMI 301 showed negative *gca* effects for flowering time. For grain yield, there were no significant *gca* effects found in lines except donor parent 863B, having significant positive *gca* and CSSL74875, having significant negative *gca*. None of the lines had significant positive *gca* effects for panicle yield and panicle harvest index and plant height except donor parent 863B showing significant positive *gca* for panicle yield. In case of panicle length, both recurrent and donor parents showed significant negative *gca* effects, anyhow recurrent parent was found to be better than donor parent by having less negative *gca*. Two recurrent parent homozygous lines 74020 and 74484 showed significant positive *gca* and two lines 74541 (recurrent parent homozygote) and CSSL 74605 exhibited significant negative *gca* for panicle length. It was also noticed that many lines (18 out of 28) maintained better panicle length than that of recurrent parent. For panicle diameter, there were no lines found to have significant *gca* effects except donor parent, possessing significant positive *gca* and two recurrent parent homozygous lines (RPHLs) 74541 and 74874 with significant negative *gca*. There were no lines found to have significant positive effects for panicle number, tiller number, biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and stover dry matter fraction. It was also observed that none of the lines performed better than recurrent parent in case of all these traits. Two CSSLs 74411 and 74875 had significant negative *gca* for panicle yield and CSSL 74449 expressed same result for stover dry matter fraction. There was only one CSSL 74618 with significant positive *gca* for 1000 grain mass and two recurrent parent homozygous lines 74412 and 74874 showed significant negative *gca* for this character. CSSL 74601 and CSSL 74875 showed significant negative *gca* effects for grain number per panicle and grain number per m² respectively. It was found that none of the lines showed better results for grain number when compared with recurrent parent.

Table 34 : Estimates of *gca* effects of lines and testers of LG4 in fully irrigated non-stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
74020	0.40	235.8	290.8	3.97	-2.74	1.24**	0.15	4.63	14.58	0.07	225.3	3.3	0.72	10.4	72.0	0.025	-0.225	141.4	4912
74015	1.85	188.4	243.8	3.86	9.48	0.36	0.01	9.52	3.47	-0.16	379.9	4.4	-0.51	353.7	166.3	-0.040	-0.076	140.7	3558
74412	-0.37	-235.8	117.1	-7.69	0.14	0.03	0.06	7.52	12.69	-0.02	-366.5	-4.9	-7.17	-434.1	-273.7	0.008	-0.480*	-112.2	-1854
74411	-1.37	-144.9	-579.2*	-0.80	-0.74	0.58	0.01	-1.70	-38.80*	-0.35	-702.5	-9.7	2.60	-261.9	-226.4	0.005	-0.364	48.6	-862
74449	0.52	-188.2	-73.3	-2.03	3.59	0.58	0.07	-3.81	-16.98	-0.05	-471.5	-9.6	1.05	-219.6	-247.3	-0.052*	-0.114	-25.4	-3012
74484	-0.04	23.8	30.7	1.53	-11.07*	0.91*	0.04	-9.37	-8.09	-0.20	-147.9	-2.5	3.27	-100.7	-125.1	-0.009	0.200	51.7	-443
74481	-0.71	161.6	7.8	4.97	7.81	-0.31	0.01	-1.14	14.69	0.18	400.2	8.7	5.16	209.3	87.6	-0.020	-0.128	67.3	2666
74526	-0.04	80.8	-29.2	4.09	3.59	0.36	-0.01	-0.81	15.13	0.22	-212.9	-3.7	-0.17	-105.2	-56.2	0.007	0.084	-30.0	585
74541	2.07	-235.6	-795.9**	-10.24*	4.37	-0.86*	-0.26**	7.97	-2.20	-0.15	-907.6	-17.4	-4.17	-223.0	-180.4	-0.012	-0.452	-103.6	-2667
74539	-0.04	111.6	138.6	2.97	-1.19	-0.64	-0.08	3.52	21.91	0.15	119.7	2.6	-0.73	37.0	62.2	0.012	-0.102	10.6	2325
74535	0.96	203.3	158.0	4.53	4.14	0.36	-0.03	3.41	2.69	-0.03	630.4	10.5	4.83	164.8	167.7	0.015	-0.344	25.7	-956
74573	0.74	-51.6	69.3	0.09	0.26	-0.20	-0.17	-7.14	5.69	-0.28	-520.6	-11.0	-5.84	-171.9	-87.8	0.018	0.100	-34.7	-288
74570	0.40	-189.7	-521.4*	-1.80	-18.52**	-0.42	0.05	-0.14	-6.98	-0.02	-1052.7	-18.9	-2.29	-440.7	-190.2	0.031	0.163	-104.7	-2937
74567	0.18	260.0	218.9	4.97	5.59	0.14	0.02	-6.14	0.47	0.23	99.1	1.6	1.27	51.5	-3.4	-0.013	-0.136	203.4	4201
74591	0.52	-118.0	181.3	-3.58	-14.18**	-0.31	0.03	-2.48	18.58	0.33	725.5	12.7	-1.06	199.3	239.8	0.030	0.399	-151.5	-2565
74574	0.85	176.3	142.5	4.75	1.14	-0.53	0.00	3.52	-15.31	-0.15	-4.9	-0.6	1.16	-167.4	-54.3	0.011	0.059	230.7	3262
74601	0.40	-273.1	166.8	-7.03	2.03	0.69	0.00	2.86	31.91	0.27	695.7	12.2	-3.06	472.6	224.5	-0.034	0.030	-271.3*	-3722
74607	0.29	-38.9	-184.1	2.97	-4.30	0.03	0.02	-1.59	-4.42	0.06	-895.1	-15.5	-1.95	-460.7	-338.5	0.011	-0.266	38.8	432
74605	0.85	-123.7	18.3	-1.80	-0.19	-0.75*	0.03	-4.81	6.24	0.19	-327.4	-6.7	1.16	-369.6	-210.7	0.025	0.098	-35.6	-734
74618	-0.60	169.4	217.4	2.86	5.26	0.14	0.01	-4.59	-0.09	0.22	867.2	17.1	3.05	463.7	345.2	0.001	0.491*	38.7	943
74614	-0.37	148.6	187.5	2.97	-1.41	0.36	0.02	-0.14	17.58	0.24	138.2	3.0	0.83	123.7	33.5	-0.011	0.045	42.5	2516
74633	-0.26	-321.9	-686**	-8.80	1.92	0.47	-0.01	-15.30**	-25.76	-0.20	-627.0	-9.3	-4.17	20.4	-18.2	-0.013	-0.055	-225.0	-4140
74692	-5.59*	137.8	193.1	2.97	1.48	-0.64	-0.03	4.30	18.24	0.12	687.0	2.3	3.83	353.7	189.4	-0.023	-0.195	42.4	2863
74685	0.96	-271.9	-111.9	-3.47	1.70	0.03	-0.04	-3.81	4.47	0.15	142.6	2.4	-0.62	-90.7	-50.0	0.006	-0.434	-132.6	-2370
74698	0.63	229.0	306.7	3.20	1.26	-0.42	-0.03	0.63	-6.98	-0.06	-256.6	-5.6	-2.29	38.1	-103.7	-0.041	-0.011	167.7	3086
74779	1.07	127.3	327.0	1.20	7.81	0.25	-0.02	1.63	15.80	0.12	938.6	15.1	2.49	371.5	307.2	0.009	-0.176	45.4	2200
74874	2.51*	-338.0	-442.9	-7.03	-3.30	-0.09	-0.28**	8.63	-25.53	-0.36	-265.0	-7.4	-2.62	145.9	196.0	0.029	-0.571*	-132.9	-3762
74875	1.07	-514.9**	-322.5	-12.35*	1.26	0.69	0.05	-4.26	-42.20*	-0.23	-187.6	-3.9	-5.73	144.8	111.9	-0.002	0.128	-224.0	-7047*
ICMB 841	-2.04	222.9	181.6	5.42	-6.41	-0.86*	-0.03	8.30	18.13	0.00	452.3	12.6	6.60	-208.5	-33.7	0.037	0.312	42.0	2519
863B	-4.81*	569.3**	549*	9.31	1.26	-1.19**	0.39**	0.86	-28.87	-0.28	444.1	18.0	4.38	93.7	-3.5	-0.010	2.017**	246.0	1293
H 77833-2	2.19**	301.3**	94.3	5.51**	9.94**	-1.56**	-0.19**	0.87	19.00**	0.17*	682.2*	8.9*	3.08*	617.4**	474.8**	0.013	-0.375**	129.9**	6209**
PPM1 301	-1.04**	145*	119.4	3.91**	-6.53**	2.80**	0.25**	-1.77	-29.00**	-0.28**	-245.0	-1.8	2.7*	-283.9**	-302.5**	-0.042**	1.567**	29.2	-3093**
RIB 3135-18	-1.15**	-446.3**	-213.7**	-9.41**	-3.41*	-1.24**	-0.06*	0.90	10.02	0.12	-437.2*	-7.1	-5.78**	-333.5**	-172.3*	0.030**	-1.192**	-159.2**	-3116**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Among three testers, RIB 3135-18 showed negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction. PPMI 301 also showed positive *gca* effects for many characters except for flowering time, plant height, panicle number, tiller number, biomass yield, vegetative growth index, fresh stover yield, dry stover yield, stover dry matter fraction and grain number per m². Tester H 77/833-2 showed positive results for most of the observed traits except for panicle length, panicle diameter, stover dry matter fraction and 1000 grain mass.

4.2.2.4.2. Terminal drought stress treatment

4.2.2.4.2(A). Analysis of variance

The analysis of variance for LG4 under terminal drought stress conditions during summer 2009 is presented in Table 35. Hybrids showed highly significant differences for all characters except for panicle yield and biomass yield. There were clear significant differences in lines for grain yield, flowering time, panicle length, panicle diameter, and 1000 grain mass and for other observed traits, there were no significant differences. As expected, effect due to testers was highly significant for all observed agronomic characters. Significant differences were observed due to line x tester effects for grain yield and panicle harvest index.

4.2.2.4.2(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG4 in drought stress treatment, during summer 2009 are presented in Table 36. For flowering time, none of the lines was found to have significant negative *gca* except donor parent 863B. There were two CSSLs 74015 and 74698 identified with significant positive *gca* and it was also observed that many of the lines had late flowering character when compared to recurrent parent ICMB 841. Among testers, PPMI 301 showed negative *gca* and H 77/833-2 expressed significant positive *gca* effects for flowering time. There was an unexpected result in this treatment that tester RIB 3135-18 showed positive *gca* effects for flowering time. There were no lines except donor parent and recurrent parent with significant positive *gca* effects for many of the observed traits, grain yield, panicle yield, panicle harvest index, plant height, panicle diameter, biomass yield, vegetative growth index, grain harvest index, stover dry matter fraction, 1000 grain mass and

Table 35 : Analyses of variance for testcross hybrids of LG4 in terminal drought stress conditions

Source of Variation	df	Time to		Panicle				Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Harvest Index (%)	Harvest Index (%)							
Replication	2	37.18**	9.24**	17.11**	3.59*	2.47	0.68	6.68**	2.18	1.55	6.14		
Hybrids	89	24266.41**	3.16**	1.04	7051028.22**	85.78**	1642.64**	14355.33**	3.62**	2.51**	6713.97**		
Lines (L)	29	5.34**	1.86**	0.92	1.34	1.22	1.59*	2.73**	1.69*	0.91	0.88		
Testers (T)	2	53.72**	72.19**	5.09**	47.5**	6.85**	189.03**	46.96**	7.03**	58.28**	37.64**		
L × T	58	1.14	1.43*	0.57	1.69**	0.95	0.79	1.2	1.1	1.11	1.09		
Error	178												

Source of Variation	df	Vegetative		Grain		Dry		Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
		Biomass Yield (kg/ha)	Growth Index (kg/ha/d)	Harvest Index (%)	Harvest Index (%)	Fresh Stover Yield (kg/ha)	Stover Yield (kg/ha)				
Replication	2	18.48**	20.36**	5.75**	20.54**	20.36**	1.82	9.59**	1.88	5.6**	
Hybrids	89	0.97	1012.63**	15971231.57**	276900.22**	1.59**	98802447.39**	85133.96**	1.91**	2.09**	
Lines (L)	29	0.91	0.89	1.07	0.93	0.93	1.07	4**	1.28	1.11	
Testers (T)	2	4.82**	3.17*	29.16**	21.67**	23.19**	6.37**	232.68**	23.11**	39.82**	
L × T	58	0.86	0.84	1.15	0.98	1.02	0.75	0.85	1.32	1.27	
Error	178										

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 36 : Estimates of *gca* effects of lines and testers of LG4 in terminal drought stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
74020	0.38	-49.6	191.8	0.13	1.256	1.1*	0.15	-0.24	-14.58	-0.11	438.4	7.1	1.63	111.0	80.2	-0.028**	0.336	18	-1481
74015	1.49**	-80.6	172.0	-2.42	3.256	0.11	0.04	6.20	-21.25	-0.28	640.3	9.5	-1.26	332.1*	302.1*	-0.010	-0.375	77	225
74412	-0.06	62.2	7.4	4.13	-1.633	0.89	0.09	-2.02	-0.14	0.05	219.7	3.7	3.96	26.5	45.7	0.016	0.043	36	900
74411	-0.51	120.9	150.1	5.91	2.256	1.1*	0.02	2.09	10.20	0.06	47.2	1.3	2.30	-40.1	-31.9	-0.001	0.120	75	2107
74449	0.60	-42.4	-175.4	-0.42	-11.967**	1.00	0.07	-4.36	-2.36	0.09	-66.8	-1.8	1.19	-64.6	-57.7	0.001	-0.506*	-2	528
74484	-0.62	116.7	130.9	5.58	-2.744	0.55	0.00	7.76	18.42	0.04	436.2	8.3	4.52	139.9	138.9	0.004	0.115	55	1927
74481	0.49	-102.6	-186.5	-3.64	1.256	-0.11	-0.08	-1.02	-0.25	0.07	-49.2	-1.3	-1.37	-26.8	-29.2	0.002	0.038	-125	-2535
74526	0.16	-186.3	-322.4	-3.87	0.922	0.66	-0.27*	-0.36	-7.58	0.03	-258.5	-4.9	-1.59	48.7	58.8	0.007	0.029	-194*	-3599
74541	0.05	89.4	208.3	3.13	1.700	-0.11	-0.02	6.09	5.42	-0.01	573.5	10.0	3.07	192.1	199.0	0.010	0.008	54	1717
74539	-0.17	-22.8	64.9	0.24	0.256	-0.56	-0.22*	5.20	-1.58	-0.09	231.4	4.2	1.96	6.5	0.1	-0.004	-0.130	-17	-151
74535	0.83	64.7	-13.4	6.36	4.144	0.33	-0.02	5.76	-14.25	-0.19	131.3	1.3	5.52	-32.4	-21.6	0.007	-0.297	171	2551
74573	0.05	62.3	236.1	0.36	1.700	0.00	-0.05	-4.91	26.70*	0.36*	513.2	8.7	1.85	122.1	110.8	-0.007	0.217	-57	943
74570	0.27	69.9	57.4	4.58	-15.189**	-0.89	-0.05	1.42	-11.69	-0.11	283.9	4.6	4.19	51.0	59.9	0.008	-0.109	120	1494
74567	0.60	110.0	-4.1	8.36	5.256	0.11	-0.01	7.31	-7.03	-0.18	69.1	0.6	1.30	129.9	105.4	-0.012	-0.245	174	2897
74591	-0.06	-113.4	-180.6	-3.31	-3.078	0.22	-0.16	7.64	6.09	-0.02	-15.1	-0.2	-1.37	123.2	128.4	0.003	-0.005	-163	-1760
74574	0.16	-200.7*	-189.4	-6.76	-3.633	0.11	0.09	-4.13	9.64	0.19	-639.7	-11.4	-4.59	-251.3	-230.1	0.016	-0.355	-195*	-3670
74601	0.05	154.7	234.3	5.69	2.144	0.00	0.00	0.53	-1.25	0.01	269.7	4.8	1.30	86.5	95.7	0.010	0.099	128	2758
74607	0.60	54.2	-263.2	-1.76	2.478	-0.45	0.00	-4.47	-6.80	0.05	-253.1	-5.3	0.30	111.0	113.3	0.002	-0.136	53	1248
74605	0.38	-63.1	-356.2	2.58	0.922	0.11	0.00	-5.69	1.20	0.14	-737.1	-13.2	-0.82	-203.5	-191.6	0.006	-0.014	-20	-1314
74618	-0.17	-222.4*	132.3	-15.42**	-1.856	-0.23	0.02	-1.36	11.31	0.17	-221.0	-3.7	-7.59**	-56.8	-70.2	-0.012	0.060	-189	-3985*
74614	-0.62	105.3	-41.2	-1.64	-1.189	-2.55**	-0.06	4.20	10.53	0.02	42.8	1.7	0.85	-65.7	-82.4	-0.012	0.326	-19	927
74633	-0.29	65.8	163.1	2.69	-0.189	-0.11	0.06	-13.10*	13.09	-0.09	506.7	9.3	2.85	178.7	177.0	0.001	0.229	4	80
74692	0.16	39.1	168.1	1.36	-0.078	-0.11	0.02	2.42	21.42	0.16	157.7	2.9	-0.93	8.7	32.1	0.019	-0.020	-17	665
74685	-0.40	-211.2*	-338.6	-4.87	0.033	0.11	0.06	-7.58	-8.36	0.07	-642.9	-11.2	-3.26	-181.3	-177.4	-0.004	-0.220	-154	-3728
74698	1.15**	24.7	-31.2	-2.09	5.811	0.11	0.05	0.87	-11.91	-0.12	-692.2	-12.8	-5.48	-209.0	-244.8	-0.020	-0.097	50	840
74779	0.16	95.4	86.8	6.02	0.589	0.44	0.01	6.53	-5.36	-0.13	-92.3	-1.8	3.07	-117.9	-112.9	-0.002	0.075	94	1804
74874	0.38	-190.4	-166.9	-8.20	-2.078	-0.34	-0.05	2.87	9.97	0.05	-421.1	-7.8	-6.92*	-235.7	-218.6	-0.001	-0.709**	-70	-1133
74875	-0.73	-80.9	159.8	-2.09	1.589	0.00	-0.24*	0.98	12.42	0.12	-179.3	-3.1	-5.37	-81.3	-80.3	0.000	-0.125	-45	-582
ICMB 841	0.27	-40.9	-186.9	-3.64	-2.078	-0.45	-0.23*	4.53	-14.03	-0.15	-243.0	-3.7	0.19	-31.3	-42.1	-0.008	-0.092	-59	-782
863B	-4.61**	372**	292.5	3.02	10.144*	-1.00	0.78**	-23.13**	-28.00*	-0.22	-49.5	4.3	0.52	-70.1	-56.8	0.010	1.739**	217**	1110
II 771833-2	0.99**	228.9**	120.4	8.66**	4.067**	-1.78**	-0.23**	4.90**	22.10**	0.14**	320.5*	4.5*	3.80**	228.2**	224.6**	0.003	0.078	107**	4333**
PPMI 301	-1.02**	55.6	37.6	1.76	-1.756	2.79**	0.24**	-2.17	-37.50**	-0.33**	-139.2	-1.3	2.65*	-192.4**	-199.2**	-0.009**	0.929**	64*	-1489*
RIB 3135-18	0.04	-284.5**	-158*	-10.42**	-2.311	-1.01**	-0.01	-2.80	15.39**	0.19**	-181.3	-3.2	-6.45**	-35.8	-25.4	0.006*	-1.007**	-171**	-2844**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

grain number. Donor parent 863B showed significant positive *gca* effects for grain yield, plant height, panicle diameter, 1000 grain mass and grain number per panicle whereas this parent exhibited significant negative *gca* for panicle number. It was noticed that recurrent parent ICMB 841 showed significant negative *gca* for panicle diameter but many lines (20 lines including donor parent) had better *gca* effect when compared to this recurrent parent. In case of panicle length, one RPHL 74020 and one CSSL 74411 showed significant positive *gca* whereas only one CSSL 74614 had significant negative *gca*. There was only one RPHL 74573 expressed significant positive *gca* effect for panicle number and tiller number and it was observed that donor parent had worst *gca* effect of all lines tested in this trial for panicle number. There was only one CSSL 74015 identified with significant positive *gca* results for fresh stover yield and dry stover yield and none of the lines found to have significant negative effects for these two traits. There were no lines found to show significant positive *gca* effect for grain mass and grain number except donor parent which was significantly positive for grain mass and grain number per panicle. In case of grain number per panicle, there were two CSSLs 74535 and 74567 having better *gca* effects than that of recurrent parent.

Among testers, RIB 3135-18 showed negative *gca* effects for all traits except for flowering time, panicle number, tiller number and stover dry matter fraction. There was an unexpected result in this treatment that this tester showed positive *gca* effects for flowering time unlike in all other treatments in the present study and anyhow this positive effect was not significant. PPMI 301 expressed positive *gca* effects for all traits except for flowering time, plant height, panicle number, tiller number, biomass yield, vegetative growth index, fresh stover yield, dry stover yield, stover dry matter fraction and grain number per m². Tester H 77/833-2 showed positive effects for all traits except for panicle length and panicle diameter.

4.2.2.4.3. Genotype x Environment interaction analysis

The results of analyses of variance and general combining ability for the grain yield and stover yield and its related traits across these two environments (Genotype x Environment interaction) are explained below.

4.2.2.4.3(A). Analyses of variance

Analysis of variance for LG4 across two moisture regimes is presented in Table 37. Significant differences were observed for grain and stover yield traits but non-significant for flowering time, plant height and tiller number between moisture treatments. Hybrids were highly significant for all observed characters. In case of environment x hybrid effects, there were clear significant differences for all characters except for panicle length, panicle number and tiller number. As expected, testers exhibited significant differences for all the observed characters and similarly, between lines also significant differences were observed for all traits except for tiller number and stover dry matter fraction. The environment x line interaction was significant for panicle harvest index, plant height, fresh stover yield and dry stover yield. The environment x tester effects had significant differences for many traits but non-significant for panicle length, panicle number, tiller number and vegetative growth index. Due to line x tester effects, significant difference was observed for grain yield, panicle yield, panicle harvest index, time to flowering, plant height, biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and grain number per m². The effects due to interaction among environment x line x tester, were significant for grain yield, panicle yield, panicle harvest index, biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and grain number per m².

4.2.2.4.3(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG4 across two moisture regimes are presented in Table 38. For flowering time, the best negative combiners among lines were 74411, donor parent 863B and recurrent parent. Even though both the parents were significantly negative, donor parent had more negative *gca* value than that of recurrent parent. It was observed that 27 lines showed late flowering when compared with recurrent parent. Among testers RIB 3135-18 and PPMI 301 showed negative *gca* effects for flowering time. For grain yield, there were two RPHLs (74020, 74412) and donor parent 863B with significant positive *gca* and three CSSLs (74449, 74685 and 74875) and one RPHL 74874 with significant negative *gca*. There were only one CSSL 74618 along with donor parent found to have significant positive *gca* effect and five CSSLs 74449, 74526, 74570, 74605 and 74685 had significant negative *gca* for panicle yield. It was noticed that none of the lines except donor parent showed better *gca* results when compared with recurrent parent for this

Table 37 : Analyses of variance for testcross hybrids of LG4 over two moisture regimes

Source of Variation	df	Time to		Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Flowering (d)									
Environments(Env)	1	0.24	348.68**	154.45**	1322.11**	0.02	14.75**	94.89**	0.15	11.4**	5.36	
Hybrids	89	6.51**	12.17**	3.59**	13.81**	5.32**	19.02**	24.3**	1.36*	3.96**	2.68**	
Lines (L)	29	7.94**	4.02**	3.32**	3.28**	2.97**	3.37**	23.69**	1.42	1.55*	1.36	
Testers (T)	2	118.07**	424.94**	58.42**	504.45**	142.73**	763.4**	705.45**	4.88**	114.79**	73.11**	
L × T	58	1.95**	2.01**	1.83**	2.15**	1.76**	1.18	1.12	1.22	1.35	0.91	
Env × Lines	29	1.06	1.4	1.01	1.59*	1.69*	0.77	1.12	0.83	0.86	0.9	
Env × Testers	2	14.93**	23.38**	3.44*	4.65*	38.21**	0.59	6.29**	2.11	0.34	2.52	
Env × Hybrids	89	1.38*	2.41**	1.6**	2.15**	2.12**	0.83	1.37*	0.8	0.7	0.95	
Env × L × T	58	1.08	2.2**	1.83**	2.35**	1.08	0.87	1.33	0.75	0.63	0.91	
Error df		355	312	332	305	351	355	347	351	349	344	

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
Hybrids	89	6.14**	4.43**	13.88**	7.62**	9.64**	1.83**	19.05**	3.63**	7.16**
Lines (L)	29	3.92**	4.8**	4.29**	3.78**	3.19**	1.08	8.21**	2.4**	1.82*
Testers (T)	2	133.02**	54.28**	495.83**	168.23**	275.44**	34.87**	692.96**	88.98**	241.23**
L × T	58	2.88**	2.53**	2.06**	4.01**	3.71**	1.06	1.23	1.3	1.76**
Env × Lines	29	1.34	1.04	1.41	1.93**	1.96**	1.06	1.33	1.21	1.41
Env × Testers	2	8.97**	1.59	4.26*	27.09**	15.87**	14.44**	39.74**	4.68**	19.06**
Env × Hybrids	89	2**	1.68**	2.1**	2.92**	2.31**	1.52**	2.03**	1.39*	2.17**
Env × L × T	58	2.09**	2.01**	2.36**	2.58**	2.02**	1.3	1.07	1.36	1.97**
Error df		298	297	275	315	315	356	355	311	311

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 38 : Estimates of *gca* effects of lines and testers of LG4 over two moisture regimes

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle Yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
74020	0.31	117.2*	117.9	3.77**	-1.94	1.14**	0.11**	1.25	-3.38	-0.08	118.0	1.1	2.20*	6.1	36.1	0.002	0.044	110*	1964*
74015	1.59**	0.2	84.5	-1.26	5.17**	0.20	-0.01	6.91*	-12.26	-0.27**	369.9**	4.3	-1.81*	381.6**	223.4*8	0.024*	-0.237	94	1161
74412	-0.30	114.8*	-61.1	2.51	-1.94	0.42	0.04	1.80	2.90	-0.04	-211.9	-3.7	1.21	-187.3*	-109.3*	0.012	-0.231	73	2638**
74411	-1.02**	33.2	-113.6	2.31	-0.44	0.80**	-0.02	-0.76	-1.49	-0.05	-311.1**	-3.8	2.41**	-252.3**	-194.1**	0.002	-0.133	66	1190
74449	0.48	-125.5**	-155.5*	-1.54	2.22	0.75**	0.03	-5.03	-13.04	-0.03	-325.7**	-6.9**	-0.77	-104.6	-119.6*	0.025**	-0.321*	3	-1045
74484	-0.41	72.8	-42.6	2.35	-1.24	0.69*	-0.02	3.93	1.79	0.00	2.4	0.4	1.44	47.4	11.0	0.002	0.146	33	561
74481	-0.19	5.5	-99.7	0.48	3.34*	-0.25	-0.07*	-2.03	3.85	0.07	-139.6	-2.1	0.18	-7.6	-36.0	0.009	-0.057	-29	-230
74526	-0.02	-85.3	-179*	-0.31	1.06	0.48	-0.06*	-1.53	13.09	0.17	-196.9	-3.8	-0.62	21.0	7.5	0.007	0.045	-128*	-1822*
74541	0.97*	-49.6	-19.0	-2.80*	1.84	-0.53	-0.05	6.08	-1.76	-0.13	-34.7	-2.1	-1.72*	12.8	20.9	0.001	-0.234	-20	-147
74539	-0.19	-59.2	-21.6	-2.35	-1.66	-0.63*	-0.06*	3.41	6.79	-0.02	1.6	0.0	-1.59	15.7	18.1	0.004	-0.128	-80	-701
74535	0.81*	30.5	-51.1	1.49	2.95	0.31	-0.06*	3.63	-9.15	-0.16	-116.3	-3.2	1.04	-104.5	-59.9	0.011	0.020	69	363
74573	0.31	29.6	29.3	1.03	-0.21	-0.14	-0.04	-0.90	12.85	0.17	116.2	1.0	0.38	109.3	104.9	0.006	0.147	-26	610
74570	0.26	8.8	-160*	3.72**	-3.29*	-0.69*	-0.04	-0.31	-12.71	-0.12	-296.3**	-5.7**	3.02**	-242**	-114.4*	0.020	0.016	69	293
74567	0.31	81.5	-16.0	2.71*	4.23**	0.09	-0.03	-0.37	-6.65	-0.03	37.7	0.3	0.05	119.1	81.5	0.013	-0.202	112*	1761
74591	0.15	-47.8	10.6	-2.47	-3.29*	-0.08	0.05	1.63	20.41*	0.20*	60.3	0.8	-2.19*	-9.5	51.2	0.017	0.185	-136**	-1754
74574	0.42	-57.8	-29.4	-1.79	-2.44	-0.25	0.01	-1.26	-6.21	-0.04	-116.0	-2.5	-1.18	-165.1*	-105.9	0.013	-0.160	4	-672
74601	0.15	-72.1	77.2	-2.24	0.90	0.31	-0.04	0.75	11.96	0.09	176.5	2.9	-1.85*	177.4*	95.2	0.012	0.053	-111*	-1072
74607	0.37	-63.6	-96.8	0.54	-2.10	-0.25	-0.03	-3.98	-8.99	0.00	-50.7	-1.6	1.03	5.2	5.5	0.007	-0.213	9	-63
74605	0.53	-40.6	-189.4*	1.22	-0.83	-0.36	-0.02	-6.20	0.35	0.11	-352.3**	-6.7**	0.64	-198.5*	-141.2*	0.015	0.030	8	-645
74618	-0.47	13.7	195.6**	-3.01*	0.51	-0.08	-0.02	-3.92	2.24	0.14	316.2**	6.1**	-1.84*	122.1	91.5	0.005	0.264	-14	-407
74614	-0.58	23.4	86.7	-0.48	-2.49	-0.05	-0.06*	1.08	10.68	0.08	158.2	3.4	-0.11	137.2	60.4	0.012	0.174	-65	-66
74633	-0.35	-44.1	60.0	-2.42	-0.33	0.14	-0.01	-3.51	5.40	0.17	106.9	3.2	-1.72*	109.4	65.8	0.006	0.076	-87	-1061
74692	-0.40	-15.1	57.3	-1.79	-0.49	-0.41	-0.04	2.41	16.46	0.09	152.6	3.6	-1.39	118.8	85.1	0.002	-0.119	-64	-24
74685	0.20	-205.7**	-250.3**	-3.59**	-0.33	0.03	-0.03	-6.64*	-5.32	0.05	-464.2**	-8.4**	-1.41	-257.3**	-198.7**	0.001	-0.338*	-121*	-2657**
74698	0.81*	63.8	140.1	1.09	2.34	-0.19	-0.03	-0.20	-12.82	-0.14	250.7*	3.4	1.57	238**	59.5	0.030*	-0.066	76	889
74779	0.53	91.2	83.5	2.10	3.01	0.31	-0.04	3.13	1.85	-0.06	142.9	1.4	1.41	42.7	50.1	0.003	-0.062	53	1814*
74874	1.36**	-116.4*	-77.4	-1.52	-3.88*	-0.25	-0.09**	4.80	5.39	-0.06	-70.5	-3.5	-1.29	-60.3	6.0	0.014	-0.652**	-4	208
74875	0.59	-155.3**	4.6	-3.59**	0.23	0.31	-0.03	-2.59	-4.74	0.04	103.5	0.9	-3.01**	68.5	85.8	0.001	-0.011	-40	-1633
ICMB 841	-1.46**	85.1	150.5	0.88	-5.43**	-0.69*	-0.04	5.47	14.34	0.03	59.6	3.4	1.67	-214**	-97.4	0.015	0.098	-6	1130
863B	-4.79**	367.1**	464.5**	4.96**	4.50**	-1.13**	0.70**	-7.07*	-31.82**	-0.16	513**	17.8**	4.29**	70.7	17.3	0.000	1.866**	155**	-586
II 77R833-2	1.51**	261.7**	152.3**	6.65**	6.55**	-1.6**	-0.22**	2.57*	23.39**	0.18**	476.7**	5.8**	2.55**	377.1**	323.2**	0.008	-0.160**	106**	5177**
PPMI 301	-1.11**	127.9**	55.9*	4.11**	-4.65**	2.76**	0.25**	-1.18	-34.23**	-0.30**	-174.3**	-1.3	4.37*8	-202.7**	-235.1**	-0.025**	1.236**	65**	-2182**
RIB 3135-18	-0.39**	-389.6**	-208.3**	-10.76**	-1.90**	-1.16**	-0.02**	-1.40	10.84**	0.12**	-302.4**	-4.5**	-6.92**	-174.4**	-88.1**	0.017**	-1.076**	-171**	-2994**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

trait. In case of panicle harvest index, one RPHL 74020, two CSSLs (74570, 74567) and 863B were significantly positive whereas one RPHL 74541 and three CSSLs, 74618, 74685 and 74875 were significantly negative. For plant height, CSSLs 74015, 74481 and 74567 showed significant positive *gca* effect like donor parent and one CSSL 74570 and two RPHLs 74591, 74874 showed significant negative *gca* effect like recurrent parent. Most of the lines (24 lines out of 29) expressed better *gca* results than recurrent parent for this trait. In case of panicle length, both recurrent and donor parents showed significant negative *gca* effects, anyhow recurrent parent was found to be better than donor parent by having less negative *gca*. Two CSSLs (74411 and 74449) and two RPHLs (74020 and 74484) showed significant positive *gca* and two CSSLs 74539 and 74570 exhibited significant negative *gca* for panicle length. It was also noticed that many lines (18 out of 28) maintained better panicle length than that of recurrent parent. For panicle diameter, there were two lines 74020 (RPHL) and 863B found to have significant positive *gca* and five CSSLs (74481, 75526, 74539, 74535 and 74614) and one RPHL 74874 with significant negative *gca*. There was only one RPHL 74591 found to have significant positive effect for panicle number and tiller number whereas donor parent was significantly negative for panicle number and CSSL 74015 was significantly negative for tiller number. For biomass yield three CSSLs (74015, 74618 and 74698) and donor parent expressed significant positive *gca* values and five CSSLs (74411, 74449, 74570, 74605 and 74685) had significant negative *gca* values. In case of vegetative growth index, CSSL 74618 and 863B were significantly positive whereas four lines 74449, 74570, 74605 and 74685 were significantly negative. There were two CSSLs (74411 and 74570), a RPHL 74020, and donor parent exhibited significant positive values and five CSSLs (74015, 74601, 74618, 74633, and 74875) and two RPHLs (74541 and 74591) showed significant negative *gca* values for grain harvest index. In case of fresh stover yield, three CSSLs 74015, 74601 and 74698 had significant positive *gca* and five CSSLs (74411, 74570, 74574, 74605 and 74685) and a RPHL 74412 had significant negative *gca* effect like recurrent parent. Only one CSSL 74015 showed significant positive combining ability whereas CSSLs 74411, 74449, 74570, 74605 and 74685 and a RPHL 74412 showed significant negative *gca* effect. It was observed that 19 lines including 13 CSSLs out of 20 and 6 RPHLs out of 8 expressed better *gca* results when compared with recurrent parent for both fresh and dry stover yield. For stover dry matter fraction, there were three CSSLs (74015, 74449 and 75698) found to have significant positive values. None of the lines except donor parent found to have significant positive values for 1000 grain mass but two CSSLs (74449 and 74685) and a RPHL 74874 showed significant negative *gca* effect. In case of

grain number per panicle, significant positive *gca* value was shown by a RPHL 74020, a CSSL 74567 and 863B and negative *gca* value was shown by CSSLs 74526, 74601 and 74685 and a RPHL 74591. There were two RPHLs (74020 and 74412) and CSSL 74779 with significant positive *gca* effect and two CSSLs 74526 and 74685 with significant negative values for grain number per m².

Among three testers, RIB 3135-18 showed negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction. PPMI 301 showed positive *gca* effects for many characters except flowering time, plant height, panicle number, tiller number, biomass yield, fresh stover yield, dry stover yield, stover dry matter fraction and grain number per m². Tester H 77/833-2 showed positive results for most of the observed traits except for panicle length, panicle diameter and 1000 grain mass.

4.2.2.5. Linkage Group 5 (LG5)

Total of 54 testcross hybrids (involving three testers) of 5 homozygous introgression lines, 8 lines heterozygous at one or more LG5 loci along with testcrosses of their donor and recurrent parents and a sampling of 3 recurrent parent homozygotes (to serve as near-isogenic controls) of LG5, which were selected based on marker genotyping of 302 plants at 10 marker loci of LG5 (done to identify CSSLs in the previous objective) were evaluated for drought tolerance in two environments *i.e.*, fully-irrigated control conditions and terminal drought stress conditions during summer 2009 at ICRISAT, Patancheru. Line x tester analysis was performed and analysis of variance and general combining ability (*gca*) effects were calculated. The results of analysis of variance and general combining ability (*gca*) for grain and stover yield and its related characters for each of two environments and across environment are presented below.

4.2.2.5.1. Fully irrigated control treatment

4.2.2.5.1(A). Analysis of variance

The analysis of variance for LG5 under fully irrigated control treatment during summer 2009 is presented in Table 39. Hybrids showed highly significant differences for all characters except for biomass yield, dry stover yield and grain number per panicle. There was clear significant difference due to lines for flowering time, panicle length, panicle diameter and 1000 grain mass. For the remaining traits, there were no significant differences among lines. As expected, effects of testers were highly significant for all observed agronomic characters except for biomass yield, vegetative growth index, and stover dry matter fraction and grain number per panicle. There were no significant differences observed for many traits due to line x tester and significant difference was noticed for flowering time, panicle length, tiller number and 1000 grain mass.

4.2.2.5.1(B). General Combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG5 in fully irrigated control conditions, during summer 2009 are presented in Table 40. For flowering time, the best negative combiners among lines were 75035, 75159 and donor parent 863B and there was only one line 75064 with significant positive *gca*. Among testers PPMI 301 followed by RIB 3135-18 showed negative *gca* effects for flowering time. There were no significant *gca* effects found in lines for grain yield, panicle yield and panicle harvest index. Similar results were noticed for plant height also but there was one line 75133 with significant negative *gca* effect. In case of panicle length, both recurrent and donor parents showed significant negative *gca* effects, anyhow recurrent parent was found have better panicle length than that of donor parent. Three lines 75048, 75073 and 75094 showed significant positive *gca* and one line 75126 exhibited significant negative *gca* for panicle length. It was also noticed that many lines (11 out of 17) maintained better panicle length than that of recurrent parent. For the trait panicle diameter, there were no lines found to have significant positive *gca* effects except donor parent and two lines 75048 and 75173 with significant negative *gca*. There was only one line 75156 obtained significant positive *gca* and also only one line 75133 found to have significant negative effects for panicle number and tiller number. For biomass yield and vegetative growth index, significant *gca* effects were not observed in any line including

Table 39 : Analyses of variance for testcross hybrids of LG5 in fully irrigated non-stress conditions

Source of Variation	df	Time to									
		75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Replication	2	3.47*	0.53	1.08	0.56	2.28	2.78	0.87	1.57	3.66*	2.13
Hybrids	53	51539.38**	1.18	1.19	10798717.52**	110.51**	8669.23**	83911.38**	2.8**	1.69*	4262.55**
Lines (L)	17	5.92**	0.83	0.76	0.79	0.96	3.58**	6.97**	0.7	1.16	1.29
Testers (T)	2	50.7**	13.15**	3.65*	9.73**	5.41**	199.85**	40.45**	1.19	12.68**	4.86**
L × T	34	1.59*	0.65	0.88	0.78	1.53	2.35**	1.09	0.72	1.13	1.59*
Error	106										

Source of Variation	df	Vegetative			Dry			Grain		
		Biomass Yield (kg/ha)	Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
Replication	2	0.68	0.55	0.93	0.94	1.55	1.8	2.13	0.43	0.16
Hybrids	53	0.75	738.08**	15953019.06**	161126.11**	1.23	10199175.65**	351963.89**	1.64*	1.29
Lines (L)	17	0.93	0.85	1.08	1.49	1.03	1.36	14.78**	1.53	1.2
Testers (T)	2	1.74	0.97	3.28**	13.31**	9.39**	0.19	533.73**	2.37	13.26**
L × T	34	0.6	0.57	0.55	0.85	0.71	1.48	2.97**	1.44	0.63
Error	106									

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 40 : Estimates of *gca* effects of lines and testers of LG5 in fully irrigated non-stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
75035	-1.10*	-247.6	-314.6	-5.22	-0.83	0.28	-0.09	3.86	0.81	-0.02	-806.6	-13.6	-2.62	-669.8*	-301.6	0.072	-0.089	-195.3	-2959
75048	0.12	-113.6	-147.6	1.34	3.94	0.95**	-0.13**	-16.20*	23.15	0.26	-424.2	-6.8	-0.95	-247.6	-226.5	-0.022	-0.806**	39.2	1910
75064	1.22**	-108.8	-378.3	-0.99	-9.72	0.17	-0.08	5.53	-4.85	-0.13	39.7	-1.7	1.72	-53.1	208.8	0.083	-0.380**	-82.2	6
75073	0.23	282.7	191.6	6.56	-2.94	0.83*	-0.08	4.31	42.37	0.44	86.4	0.9	1.83	-219.8	23.5	0.057	-0.053	-5.0	3749
75076	0.78	-135.8	154.6	-4.22	2.72	-0.05	0.02	-6.58	-1.52	-0.37	-1138.9	-21.2	-13.61**	-766.5**	-380.1	0.038	-0.108	-14.3	-2131
75094	-0.55	-327.6	-400.3	-6.55	6.83	1.39**	0.07	0.09	-14.30	0.03	-139.3	-1.3	-0.84	21.3	178.8	0.052	0.029	-175.3	-4980
75132	0.45	289.3	340.0	4.45	2.06	-0.38	-0.06	-0.36	0.37	0.12	1072.2	18.7	5.61	550.2	331.7	-0.013	-0.138	233.1	4611
75133	0.45	-273.4	-297.0	-8.77	-12.16*	0.28	0.03	7.64	-53.90*	-0.50*	-749.1	-14.4	-7.84	186.9	-143.0	-0.059	0.293*	-324*	-5543
75126	0.78	-144.5	135.6	-3.55	-1.72	-0.82*	0.00	6.53	7.93	0.02	644.7	10.2	1.16	176.9	108.4	-0.008	-0.410**	4.7	-261
75134	0.78	24.0	4.2	3.01	0.50	-0.60	-0.06	2.42	-0.07	0.10	502.2	7.8	5.05	22.4	97.9	0.033	0.074	58.3	-486
75146	0.45	-126.2	-128.4	-4.99	4.83	0.17	0.06	-3.03	-26.85	-0.07	297.4	4.4	-0.17	378.0	266.0	-0.004	-0.032	-23.5	-1649
75159	-1.66**	45.6	18.9	2.78	-2.72	-0.49	-0.01	-5.58	-0.30	-0.25	394.0	10.3	6.05	-218.7	-25.3	0.048	-0.129	159.0	1425
75156	-0.33	167.8	220.5	3.12	-1.28	0.17	-0.04	-1.25	44.00*	0.59*	890.7	16.7	4.83	381.3	269.9	0.004	-0.303*	9.8	3870
75164	0.56	118.8	181.6	2.56	-2.83	-0.05	-0.03	3.20	6.48	0.06	189.0	2.2	-1.17	178.0	62.7	-0.093*	0.099	61.1	950
75173	-0.10	162.3	97.2	5.34	0.39	0.62	-0.19**	-11.91	-25.63	-0.41	657.6	12.4	6.49	340.2	159.9	-0.015	0.356*	238.4	1225
75180	0.45	279.0	331.7	4.34	-2.83	-0.38	-0.07	1.42	13.81	0.26	137.0	1.5	1.72	136.9	-46.3	-0.057	-0.250	181.8	5245
ICMB 841	0.67	172.9	168.3	4.23	7.50	-0.93**	0.02	3.86	6.93	0.08	-620.9	-11.9	-3.84	-205.4	-336.8	-0.045	-0.003	104.4	2430
863B	-3.21**	-64.8	-178.0	-3.44	8.28	-1.16**	0.65**	6.09	-18.41	-0.22	-1031.9	-14.2	-3.40	9.1	-247.8	-0.071	1.851**	-270.2*	-7411*
H 77/833-2	1.30**	230.7**	219.1	3.77	6.09**	-1.4**	-0.14**	3.99	30.10**	0.22*	429.5	5.7	-0.45	478.1**	313.6**	-0.002	-0.391**	93.2	5160**
PPM1 301	-1.27**	137.8	-7.8	4.03	-3.20	2.39**	0.19**	-2.36	-33.40**	-0.22*	-215.9	-1.7	3.88	-338**	-219.7*	-0.007	1.459**	-31.6	-4504**
RIB 3135-18	-0.03	-368.5**	-211.3	-7.79**	-2.89	-0.92**	-0.04	-1.64	3.22	0.00	-213.6	-4.0	-3.43	-140.2	-93.9*	0.009	-1.067**	-61.6	-655

** Significant at 0.01 level of probability, * Significant at 0.05 level of probability

parents. None of the lines found to have significant positive *gca* for grain harvest index, fresh stover yield, dry stover yield and stover dry matter fraction but there were two lines 75035 and 75076 had significant negative *gca* for fresh stover yield and the later line was significantly negative for grain harvest index also. There was only one line 75164 with significant negative *gca* for stover dry matter fraction and it was also observed that 7 lines for dry stover yield and 5 lines for stover dry matter fraction obtained better results when compared with recurrent parent. There were three lines 75133, 75173 and donor parent with significant positive *gca* for 1000 grain mass and four lines 75048, 75064, 75126 and 75156 showed significant negative *gca* for this character. Lines did not show any significant *gca* effects for grain number per panicle except lines 75133 and 863B, which were significantly negative. Similar results were observed for grain number per m² also but here line 75133 did not show significant negative *gca*, anyhow it had negative *gca* effect. It was found that none of the lines showed better results for grain number when compared with recurrent parent.

Among three testers, RIB 3135-18 showed negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction. Tester H 77/833-2 showed positive results for most of the observed traits except for panicle length, panicle diameter, stover dry matter fraction and 1000 grain mass whereas PPMI 301 showed significant positive *gca* effects for panicle length, panicle diameter and 1000 grain mass whereas it expressed significant negative *gca* effects for flowering time, panicle number, tiller number, fresh stover yield, dry stover yield and grain number per m².

4.2.2.5.2. Terminal drought stress treatment

4.2.2.5.2(A). Analysis of variance

The analysis of variance for LG5 under terminal drought stress conditions during summer 2009 is presented in Table 41. Hybrids showed highly significant differences for all characters except for panicle yield, biomass yield and grain number. There was clear significant difference in lines for flowering time, panicle length, panicle diameter, stover dry matter fraction and 1000 grain mass and for other observed traits, there was no significant difference. As expected, effect due to testers was highly significant for all observed

Table 41 : Analyses of variance for testcross hybrids of LG5 in terminal drought stress conditions

Source of Variation	df	Time to		Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Flowering (d)									
Replication	2	2.19	0.35	0.5	0.42	0.3	0.54	0.3	1.92	0.71	0.85	
Hybrids	53	12065.68**	1.63*	0.98	7716637.19**	111.66**	638.41**	10914.56**	3.25**	1.97**	5209.27**	
Lines (L)	17	4.6**	0.71	0.88	0.45	1.59	1.93*	3.09**	0.64	1.24	0.9	
Testers (T)	2	23.83**	25.69**	2.21	20.17**	18.42**	19.87**	13.06**	5.58**	19.5**	7.49**	
L × T	34	1.36	0.68	0.69	0.78	1.14	0.94	1.07	1.29	1.14	1.05	
Error	106											

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	1000-Grain Mass (g)	Stover Dry Matter Fraction	Grain Number/Panicle	Grain Number/m ²
Replication	2	2.8	2.17	0.68	2.61	2.42	4.18*	2.45	2.24	1.7
Hybrids	53	1	932.85**	18137105.41**	250580.27**	2.09**	72206.23**	115635596.99**	1.21	1.3
Lines (L)	17	0.72	0.72	0.41	0.83	0.92	3.53**	2.66**	0.62	0.66
Testers (T)	2	4.12*	1.89	19.43**	21.46**	22.94**	109.11**	11.42**	12.51**	18.41**
L × T	34	0.95	0.91	0.78	1.02	1.27	1.69*	0.99	0.64	0.61
Error	106									

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

agronomic characters except for panicle yield and vegetative growth index. There were no significant differences observed due to line x tester effects except for 1000 grain mass.

4.2.2.5.2(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG5 in terminal drought stress treatment, during summer 2009 are presented in Table 42. For flowering time, the best negative combiners among lines were 75159 and donor parent 863B and there were five lines 75076, 75132, 75126, 75134 and 75180 with significant positive *gca* which showed late flowering character. Among testers PPMI 301 followed by RIB 3135-18 were best negative combiners for flowering time and H 77/833-2 contributed late flowering nature to the testcross hybrids. There were no significant *gca* effects found in lines for grain yield, panicle yield and panicle harvest index observed in control treatment. For plant height, donor parent had significant positive *gca* and there was one line 75048 with significant negative *gca* effect. In case of panicle length, one line 75094 showed significant positive *gca* and two lines 75076 and 75126 exhibited significant negative *gca* for panicle length. For panicle diameter, there were no lines found to have significant positive *gca* effects except donor parent and two lines 75048 and 75094 with significant negative *gca*. For panicle number and tiller number, significant positive *gca* effects were not observed in any line and there was only one line 75173 found to have significant negative effect. It was observed that lines 75048 and 75180 exhibited better *gca* effects for tiller number when compare to recurrent parent. Significant *gca* effects were not observed in lines for biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and stover dry matter fraction. Only donor parent showed significant negative *gca* for stover dry matter fraction. It was also observed that 7 lines obtained better results when compared with recurrent parent for this trait. None of the lines showed significant *gca* effects for grain number and grain mass except donor parent which was significantly positive for 1000 grain mass. There were also no lines found to have better results for these traits when compared with recurrent parent ICMB 841.

Among three testers, RIB 3135-18 showed negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction. PPMI 301 showed significant positive *gca* effects for panicle length, panicle diameter, grain harvest

Table 42 : Estimates of *gca* effects of lines and testers of LG5 in terminal drought stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover-Dry Matter Fraction (%)	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/ m ²
75035	-1.12	-18.7	-9.0	1.38	-2.099	0.70	0.05	-10.38	32.22	-0.01	69.2	2.5	1.32	-58.8	-25.7	0.017	-0.332	-16	1194
75048	-0.79	-113.5	-309.0	-2.17	-16.21**	1.25	-0.40**	0.84	33.33	0.35	-265.7	-3.6	-1.46	-101.0	-60.7	0.019	-0.763**	-70	325
75064	0.88	-28.4	-169.0	-1.06	2.235	1.14	0.03	-0.05	-10.33	0.06	-109.3	-3.1	-0.91	109.0	117.8	0.009	0.005	-44	115
75073	0.21	-77.7	-107.9	0.27	0.679	0.70	0.03	-0.83	7.33	0.19	-135.8	-2.5	1.20	-169.9	-132.0	0.015	0.118	-64	-1445
75076	1.54*	7.3	-23.8	3.16	3.123	-2.41*	-0.18	-2.27	-30.44	-0.18	82.3	-0.4	2.20	9.0	2.2	-0.010	-0.647**	129	-691
75094	-1.01	-36.6	-3.9	0.27	3.457	2.69**	-0.38**	-3.16	-4.89	0.09	-419.6	-6.1	-2.57	-244.3	-224.1	0.001	-0.003	15	-476
75132	1.65*	-1.6	103.7	0.38	-0.099	-0.08	0.03	4.62	7.00	0.01	407.0	5.2	0.20	197.9	199.2	0.004	-0.342	50	1373
75133	-0.46	26.9	-16.0	-0.51	2.346	0.59	-0.18	0.28	2.11	0.07	89.5	2.2	0.09	175.7	171.0	0.001	0.087	28	750
75126	1.87**	112.5	190.9	3.72	1.790	-2.96**	0.06	6.06	13.67	0.04	-0.6	-2.4	-0.24	-13.2	-33.2	-0.013	-0.170	115	3422
75134	1.32*	-175.0	-78.9	-5.06	1.568	0.59	-0.01	1.06	-2.00	0.02	-388.3	-8.3	-2.69	-152.1	-155.4	-0.005	0.062	-114	-3557
75146	0.21	-12.7	11.6	1.05	2.123	-0.41	0.02	2.95	-1.33	-0.03	186.4	3.0	0.87	62.3	71.0	0.008	-0.191	41	603
75159	-1.56*	-122.3	-260.2	-3.17	-1.765	-0.41	0.06	-6.72	-4.89	0.14	-421.6	-5.9	-0.80	-97.7	-65.8	0.017	0.096	-114	-2079
75156	-0.79	15.4	-190.0	1.94	-3.321	0.36	0.06	4.84	-11.00	-0.15	-189.3	-2.8	1.54	-117.7	-103.2	0.004	0.133	39	-26
75164	0.54	49.5	129.5	2.16	1.012	0.36	-0.01	2.28	4.33	0.04	-79.8	-2.5	-1.13	-18.8	-2.0	0.007	0.096	49	1364
75173	0.10	-90.5	-256.0	-7.06	-0.432	0.25	0.14	7.17	-40.00*	-0.44*	-364.4	-7.1	-3.46	11.2	0.1	-0.008	0.296	-131	-2466
75180	1.65*	36.8	244.8	-0.95	-1.765	0.03	0.01	-1.05	20.00	0.26	526.5	7.4	-0.35	185.7	177.8	0.001	-0.082	4	1304
ICMB 841	-0.67	220.7	355.5	5.27	-2.321	-0.86	-0.04	-6.16	14.89	-0.18	608.8	12.0	4.09	190.1	149.6	-0.015	0.350	111	3115
863B	-3.56**	208.0	387.6	0.38	9.679*	-1.52	0.72*8	0.51	-30.00	-0.26	404.6	12.4	2.09	32.3	-86.9	-0.051**	1.290**	-28	-2824
H 77/833-2	1.45**	197.3**	113.5	6.21**	8.031**	-1.13**	-0.16**	6.30**	23.30**	0.12	367.1*	4.7	2.64*	305.9**	267.8**	-0.007	0.074	94*	4399**
PPMI 301	-1.08**	90.9	31.7	3.12	-3.802*	2.01**	0.21**	-3.88	-36.90**	-0.25**	-199.3	-2.2	3.57**	-241**	-235.9**	-0.009*	0.951**	74	-578
RIB 3135-18	-0.36	-288.3**	-145.2	-9.34**	-4.228**	-0.87*	-0.05	-2.42	13.61	0.14	-167.9	-2.5	-6.22**	-64.9	-31.9	0.016**	-1.024**	-167**	-3822**

** Significant at 0.01 level of probability, * Significant at 0.05 level of probability

index and 1000 grain mass whereas it expressed significant negative *gca* effects for flowering time, plant height, panicle number, tiller number, fresh stover yield, dry stover yield and stover dry matter fraction. Tester H 77/833-2 showed positive results for most of the observed traits except for panicle length, panicle diameter and stover dry matter fraction.

4.2.2.5.3. Genotype x Environment interaction analysis

The results of analyses of variance and general combining ability for the grain yield and stover yield and its related traits across two environments (Genotype x Environment interaction) are explained below.

4.2.2.5.3(A). Analyses of variance

Analysis of variance of LG5 across two moisture regimes is presented in Table 43. Significant differences were observed for most of the traits except for flowering time and panicle number due to different moisture treatments. Hybrids were highly significant for all observed characters. In case of environment x hybrid effects, there was clear significant difference for traits, panicle harvest index, tiller number, grain harvest index, fresh stover yield, dry stover yield, stover dry matter fraction, grain mass and grain number. As expected, all the three testers used for testcross hybrid development were significantly different for all observed agronomic characters and similarly, lines were also observed as significantly different for all traits. The environment x line interaction was significant for panicle yield, panicle number, fresh stover yield, stover dry matter fraction and grain number per panicle. The environment x tester effect had significant differences for panicle harvest index, grain harvest index, fresh stover yield, dry stover yield, stover dry matter fraction, grain mass and grain number. Due to line x tester, significant difference was observed for many traits except for plant height, biomass yield, fresh and dry stover yield and stover dry matter fraction. The effect due to interaction among environment x line x tester, was significant only for grain mass and grain number.

Table 43 : Analyses of variance for testcross hybrids of LG5 over two moisture regimes

Source of Variation	df	Time to		Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Grain Yield (kg/ha)							
Environments(Env)	1	0.46	1437.2**	965.44**	176.99**	72.26**	139.35**	15.45**	4.02	8.41**
Hybrids	53	6.63**	8.65**	9.33**	4.77**	20.02**	26.61**	1.49*	4.45**	3.21**
Lines (L)	17	9.17**	6.55**	4.59**	3.26**	8.67**	38.96**	0.49	1.96*	2.42**
Testers (T)	2	63.69**	135.41**	177.3**	77.23**	413.63**	341.81**	8.73**	66.09**	21.49**
L × T	34	2.01**	2.24**	1.49*	1.26	2.54**	1.9**	1.56*	2.07**	2.53**
Env × Lines	17	0.87	1.17	1.68*	1.43	0.92	1.1	0.92	1.85*	1.64
Env × Testers	2	0.8	0.42	2.13	1.33	1.16	1.56	0.44	0.06	0.24
Env × Hybrids	53	0.86	1.18	1.23	1.36	1.01	1.27	1.34	1.39	1.46*
Env × L × T	34	0.86	1.23	0.95	1.33	1.05	1.33	1.6*	1.23	1.44
Error df		212	189	198	208	210	206	206	201	195

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
Hybrids	53	4.74**	4.07**	8.18**	5.99**	7.4**	2.25**	35.58**	3.33**	5.6**
Lines (L)	17	3.49**	4.59**	5.69**	4.57**	2.24**	3.8**	25.75**	2.92**	2.17**
Testers (T)	2	71.83**	39.11**	139.47**	97.82**	152.79**	4.72**	675.3**	19.68**	97.76**
L × T	34	1.42	1.76**	1.70*	1.29	1.43	1.33	2.86**	2.58**	1.9**
Env × Lines	17	1.2	1.14	1.35	1.77*	1.27	2.11**	1.08	1.9*	1.18
Env × Testers	2	3.03	2.01	31.36**	7.28**	8.64**	5.11**	35.91**	10.64**	30.26**
Env × Hybrids	53	1.23	1.23	2.32**	1.67**	1.64**	1.81**	2.73**	2.14**	2.48**
Env × L × T	34	1.14	1.23	1.1	1.29	1.42	1.47	1.6*	1.75**	1.49*
Error df		181	181	174	195	191	207	210	186	187

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

4.2.2.5.3(B). General combining Ability (ga)

Estimates of *gca* effects of lines and testers for LG5 across two moisture regimes are presented in Table 44. For flowering time, the best negative combiners among lines were 75035, 75094, 75159 and donor parent 863B and there were six lines 75064, 75076, 75132, 75126, 75134 and 75180 with significant positive *gca*. Among testers, only PPMI 301 showed significant negative *gca* effect for flowering time. There were two lines 75180 and donor parent exhibited significant positive *gca* effect and four lines 75048, 75094, 75126 and 75134 showed significant negative *gca* effect for grain yield. In case of panicle yield, only donor parent showed significant positive *gca* value whereas two lines 75048 and 75134 were significantly negative. For panicle harvest index, lines 75173 and 863B found to have significant positive *gca* and four lines 75094, 75126, 75134 and 75146 had significant negative *gca*. There were three lines 75073, 75094 and donor parent exhibiting significant positive *gca* value and there were three lines 75159, 75156 and 75180 exhibiting significant negative *gca* value for plant height. In case of panicle length, both recurrent and donor parents showed significant negative *gca* effects, anyhow recurrent parent was found to have better panicle length than that of donor parent. Three lines 75048, 75073 and 75094 showed significant positive *gca* and two lines 75126 and 75159 exhibited significant negative *gca* for panicle length. It was also noticed that many lines (14 lines) maintained better panicle length than that of recurrent parent. For the trait panicle diameter, line 75173 and 863B found to have significant positive *gca* effects and eight lines 75035, 75048, 75064, 75073, 75132, 75134, 75164 and 75180 with significant negative *gca*. There were two lines 75035 and 75048 obtained significant positive *gca* and only one line 75076 found to have significant negative effects for panicle number. In case of tiller number, only one line 75048 was significantly positive and two lines 75026 and 75133 were significantly negative. For biomass yield and vegetative growth index, significant positive *gca* effects were not observed in any line except donor parents. Four lines 75035, 75048, 75134 and 75159 for panicle number and one line 75134 for tiller number had significant negative *gca* effects. In case of grain harvest index, two lines 75173 and donor parent showed significant positive effect whereas five lines 75094, 75126, 75134, 75146 and 75164 showed significant negative value. For fresh stover yield, four lines 75132, 75164, 75180 including donor parent significantly negative. There was only one line 75132 with significant positive *gca* and there were two lines 75035 and 75159 with significant negative values for dry stover yield. In case of stover dry matter fraction, four lines 75035, 75048, 75064 and 75073 were found to have significant

Table 44 : Estimates of *gca* effects of lines and testers of LG5 over two moisture regimes

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
75035	-1.11**	-104.3	-101.1	-1.41	-2.87	0.34	-0.06*	0.46	21.29**	0.11	-291.6*	-3.7	0.51	-387.7**	-198.9**	0.033**	-0.238*	-115*	-1045
75048	-0.34	-170.2**	-247.3**	-1.30	-0.91	0.95**	-0.19**	-4.75	19.46*	0.31*	-300.4*	-4.4	-1.27	-219.4*	-64.3	0.031*	-0.813**	-38	435
75064	1.05**	-20.6	-78.4	0.82	1.39	0.51	-0.06*	0.62	13.27	0.16	-31.5	-2.6	-0.16	-98.3	41.0	0.035**	-0.216*	-38	1041
75073	0.22	47.8	-93.7	2.57	3.96*	0.62*	-0.06*	-0.38	16.07	0.20	-215.6	-4.3	1.60	-173.5	-73.8	0.026*	0.004*	-71	286
75076	1.16**	-27.8	-70.1	0.85	1.52	0.00	0.02	0.56	-24.77**	-0.30*	-42.6	-2.4	-0.03	15.6	63.3	0.004	-0.216*	198**	1547
75094	-0.78*	-144.3*	-97.8	-3.12*	3.74*	1.89**	0.03	-3.66	0.10	0.11	-174.2	-1.9	-2.04*	-146.2	-58.9	0.016	-0.016	-94*	-2349*
75132	1.05**	30.5	86.3	-1.11	-0.42	-0.38	-0.05*	0.01	-5.10	-0.05	244.1	2.8	-1.18	247.8**	143.1*	-0.014	-0.268**	56	993
75133	0.00	78.2	65.0	0.69	-0.75	0.29	0.01	1.84	-14.14	-0.21*	58.5	0.9	0.34	55.0	40.3	0.000	0.162	52	299
75126	1.33**	-129.3*	27.7	-3.44**	-1.37	-0.78**	-0.01	4.18	2.01	-0.09	62.4	-0.8	-2.19*	51.9	-1.5	-0.021	-0.318**	-25	-419
75134	1.05**	-170.5**	-172.9*	-3.46**	-0.37	-0.16	-0.07*	-0.38	-9.82	-0.06	-279.3*	-6.4**	-2.27**	-111.5	-80.5	0.004	0.040	-99*	-3678**
75146	0.33	-75.6	31.9	-2.8*	2.08	-0.27	0.00	-2.16	-8.82	0.00	80.4	0.4	-1.90*	93.9	46.3	-0.008	-0.140	-6	-755
75159	-1.61**	-94.2	-125.2	-1.27	-3.64*	-0.60*	-0.02	-1.31	4.40	0.10	-285.8*	-3.0	-0.05	-284.4**	-167.8**	0.022	-0.045	-26	-1286
75156	-0.56	16.2	1.0	0.79	-3.70*	0.12	-0.03	-0.32	7.73	0.10	53.0	1.5	0.17	89.6	40.2	-0.006	-0.113	-27	712
75164	0.55	-29.1	20.0	-1.17	-2.31	0.01	-0.06*	0.62	-3.38	-0.07	56.8	-0.2	-1.97*	200.5*	81.6	-0.021	0.069	-30	-842
75173	0.00	113.0	95.6	2.75*	-1.42	0.29	0.06*	2.28	-12.93	-0.20	122.6	2.0	1.90*	122.4	24.8	-0.022	0.297**	103*	865
75180	1.05*	121.6*	152.7	0.98	-3.70*	-0.32	-0.07*	-1.93	8.12	0.14	174.7	1.3	0.72	246.5**	61.5	-0.038**	-0.194	61	2823**
ICMB 841	0.00	83.5	126.3	1.22	1.19	-1.04**	-0.05	3.13	2.12	-0.05	174.9	3.4	0.68	6.8	7.4	-0.001	0.145	22	773
863B	-3.39**	475*8	379.7**	8.40**	7.57*8	-1.48**	0.65*	1.18	-15.62	-0.20	593.7**	17.3**	7.16**	291.1**	96.2	-0.041**	1.861**	77	601
H 77/833-2	1.37**	220.5**	152**	5.02**	6.75**	-1.45**	-0.17**	4.21**	26.33**	0.14**	497.2**	6.8**	1.33**	416.6**	341.2**	0.005	-0.187**	76**	4661**
PPMI 301	-1.17**	97.3**	37.1	3.30**	-4.90**	2.49**	0.22**	-3.32*	-33.78**	-0.23**	-219.4**	-2.0*	3.66**	-270**	-246.5**	0.012*	1.176**	16	-3114**
RIB 3135-18	-0.20	-317.9**	-189.1**	-8.32**	-1.84**	-1.04**	-0.04**	-0.89	7.44*	0.09*	-277.8**	-4.8*	-4.99**	-146.7**	-94.7**	0.007	-0.980**	-91**	-1547**

** Significant at 0.01 level of probability, * Significant at 0.05 level of probability

positive *gca* effect and two lines 75180 and donor parent were observed as significantly negative. There were two lines 75173 and donor parent with significant positive *gca* and six lines 75035, 75048, 75064, 75076, 75132 and 75126 with significant negative *gca* for 1000 grain mass. In case of grain number per panicle, two lines 75076 and 75173 showed significant positive and three lines 75035, 75094 and 75134 showed significant negative *gca* effect. There was only one line 75180 with significant positive *gca* value whereas two lines 75094 and 75134 with significant negative value for grain number per m².

Among three testers, RIB 3135-18 showed negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction. Tester H 77/833-2 showed positive results for most of the observed traits except for panicle length, panicle diameter and 1000 grain mass whereas PPMI 301 showed significant positive *gca* effects for grain yield, panicle harvest index, panicle length, panicle diameter, grain harvest index, stover dry matter fraction and 1000 grain mass whereas it expressed significant negative *gca* effects for flowering time, plant height, panicle number, tiller number, biomass yield, vegetative growth index, fresh stover yield, dry stover yield and grain number per m².

4.2.2.6. Linkage Group 6 (LG6)

A total 42 testcross hybrids (involving three testers) of 10 homozygous segmental introgression lines and a sampling of 2 recurrent parent homozygotes (to serve as near-isogenic controls) of LG6, which were selected based on marker genotyping of 102 plants at 9 marker loci of LG6 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in three environments *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions during summer 2010 at ICRISAT, Patancheru. Line x tester analysis was performed and analysis of variance and general combining ability (*gca*) effects were calculated. The results of analysis of variance and general combining ability (*gca*) for grain and stover yield related characters for each of three moisture regimes and across these three moisture regimes are presented below.

4.2.2.6.1. Fully irrigated control treatment

4.2.2.6.1(A). Analysis of variance

The analysis of variance for LG6 under fully irrigated control treatment during summer 2010 is presented in Table 45. Hybrids showed highly significant differences for all characters except for biomass yield and dry stover yield. There were clear significant differences between lines for grain yield and its related traits *viz.*, time to 75% flowering, panicle yield, panicle harvest index, plant height, panicle diameter, panicle number, 1000 grain mass and grain number. But results were non-significant for panicle length, tiller number, biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and stover dry matter fraction. As expected, effects of testers were highly significant for all observed agronomic characters but not for biomass yield, vegetative growth index, fresh stover yield and dry stover yield. Lines x tester effects were significant for flowering time, plant height, panicle diameter and 1000 grain mass.

4.2.2.6.1(B). General Combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG6 in fully irrigated non- stress conditions during summer 2010 are presented in Table 46. For flowering time, the best negative combiners among lines were a recurrent parent homozygous line (RPHL) 86083 and donor parent 863B and line 86053 showed significant positive *gca*. Among testers RIB 3135-18 and PPMI 301 showed negative *gca* effects for flowering time. For grain yield, CSSL 86053 and donor parent 863B were significantly positive whereas CSSLs 86025, 86083 and recurrent parent showed significantly negative *gca*. Six CSSLs (86019, 86027, 86053, 86064, 86088 and 86096) out of 10 exhibited better grain yield *gca* than that of recurrent parent. CSSL 86053 and donor parent expressed significant positive *gca* and RPHL 86083 had significant negative *gca* for panicle yield. Four CSSLs (86019, 86027, 86053 and 86096) out of 10 exhibited better panicle yield *gca* than that of recurrent parent. There were no lines with significant positive *gca* except donor parent for panicle harvest index and there was only one RPHL 86083 with significant negative *gca* for this trait. Four CSSLs (86019, 86053, 86064 and 86096) and one RPHL 86015 exhibited better *gca* for panicle harvest index than that of recurrent parent. In case of plant height, CSSL 86053 and donor parent 863B showed significant positive *gca* effects whereas two CSSLs 86019 and 86042 and one RPHL 86083

Table 45 : Analyses of variance for testcross hybrids of LG6 in fully irrigated non-stress conditions

Source of Variation	df	Time to									
		75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Replication	2	8.64**	11.07**	1.08	10.41**	29.13**	0.24	29.27**	1.83	0.39	0.44
Hybrids	41	123838.5**	4.71**	4.45**	24173780.14**	911.26**	4197.92**	114676.85**	4.62**	2.9**	15602.23**
Lines (L)	13	7.2**	3.43**	2.4**	2.05*	8.78**	1.55	18.69**	0.98	2.36**	1.5
Testers (T)	2	14.85**	60.18**	14.87**	49.17**	18.05**	75.48**	56.7**	2.22	23.98**	17.48**
L × T	26	2.61**	1.07	1.02	1.15	2.07**	1.13	1.77*	1.12	1.29	1.41
Error	82										

Source of Variation	df	Biomass		Vegetative		Dry		Stover		Grain	
		Yield (kg/ha)	Yield (kg/ha)	Index (kg/ha/d)	Index (%)	Fresh Stover Yield (kg/ha)	Stover Yield (kg/ha)	Matter Fraction	1000-Grain Mass (g)	Number/Panicle	Number/m ²
Replication	2	0.6	0.6	0.36	1.41	1.97	1.49	4.23*	7.92**	6.16**	5.99**
Hybrids	41	0.68	589.14**	20694311.89**	31007.51**	0.98	29802922.27**	333940.32**	3.25**	3.03**	3.03**
Lines (L)	13	0.56	0.56	0.36	0.76	0.83	0.84	22.21**	2.6**	2.82**	2.82**
Testers (T)	2	0.17	0.17	9.33**	2.22	0.35	9.22**	362.78**	27.18**	27.64**	27.64**
L × T	26	0.78	0.8	0.83	0.66	1.03	1.22	2.14**	1.05	1.23	1.23
Error	82										

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 46 : Estimates of *gca* effects of lines and testers of LG6 in fully irrigated non-stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/ m ²
86014	-0.48	-117.9	-5.2	-2.37	-3.03	0.15	-0.11	1.91	-1.74	-0.05	387.5	9.0	1.81	755.4	100.3	-0.042	-0.427**	-17	-21
86015	0.18	-38.0	-150.2	1.41	-2.03	-1.18*	-0.17**	3.79	11.04	0.02	-928.8	-18.5	0.92	-1096.8	-569.2*	-0.017	-0.237	-43	194
86019	-0.26	127.9	174.5	0.74	-6.92**	-0.52	-0.03	-1.76	16.15	0.12	739.1	15.4	3.14	142.1	272.2	0.047	-0.444**	66	1346
86025	0.18	-400.4*	-192.9	-5.93	0.97	0.60	0.21**	-6.65	-0.07	0.13	233.2	4.2	-1.30	219.8	133.4	0.019	0.034	-162*	-2075*
86027	-0.04	132.3	141.0	0.52	-1.14	-0.29	0.15**	5.57	48.10**	0.19	-870.6	-17.3	-5.97	-446.8	-313.4	-0.014	-0.458**	-59	1224
86042	-0.04	-54.4	-76.4	0.07	-3.92*	0.04	-0.22**	-9.65	-3.63	0.10	-221.0	-4.3	-2.52	92.1	19.6	0.006	-0.363*	14	364
86052	-0.26	-175.8	-77.5	-2.26	0.97	-0.18	0.18**	3.13	12.60	0.01	342.0	7.5	0.92	483.2	127.2	-0.017	0.528**	-117	-1126
86053	2.29**	445.3*	296*	6.85	5.30**	0.04	0.15**	-7.32	-24.07	-0.09	315.4	0.1	0.03	388.7	302.9	-0.026	0.102	239**	1941*
86064	0.29	183.0	-114.5	6.52	-0.92	-0.18	-0.17**	4.46	2.48	-0.08	-190.9	-5.0	0.92	624.3	103.9	-0.037	-0.407*	162*	1550
86083	-0.82**	-623.2**	-460.1**	-9.71**	-9.58**	-0.63	-0.26**	4.46	-5.96	-0.11	-403.9	-5.9	-2.52	-645.7	-236.2	0.023	-0.148	-232**	-2856**
86088	0.18	49.6	80.6	-0.37	2.86	1.48**	0.01	-5.98	6.82	0.12	719.3	13.6	1.92	436.5	346.4	0.026	-0.206	0	434
86096	-0.04	288.3	234.8	3.29	3.75	0.15	-0.10**	7.46	30.15	0.10	-155.7	-2.5	-0.30	-435.7	-78.2	0.023	0.113	-18	1128
ICMB 841	-0.04	-362.1*	-194.8	-6.37	-0.81	-0.29	-0.18**	-6.21	-16.52	0.07	41.1	0.9	-0.41	-201.3	-56.5	0.021	-0.508**	-18	-1288
863B	-1.15**	545.3**	344.7*	7.63*	14.52**	0.82	0.64**	6.79	-75.40**	-0.52**	-6.7	2.9	3.37	-315.7	-152.5	-0.012	2.420**	186*	-815
H 77/833-2	0.60**	281.1**	109.8	5.45**	2.12*	-1.41**	-0.18**	3.16	28.90**	0.13*	44.6	-0.7	1.30	203.3	88.18	-0.005	-0.486**	98**	2187**
PPMI 301	-0.29*	441.6**	185.2**	7.91**	2.31**	2.43**	0.21**	1.59	-47.80**	-0.32**	101.6	2.9	5.08**	285.7	-45.89	-0.038**	1.593**	128**	-202
RIB 3135-18	-0.31*	-722.7**	-295.1**	-13.36**	-4.43**	-1.02**	-0.03	-4.75	18.90*	0.18**	-146.2	-2.2	-6.38**	-489.0	-42.29	0.044**	-1.107**	-226**	-1985**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

had significant negative *gca*. For panicle length, there was one CSSL 86088 with significant positive *gca* and also only one RPHL 86015 with significant negative *gca*. There were four CSSLs (86025, 86027, 86052, and 86053) and donor parent exhibited significant positive *gca* whereas three CSSLs (86042, 86064 and 86096), two RPHLs (86015 and 86083) and recurrent parent showed significant negative *gca* for panicle diameter. There was only one CSSL 86027 found to have significant positive *gca* for panicle number and there were no lines with significant *gca* effects for tiller number but donor parent showed significant negative *gca* effects for both these traits. There were no significant *gca* effects noticed among lines including both the parents for biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and stover dry matter fraction but there was only one RPHL 86015 had significant negative *gca* for dry stover yield. For 1000 grain mass, two lines 86052 and donor parent 863B had significant positive *gca* but six CSSLs (86014, 86019, 86027, 86042 and 86064) including recurrent parent showed negative *gca* results. For grain number per panicle, two CSSLs 86053, 86064 and donor parent exhibited significant positive *gca* effects whereas line 86053 showed similar results for grain number per m² also. Lines 86025 and 86083 expressed significant negative *gca* effects for both grain number per panicle and grain number per m². It was observed that six CSSLs (86019, 86027, 86053, 86064, 86088 and 86096) exhibited better *gca* effects for grain number per m² when compared with ICMB 841.

Among testers, RIB 3135-18 showed negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction where as PPMI 301 showed exactly with opposite effects (positive *gca*) of RIB 3135-18 except for flowering time for which it also showed significant negative *gca* like RIB 3135-18. Tester H 77/833-2 exhibited positive *gca* effects for all observed traits except for panicle length, panicle diameter and 1000 grain mass.

4.2.2.6.2. Early drought stress treatment

4.2.2.6.2(A). Analysis of variance

The analysis of variance for LG6 under early onset terminal drought stress conditions during summer 2010 are presented in Table 47. Hybrids showed highly significant differences for all

Table 47 : Analyses of variance for testcross hybrids of LG6 in early-onset terminal drought stress conditions

Source of Variation	df	Time to									
		75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Replication	2	2.82	14.24**	16.24**	10.3**	2.89	0.4	11.22**	0.8	1.05	2.34
Hybrids	41	74262.51**	4.06**	2.97**	16678044.62**	415.21**	3581.97**	50396.27**	3.22**	3.08**	26650.91**
Lines (L)	13	6.75**	1.99*	1.91*	2.36**	6.43**	2.21*	9.04**	1.42	2.15**	2.16*
Testers (T)	2	18.25**	58.04**	11.66**	74.68**	15.31**	80.21**	40.52**	0.52	31.71**	26.07**
L × T	26	2.13**	0.94	1.03	1.18	1.06	0.87	0.71	1.34	1.18	1.64*
Error	82										

Source of Variation	df	Biomass		Vegetative		Dry		Grain		
		Yield (kg/ha)	Yield Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
Replication	2	2.6	3	9.77**	23.61**	3.45*	107.73**	8.33**	9.49**	11.1**
Hybrids	41	1.03	856.83**	20500600.34**	47105.17**	1.85**	21939258.15**	41670.82**	4.44**	3.07**
Lines (L)	13	0.96	0.96	2.03*	1.19	1.2	0.5	2.6**	2.15*	1.58
Testers (T)	2	1.27	0.55	54.06**	20.14**	11.78**	38.76**	43.39**	58.76**	35.12**
L × T	26	1.04	1.07	0.65	1.18	1.33	0.75	0.52	0.96	1.32
Error	82									

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

characters except for biomass yield. There were clear significant differences between lines for grain yield related traits but non-significant for biomass yield, vegetative growth index, fresh stover yield, dry stover yield and grain number per m². As expected, effects of testers were highly significant for all observed agronomic characters but not for biomass yield and vegetative growth index. Lines x tester effects were significant only for flowering time and tiller number.

4.2.2.6.2(B). General Combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG6 in early-onset drought stress conditions during summer 2010 are presented in Table 48. For flowering time, the best negative combiners among lines were a RPHL 86083 and donor parent 863B and CSSL 86053 showed significant positive *gca*. Among testers RIB 3135-18 followed by PPMI 301 showed significant negative *gca* effects for flowering time. For grain yield, CSSLs 86025, 86053 and donor parent 863B exhibited better *gca* effects whereas other lines did not show any significant results. CSSL 86025 and donor parent expressed significant positive *gca* and CSSL 86064 had significant negative *gca* for panicle yield. There were no lines with significant *gca* effects except donor parent which was significantly positive for panicle harvest index. In case of plant height, CSSLs 86053, 86088 and donor parent 863B showed significant positive *gca* effects whereas only one RPHL 86083 had significant negative *gca*. For panicle length, there was one CSSL 86088 with significant positive *gca* unlike donor parent which had significant negative *gca*. There were four CSSLs (86019, 86025, 86052, and 86053) and donor parent 863B exhibited better *gca* whereas three CSSLs (86015, 86064 and 86096) and one RPHL 86083 showed worse *gca* effects when compared with that of recurrent parent for panicle diameter. There was only one RPHL 86083 found to have significant positive *gca* for panicle number and tiller number unlike donor parent, which had significant negative *gca* for these traits. One CSSL 86014 also showed significant negative *gca* for tiller number. For biomass and vegetative growth index, there were no lines with significant positive *gca* effect but there was only one CSSL 86014 with significant negative *gca*. In case of grain harvest index, CSSL 86025 and donor parent 863B had significant positive *gca* results. For fresh stover yield and dry stover yield, line 86053 exhibited significant positive effects and CSSL 86014 was significantly negative for dry stover yield. There were no significant *gca* effects observed in lines for stover dry matter fraction. For

Table 48 : Estimates of *gca* effects of lines and testers of LG6 in early-onset terminal drought stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number m ²
86014	0.08	-2.5	1.0	-0.79	-0.95	0.08	-0.13	17.50**	-26.41	-0.30*	-662.9*	-13.4*	-3.60	-724.6**	-247.6	0.008	-0.320	77	90
86015	-0.03	-232.8	-266.4	-5.13	-4.29	0.08	-0.17*	7.02	-18.19	-0.16	-279.0	-5.4	-3.05	-133.5	-67.3	0.016	-0.442	-75	-1129
86019	-0.03	-82.2	-47.6	-3.68	-1.62	-0.59	0.16*	-2.20	5.14	0.05	-89.2	-1.6	-1.16	-43.5	-95.8	-0.020	-0.286	-42	-297
86025	-0.25	265.5*	315.8*	5.54	-3.62	0.75	0.18**	1.58	-20.08	-0.15	261.3	5.6	4.73*	97.6	-109.0	-0.036	0.200	19	1787*
86027	-0.03	-57.3	-79.8	-0.24	-3.84	-0.37	0.06	5.36	4.48	-0.03	29.5	0.6	-0.60	17.6	54.9	0.002	-0.451	19	596
86042	-0.25	-172.3	73.2	-6.02	-0.95	-0.25	-0.13*	2.80	13.92	0.04	178.6	4.0	-2.71	-71.3	50.9	0.017	-0.170	-114	-1026
86052	-0.03	1.9	13.3	0.43	-1.06	0.75	0.17*	-2.09	11.37	0.06	44.3	0.8	1.06	-3.5	-23.6	-0.005	0.032	-2	147
86053	2.07**	153.7	-38.2	6.10	8.27**	0.41	0.12	-15.10*	-23.30	0.02	468.5	5.0	1.73	661*	452**	0.008	-0.181	165*	1622*
86064	0.52	-21.8	-408.9**	5.76	2.27	-0.25	-0.20**	0.80	12.14	0.05	-379.8	-8.1	1.73	-109.0	-25.4	-0.001	-0.124	7	319
86083	-0.92**	-4.8	-36.6	1.65	-10.84**	-0.70	-0.22**	-2.87	45.20*	0.34**	-112.9	-0.5	1.40	-37.9	-130.8	-0.031	0.543	-92	-483
86088	-0.03	3.1	103.1	-1.91	5.159*	1.30**	-0.11	-0.20	33.03	0.18	212.5	4.3	-0.38	36.5	55.2	0.013	-0.183	-25	33
86096	0.19	-141.8	-28.5	-6.46	2.94	-0.14	-0.18**	-0.31	26.14	0.15	-70.1	-1.9	-4.16	354.3	195.8	0.023	-0.139	-135	-1040
ICMB 841	0.08	-143.9	72.1	-5.13	-3.29	0.19	-0.02	-1.42	12.81	0.12	81.5	1.4	-2.16	-115.7	-45.0	0.013	-0.156	-70	-602
863B	-1.36**	435.3**	327.5*	9.87**	11.82**	-1.25**	0.49**	-10.87	-76.30**	-0.36**	317.6	9.2	7.17**	72.1	-64.4	-0.007	1.675**	267**	-15
H 77/833-2	0.66**	-10.3	-123.3	1.82	2.76**	-1.20**	-0.15**	0.67	5.13	0.04	183.4	2.4	5.90,2**	590.2**	252.1**	-0.029*	-0.160	-17	446
PPM1 301	-0.30*	454.1**	253.1**	12.5**	1.95	2.12**	0.22**	-2.41	-57.00**	-0.32**	-87.1	-1.2	7.73**	-45.5	-231.2**	-0.054*	1.104**	274**	1690**
RIB 3135-18	-0.35**	-443.8**	-129.8*	-14.32**	-4.71**	-0.92**	-0.07*	1.74	51.90**	0.28**	-96.3	-1.2	-7.73**	-544.8**	-20.9	0.084**	-0.943**	-258**	-2136**

** Significant at 0.01 level of probability. * Significant at 0.05 level of probability

1000 grain mass, only donor parent 863B had significant positive *gca* and for grain number per panicle, CSSL 86053 and donor parent exhibited significant positive *gca* effects. Two CSSLs 86025 and 86053 expressed significant positive *gca* effects for grain number per m².

Among testers, RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction whereas PPMI 301 showed exactly opposite effects (positive *gca*) of RIB 3135-18 except for flowering time and dry stover yield for which it showed negative *gca* effects like RIB 3135-18. Tester H 77/833-2 exhibited significant positive *gca* effects for flowering time, plant height, fresh stover yield and dry stover yield. It showed significant negative *gca* effects for panicle length, panicle diameter and stover dry matter fraction.

4.2.2.6.3. Late drought stress treatment

4.2.2.6.3(A). Analysis of variance

The analysis of variance for LG6 under late-onset terminal drought stress conditions during summer 2010 is presented in Table 49. Hybrids showed highly significant differences for all characters except for biomass yield and dry stover yield. There were clear significant difference between lines for grain yield, panicle yield, time to 75% flowering, plant height, panicle length, panicle diameter, panicle number, tiller number and 1000 grain mass but non-significant for panicle harvest index, biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield, stover dry matter fraction and grain number. Effects of testers were highly significant for all observed agronomic characters but not for biomass yield and vegetative growth index. Lines x tester effects were significant only for panicle length.

4.2.2.6.3(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG6 in late-onset terminal drought stress conditions, during summer 2010 are presented in Table 50. For flowering time, the best negative combiners among lines were a RPHL 86083 and donor parent 863B and one CSSL

Table 49 : Analyses of variance for testcross hybrids of LG6 in late-onset terminal drought stress conditions

Source of Variation	df	Time to									
		75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Replication	2	0.25	6.46**	8.66**	0.52	10.28**	2.37	59.82**	4.04*	1.61	0.83
Hybrids	41	73598.66**	4.81**	3.21**	27372971.37**	642.62**	7117.37**	138171.07**	3.61**	1.84**	12490.81**
Lines (L)	13	4.34**	1.86*	3.29**	0.65	7.58**	2.58**	21.56**	0.92	1.87*	1.87*
Testers (T)	2	9.94**	74.5**	3.73*	56.46**	13.17**	103.63**	96.79**	1.1	15.05**	10.74**
L × T	26	1.34	0.92	1.44	1.01	1.5	1.96*	1.41	1.11	0.6	0.93
Error	82										

Source of Variation	df	Vegetative									
		Biomass Yield (kg/ha)	Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²	
Replication	2	3.28*	3.11	2.33	18.52**	3.16*	49.94**	3.14*	0.9	4.52*	
Hybrids	41	1	873.54**	34899418.53**	91687.43**	1.12	29973304.53**	354466.61**	1.8*	2.16**	
Lines (L)	13	1.42	1.47	0.93	1.62	1.08	1.58	21.57**	0.75	1.64	
Testers (T)	2	1.44	1.06	12.1**	12.21**	4.15*	40.25**	340.7**	15.5**	19.35**	
L × T	26	0.76	0.78	0.95	0.71	0.78	0.93	1.91*	0.51	1.08	
Error	82										

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 50 : Estimates of *gca* effects of lines and testers of LG6 in late-onset terminal drought stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
860014	-0.12	-157.8	-66.5	-2.62	1.40	0.40	-0.10*	0.83	2.00	-0.02	-331.3	-6.4	-3.06	-407.0	-138.5	-0.004	-0.318*	-23	-474
860015	-0.23	92.7	147.1	1.60	-3.83	-0.48	-0.13**	0.72	28.00	0.19	664.5	13.6	4.61	84.1	165.3	0.028	-0.133	-18	800
860019	-0.01	30.5	67.4	0.94	-3.38	-0.37	0.10	1.28	17.56	0.07	47.6	0.7	0.06	-83.7	-7.6	0.044	-0.526**	22	1167
860025	-0.01	122.0	341*	-0.51	1.62	0.63	0.21**	10.17	6.33	-0.12	1043.4	20.6	3.17	685.2	350.4	-0.031	0.098	5	443
860027	-0.01	-120.9	-346.9*	2.60	-6.38**	-0.81*	0.10*	6.83	-16.33	-0.19	-567.8	-11.3	1.39	-500.3	-168.2	0.016	-0.078	-29	-519
860042	-0.56	-101.5	-93.6	-0.62	-5.71*	0.18	-0.14**	10.06	11.89	-0.04	327.2	7.8	3.06	26.3	68.4	0.001	-0.350*	-63	-84
860052	0.10	79.1	-464.7**	-3.73	-4.27	-0.26	0.10*	2.06	-16.44	-0.12	-1008.1	-20.3	-2.17	-392.5	-202.2	-0.009	0.167	86	142
860053	1.99**	92.6	210.5	0.83	6.39**	0.52	0.09	-5.50	11.33	0.18	653.7	8.1	-2.06	599.7	471.8	0.021	-0.293	46	1070
860064	0.10	71.2	-101.2	4.60	-0.60	-0.81*	-0.24**	-10.94	34.89	0.37**	-678.4	-12.7	-2.50	141.9	-192.6	-0.051	-0.337*	3	1045
860083	-0.89*	-231.5	-287.6	-1.29	-8.71**	-0.37	-0.23**	1.61	-39.22	-0.28	-1254.1	-23.5	-3.61	-1033.7**	-563.6*	0.025	-0.057	-13	-1268
860088	0.33	-122.2	63.8	-3.29	4.50*	1.40**	-0.10*	-1.72	32.22	0.22	686.9	12.7	0.94	184.1	271.4	0.030	-0.379*	-103	-328
860096	0.66	-161.5	-142.4	-1.29	2.40	0.29	-0.11*	0.83	2.11	0.00	-701.7	-15.0	-6.50	-311.4	-175.7	0.040	0.172	-105	-1109
ICMB 841	-0.12	-73.9	-20.2	-1.40	1.84	0.07	-0.14*	-7.17	-11.56	0.02	-241.5	-4.4	-0.61	-114.8	-193.3	-0.016	-0.464**	35	186
863B	-1.23**	481.1**	693.3**	4.16	14.73**	-0.37	0.64**	-9.06	-62.70**	-0.28*	1359.7*	30.1*	7.28	1121.9**	314.3	-0.094**	2.499**	155	-1072
H 77/833-2	0.60**	105.7	92.8	3.03*	1.96	-1.27**	-0.18**	3.75	24.20**	0.10	425.0	7.1	0.68	643**	280.7*	-0.022	-0.311**	36	1243**
PPMI 301	-0.27	469.9**	75.0	9.36**	2.34*	2.07**	0.25**	-1.35	-41.40**	-0.24**	-287.3	-5.0	5.79**	97.8	-229.5	-0.077**	1.517**	125**	157
RIB 3135-18	-0.32*	-575.6**	-167.8*	-12.39**	-4.30**	-0.80**	-0.07**	-2.40	17.20	0.14*	-137.7	-2.0	-6.46**	-740.8*8	-51.2	0.099**	-1.206**	-160**	-1400**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

86053 showed significant positive *gca*. Among testers, RIB 3135-18 showed significant negative *gca* effects for flowering time. For grain yield, only donor parent 863B exhibited significant positive *gca* effects whereas other lines did not show any significant results. One CSSL 86025 and donor parent expressed significant positive *gca* and CSSLs 86027 and 86052 had significant negative *gca* for panicle yield. There were no lines with significant *gca* effects for panicle harvest index. In case of plant height, CSSLs 86053, 86088 and donor parent 863B showed significant positive *gca* effects whereas two CSSLs (86027 and 86042) and one RPHL 86083 had significant negative *gca*. For panicle length, there was one CSSL 86088 with significant positive *gca* and lines 86027 and 86064 with significant negative *gca*. For panicle diameter, there were four lines (86025, 86027, 86052 and donor parent 863B) exhibited significant positive *gca* whereas eight lines (86014, 86015, 86042, 86064, 86083, 86088, 86096 and recurrent parent ICMB 841) showed significant negative *gca* effects. There was only one CSSL 86064 found to have significant positive *gca* for panicle number and donor parent exhibited significant negative *gca* for both panicle number and tiller number. For biomass and vegetative growth index, there was no line with significant positive *gca* effect except donor parent. In case of grain harvest index, lines did not show any significant *gca* results. For fresh stover yield, only donor parent had significant positive *gca* where line 86083 had significant negative *gca* for both fresh stover and dry stover yield. There were no significant *gca* effects observed in lines for stover dry matter fraction except donor parent which showed significant negative *gca* effect. For 1000 grain mass, only donor parent 863B had significant positive *gca* whereas five CSSLs (86014, 86019, 86042, 86064 and 86088) and recurrent parent had significant negative *gca* for this trait. Significant *gca* effects were not observed in lines for both grain number per panicle and grain number per m².

Among testers, RIB 3135-18 showed negative *gca* effects for all grain yield and its related traits except for tiller number and stover dry matter fraction. PPMI 301 showed significant positive *gca* grain yield, panicle harvest index, plant height, panicle length, panicle diameter, grain harvest index, 1000 grain mass and grain number per panicle but it exhibited significant negative *gca* effects for panicle number, tiller number and stover dry matter fraction. Tester H 77/833-2 exhibited positive *gca* effects for all traits except for panicle length, panicle diameter, stover dry matter fraction and 1000 grain mass.

4.2.2.6.4. Genotype x Environment interaction analysis

The results of analyses of variance and general combining ability for the grain yield and stover yield and its related traits across these three environments (Genotype x Environment interaction) are explained below.

4.2.2.6.4(A). Analyses of variance

Analysis of variance for LG6 across three moisture regimes during summer 2010 is presented in Table 51. Significant differences were observed for grain and stover yield traits but non-significant for flowering time, plant height, panicle diameter, panicle number and stover dry matter fraction between moisture treatments. Hybrids were highly significant for all observed characters. In case of environment x hybrid effects, there were clear significant differences for grain yield, panicle harvest index, biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and grain number. As expected, testers were significantly different for all the observed agronomic characters and similarly, lines were also significantly different for all traits except for stover dry matter fraction. The environment x line interactions were significant for grain yield, panicle yield, panicle harvest index, biomass yield, vegetative growth index, grain harvest index, fresh stover yield, dry stover yield and grain number. The environment x tester effects had significant differences for many traits but non-significant for flowering time, plant height, panicle length, and panicle diameter and tiller number. Due to line x tester, significant difference was observed for grain yield, panicle harvest index, time to flowering, panicle length, panicle diameter, biomass yield, vegetative growth index, grain harvest index, dry stover yield, grain mass and grain number. The effects due to interaction among environment x line x tester, were significant for panicle harvest index, plant height, grain harvest index and dry stover yield.

4.2.2.6.4(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG6 across three moisture regimes are presented in Table 52. For flowering time, the best negative combiners among lines were a RPHL 86083 and donor parent 863B and CSSL 86053 showed significant positive *gca*. Among testers, RIB 3135-18 followed by PPMI 301 showed significant negative *gca* effects

Table 51 : Analyses of variance for testcross hybrids of LG6 over three moisture regimes

Source of Variation	df	Time to		Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Grain Yield (kg/ha)							
Environments (Env)	2	0.16	67.94**	102.56**	0.02	5.36*	2.97	4.66	1.1	9.11*
Hybrids	41	10.03**	27.82**	54.19**	9.7**	14.68**	23.77**	1.29	5.91**	4.56**
Lines (L)	13	16.66**	14.8**	19.53**	20.48**	4.32**	43.46**	1.4	4.58**	2.99**
Testers (T)	2	41.01**	447.5**	934.54**	45.62**	249.81**	175.38**	1.71	73.39**	55.03**
L × T	26	4.34**	2.05**	3.81**	1.54	1.77*	2.26**	1.2	1.38	1.46
Env × Lines	26	0.43	2.36**	3.21**	0.98	0.85	0.93	0.96	1.21	1.37
Env × Testers	4	0.06	13.84**	14.69**	0.14	0.66	1.05	1	3.24**	1.03
Env × Hybrids	82	0.59	2.21**	2.88**	1.25	0.93	0.79	1.1	1.08	1.27
Env × L × T	52	0.72	1.23	1.81**	1.47*	0.98	0.69	1.19	0.86	1.25
Error df		246	233	232	246	246	246	246	245	245

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
Hybrids	41	5.03**	4.2**	46.41**	8.53**	6.28**	6.6**	59.01**	13.45**	10.58**
Lines (L)	13	6.77**	5.3**	16.59**	5.43**	8.65**	1.73	56.64**	9.58**	4.26**
Testers (T)	2	32.76**	22.26**	791.3**	122.58**	47.1**	109.56**	801.94**	187.07**	155.61**
L × T	26	2.02**	2.26**	4.02**	1.31	1.96**	1.11	3.04**	2.03**	2.59**
Env × Lines	26	2.05**	1.96**	2.97**	2.69**	1.69*	1.05	1.15	1.62*	2.09**
Env × Testers	4	3*	2.63*	14.92**	2.73*	2.6*	3.71**	4.44**	12.75**	21.14**
Env × Hybrids	82	1.57**	1.5**	3.14**	1.69**	1.74**	1.08	1.32	1.99**	2.45**
Env × L × T	52	1.22	1.19	2.32**	1.1	1.69**	0.89	1.17	1.35	1.19
Error df		223	223	211	226	224	243	245	232	233

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table S2 : Estimates of *gca* effects of lines and testers of LG6 over three moisture regimes

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
86014	-0.17	-144.4*	-33.7	-3.53**	-0.86	0.21	-0.11**	6.77	-9.68	-0.13	-229.1	-4.1	-1.81**	-129.1	-117.0	-0.017	-0.380**	-15	-413
86015	-0.03	-103.8	-99.9	-1.81*	-3.38**	-0.52*	-0.16**	3.85	5.98	0.01	-315.5**	-6.0*	-0.40	-425.7**	-207.6**	0.005	-0.295**	-64*	-192
86019	-0.10	-6.2	54.6	-1.34	-3.97**	-0.49	0.07*	-0.89	11.98	0.07	85.4	2.0	-0.40	-96.7	0.8	0.020	-0.443**	3	658*
86025	-0.03	36.0	144.5	0.44	-0.34	0.65*	0.20**	1.70	7.96	0.02	110.2	2.3	0.63	67.0	-30.7	-0.020	0.086	-12	264
86027	-0.03	-67.0	-105.4	-0.64	-3.78**	-0.49	0.10**	5.92	11.13	-0.02	-55.3	-1.3	-0.76	113.1	27.8	-0.003	-0.353**	-51	155
86042	-0.29	-142.8*	-42.4	-3.25**	-3.52**	-0.01	-0.17**	1.07	6.43	0.02	-13.1	0.4	-1.90**	25.7	18.7	0.004	-0.319**	-72*	-399
86052	-0.06	36.3	-44.8	2.27*	-1.46	0.10	0.15**	1.03	1.54	-0.02	-170.0	-3.3	1.43*	-110.8	-112.2	-0.015	0.217*	13	-23
86053	2.12**	178.8**	146.0	2.98**	6.65**	0.32	0.12**	-9.30*	-12.98	0.03	606.1**	6.6**	0.19	691.2**	466.2**	0.015	-0.149	122**	1266**
86064	0.31	25.7	-218.4**	4.02**	0.25	-0.42	-0.20**	-1.89	15.54	0.11	-97.1	-2.3	0.96	124.6	110.2	0.008	-0.314**	29	693*
86083	-0.87**	-280.1**	-271.5**	-2.80**	-9.71**	-0.56*	-0.24**	1.07	-0.94	-0.02	-574.2**	-9.5**	-1.36*	-577.5**	-296.6**	0.002	0.088	-114**	-1400**
86088	0.16	-74.9	72.4	-3.45**	4.17**	1.39**	-0.06*	-2.64	23.00*	0.16*	137.1	2.3	-2.06**	-48.2	68.7	0.018	-0.280**	-70*	-232
86096	0.27	43.1	11.2	0.00	3.02*	0.10	-0.16**	2.66	18.50	0.08	259.8*	4.6	-0.95	326.3*	236.3**	0.024	0.024	-62	-14
ICMB 841	-0.03	-141.8*	-57.8	-3.52**	-0.75	-0.01	-0.12**	-4.93	-6.05	0.06	-203.3	-3.9	-1.31*	-247.2	-157.9*	0.002	-0.400**	2	-271
863B	-1.24**	641.3**	445.1**	10.63**	13.69**	-0.27	0.59**	-4.38	-72.40**	-0.39**	459.3**	12.2**	7.76**	287.3*	-6.7	-0.042**	2.518**	291**	-92
H 77/833-2	0.62**	154.9**	16.3	3.9**	2.28**	-1.29**	-0.17**	2.52	21.40**	0.10**	272**	3.9**	1.23**	574.8**	238.2**	-0.023**	-0.343**	55**	1453**
PPMI 301	-0.29**	457**	191.3**	10.29**	2.20**	2.21**	0.23**	-0.72	-49.70**	-0.30**	73.9	2.1	7.25**	204.8**	-115.4**	-0.052**	1.454**	175**	485**
RIB 3135-18	-0.33**	-611.9**	-207.7**	-14.19**	-4.48**	-0.91**	-0.05**	-1.80	28.30**	0.20**	-345.9**	-6.0**	-8.49**	-779.6**	-122.8**	0.076**	-1.110**	-230**	-1939**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

for flowering time. For grain yield, a CSSL 86053 and donor parent 863B were significantly positive whereas lines 86014, 86042, 86083 and recurrent parent showed significantly negative *gca*. Six CSSLs (86019, 86025, 86052, 86053, 86064 and 86096) out of 10 exhibited better grain yield *gca* than that of recurrent parent. Only donor parent expressed significant positive *gca* and lines 86064 and 86083 had significant negative *gca* for panicle yield. Three CSSLs 86052, 86053, 86064 and donor parent had significant positive *gca* for panicle harvest index and there were three CSSLs (86014, 86042 and 86088), two RPHLs (86015 and 86083) and recurrent parent with significant negative *gca* for this trait. But, it was observed that seven CSSLs (86019, 86025, 86027, 86052, 86053, 86064 and 86096) out of 10 exhibited better *gca* for panicle harvest index than that of recurrent parent. In case of plant height, CSSLs 86053, 86088, 86096 and donor parent 863B showed significant positive *gca* effects whereas three CSSLs (86019, 86027 and 86042) and two RPHLs (86015 and 86083) had significant negative *gca*. For panicle length, there were two CSSLs 86025 and 86088 with significant positive *gca* and two RPHLs 86015 and 86083 with significant negative *gca*. There were six lines (86019, 86025, 86027, 86052, 86053 and donor parent 863B) exhibited significant positive *gca* whereas eight lines (86014, 86015, 86042, 86064, 86083, 86088, 86096 and recurrent parent) showed significant negative *gca* for panicle diameter. There was only one CSSL 86088 found to have significant positive *gca* for both panicle number and tiller number but donor parent showed significant negative *gca* effects for both these traits. For biomass yield and vegetative growth index, there were six CSSLs (86019, 86025, 86053, 86088 and 86096) including donor parent expressed better *gca* effects when compared with that of recurrent parent whereas two RPHLs 86015 and 86083 were significantly negative combiners for these two traits. In case of grain harvest index, CSSL 86052 and donor parent showed significant positive *gca* results and three CSSLs (86014, 86042 and 86088), one RPHL 86083 and recurrent parent showed significant negative *gca* effects. For fresh stover and dry stover yield, CSSLs 86053 and 86096 had significant positive and RPHLs 86015 and 86083 had significant negative *gca* effects. Donor parent 863B expressed significant positive results in case of fresh stover yield but not for dry stover yield. It was observed that six CSSLs (86025, 86027, 86042, 86053, 86064 and 86096) for fresh stover yield and 7 CSSLs (86019, 86027, 86042, 86053, 86064, 86088 and 86096) out of 10 for dry stover yield exhibited better *gca* effects than that of recurrent parent. There were no lines found to have any significant *gca* effects for stover dry matter fraction except donor parent which was significantly negative for this trait. For 1000 grain mass, two lines 86052 and donor parent 863B had significant positive *gca* but eight lines (86014, 86015, 86019, 86027, 86042, 86064

and 86088) including recurrent parent showed negative *gca* results. For grain number per panicle, CSSL 86053 and donor parent exhibited significant positive *gca* effects whereas lines 86015, 86042, 86083 and 86088 expressed significant negative *gca* effects. There were three CSSLs 86019, 86053 and 86064 with significant positive *gca* and there was only one RPHL 86083 with significant negative *gca* in case of grain number per m².

Among testers, RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number and tiller number where as PPMI 301 showed exactly opposite effects (positive *gca*) of RIB 3135-18 except for flowering time, dry stover yield and stover dry matter fraction to which it also showed significant negative *gca* like RIB 3135-18. Tester H 77/833-2 exhibited positive *gca* effects for all observed traits except panicle length, panicle diameter and 1000 grain mass.

4.2.2.7. Linkage Group 7 (LG7)

A total 81 testcross hybrids (involving three testers) of 14 homozygous segmental introgression lines and a sampling of 11 recurrent parent homozygotes (to serve as near-isogenic controls) of LG7, which were selected based on marker genotyping of 227 plants at 10 marker loci of LG7 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in three environments *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions during summer 2010 at ICRISAT, Patancheru. Line x tester analysis was performed and analysis of variance and general combining ability (*gca*) effects were calculated. The results of analysis of variance and general combining ability (*gca*) for grain and stover yield related characters for each of three moisture regimes and across these three moisture regimes are presented below.

4.2.2.7.1. Fully irrigated control treatment

4.2.2.7.1(A). Analysis of variance

The analysis of variance for LG7 under fully irrigated control treatment during summer 2010 is presented in Table 53. Hybrids showed highly significant differences for all characters except for biomass yield and dry stover yield. There were clear significant differences between lines for all traits except for panicle yield, biomass yield, vegetative growth index, fresh stover yield, dry stover yield, stover dry matter fraction and grain number per m². As expected, effects of testers were highly significant for all observed agronomic characters. Significant differences were found due to line x tester effects in case of flowering time, panicle length and 1000 grain mass.

4.2.2.7.1(B). General Combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG7 in fully irrigated non-stress conditions, during summer 2010 are presented in Table 54. For flowering time, the best negative combiners among lines were 87005, 87032, 87157, 87188, 87195 and donor parent 863B. Among testers both RIB 3135-18 and PPMI 301 showed negative *gca* effects for flowering time. Lines 87001, 87035, 87150, 87217 and 87226 found to have significant positive *gca* for flowering time. The combining ability for grain yield revealed that only one introgression line 87081 and donor parent were significantly positive and only one line 87104 was significantly negative. There were no lines except donor parent found to have significant positive *gca* effects for panicle yield and two lines 87104 and 87217 showed significant negative *gca* effects for this trait. In case of panicle harvest index, line 87150 and donor parent had significant positive *gca* and lines 87104 and 87174 had significant negative *gca*. There were four lines 87001, 87035, 87081 and 863B observed to have significant positive *gca* effects whereas five lines 87005, 87016, 87032, 87174 and 87214 exhibited significant negative *gca* for plant height. Four lines (87035, 87081, 87150 and recurrent parent) showed significant positive *gca* effects and five lines, 87005, 87157, 87174, 87188 and 87214 showed significant negative effects for panicle length. It was also observed that many lines 12 out of 20 obtained worse *gca* results than that of recurrent parent for this trait. In case of panicle diameter, lines 87002, 87195 and 863B showed significant positive *gca* effects whereas lines 87016, 87056, 87071 and 87117 showed significant negative *gca* effects. There were two

Table 53 : Analyses of variance for testcross hybrids of LG7 in fully irrigated non-stress conditions

Source of Variation	df	Time to									
		75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Replication	2	17.48**	0.31	1.86	2.05	4.01*	0.93	57.9**	6.48**	0.36	0.81
Hybrids	80	241925.74**	5.5**	2.97**	35921184.82**	756.7**	6762.7**	159240.55**	5.75**	2.63**	14829.1**
Lines (L)	26	5.78**	2.79**	1.41	2.95**	5.94**	3.87**	8.8**	0.71	2.82**	2.16**
Testers (T)	2	43.56**	152.76**	21.97**	150.73**	25.26**	124.33**	136.8**	3.7*	28.79**	14.06**
L × T	52	3.49**	1.2	1.02	1.13	1.03	1.46*	1.42	1.51*	1.19	1.38
Error	160										

Source of Variation	df	Biomass		Vegetative		Dry		Stover		Grain	
		Yield (kg/ha)	Yield (kg/ha)	Growth Index (kg/ha/d)	Index (%)	Yield (kg/ha)	Yield (kg/ha)	Matter Fraction	1000-Grain Mass (g)	Number/Panicle	Number/m ²
Replication	2	3.27*	3.27*	2.48	0.28	11.25**	4.38*	11.47**	2.23	0.06	0.02
Hybrids	80	1.07	1.07	915.38**	40322125.26**	61986.2**	1.33	2821690.69**	296168.99**	4.2**	3.13**
Lines (L)	26	1.12	1.12	1.24	1.71*	1.02	1.01	1.37	11.93**	2.57**	1.08
Testers (T)	2	4.66*	4.66*	4.03*	59.28**	15.48**	5.82**	23.26**	422.5**	88.29**	82.45**
L × T	52	0.91	0.91	0.88	0.74	1.03	1.21	0.74	2.04**	1.05	1.09
Error	160										

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Table 54 : Estimates of *gca* effects of lines and testers of LG7 in fully irrigated non-stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Panicle Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
87001	0.53*	163.2	70.6	3.25	6.20**	0.63	-0.08	4.48	8.63	-0.02	-227.5	-5.9	1.49	-5.8	-122.5	-0.005	0.114	7	636
87002	0.20	-300.4	-102.3	-5.53	0.20	0.07	0.11*	1.37	-13.59	-0.12	133.9	2.0	-2.18	52.0	34.4	-0.005	-0.154	-71	-1186
87005	-0.58*	21.2	92.2	-0.19	-7.35**	-1.26**	0.06	-3.08	-2.15	0.03	277.2	6.9	1.93	306.4	-16.4	-0.022	-0.259	38	612
87016	0.20	-26.8	-157.2	2.25	-5.13*	-0.60	-0.26**	-1.41	29.85	0.21	125.0	1.9	2.38	243.1	80.6	-0.007	-0.549**	-25	941
87032	-0.47*	100.2	109.5	0.70	-5.46*	0.18	0.02	1.92	8.85	0.01	-1242.9*	-23.5*	-4.62	-949.1*	-536.0*	-0.014	-0.334*	49	944
87035	0.98**	-74.4	35.5	-1.97	7.98**	1.73**	0.07	8.03	-30.04	-0.29**	-1232.6*	-26.5*	-8.39**	-501.4	-488.7*	-0.056	0.247	29	-866
87041	-0.02	52.7	75.5	1.58	1.20	0.85	-0.07	-0.86	4.52	0.04	481.0	9.5	1.82	543.1	203.7	-0.014	0.022	11	241
87056	-0.02	-127.5	-268.4	1.25	-0.58	-0.49	-0.18**	3.70	-25.15	-0.20	-795.3	-15.6	-0.96	-715.8	-358.1	-0.053	-0.185	65	-270
87071	-0.14	-54.7	-262.7	2.47	-1.02	-0.26	-0.12*	-3.75	-15.26	-0.04	-21.1	-0.3	2.49	-244.7	40	0.031	-0.387*	70	485
87081	0.09	344.1*	224.2	5.47	7.42**	1.18**	0.01	-6.75	21.74	0.23	211.7	3.4	1.05	53.1	191.5	0.047	0.337*	23	1156
87104	0.09	-662.7**	-403.4*	-17.53**	3.31	0.74	-0.09	12.40*	18.19	-0.05	-401.7	-8.3	-8.28**	254.2	59.8	-0.007	-0.817**	-267**	-2359**
87117	-0.14	-309.0	-251.0	-5.75	-2.24	0.29	-0.14**	0.14	9.41	0.04	-98.5	-1.7	-1.96	-188.8	-49.3	0.016	-0.331	-181*	-1336
87130	0.09	-18.1	-34.4	1.36	2.65	0.40	-0.02	-1.30	-17.04	-0.09	507.0	9.6	0.93	174.2	339.7	0.041	-0.045	73	112
87134	-0.14	-103.6	252.6	-4.19	-4.13	-0.26	-0.08	0.59	13.19	0.06	529.2	10.8	-0.51	-150.2	74.8	0.032	-0.012	-59	-303
87148	-0.14	-207.1	-131.3	-3.42	0.98	0.29	-0.07	-1.86	54.50**	0.39**	-96.6	-1.6	0.16	-609.1	-166.7	0.034	-0.423*	-164*	-517
87150	1.20**	130.5	-174.0	7.36*	3.42	1.29**	-0.02	-4.75	-63.30**	-0.35**	-647.1	-14.2	1.27	-760.2	-279.9	0.009	0.344*	230**	127
87157	-0.69**	291.6	366.9	2.81	-4.02	-1.37**	0.04	1.92	20.63	0.11	574.2	13.3	4.38	525.3	5.9	-0.051	0.077	75	1321
87167	0.09	161.3	-5.3	4.14	0.87	0.29	-0.08	0.70	-18.26	-0.09	475.3	8.9	2.93	292.0	279.1	0.023	-0.005	111	767
87170	-0.36	179.3	296.5	0.36	-3.47	-0.82	0.10	0.03	35.90*	0.23	465.2	10.1	3.16	283.1	-33	-0.024	0.051	-54	699
87174	-0.25	-264.5	47.2	-6.86**	-6.68**	-2.26**	-0.01	-3.19	0.85	0.06	-412.7	-7.8	-6.62*	-221.4	-237	-0.024	-0.504**	-64	-343
87188	-0.47**	132.3	-35.0	3.14	-3.24	-0.93**	-0.03	0.59	26.74	0.15	304.4	7.1	3.05	474.2	137.8	-0.014	0.091	-50	384
87195	-0.58*	26.2	2.7	1.36	-0.80	-0.26	0.11*	7.37	11.30	-0.04	210.6	5.5	2.71	36.4	6.5	-0.002	0.181	-56	-246
87214	-0.25	-101.6	54.0	-2.19	-9.91**	-0.93**	0.02	-14.80*	-8.48	0.19	454.6	9.5	-0.07	208.6	199.4	0.013	-0.094	-2	-281
87217	0.64**	-279.9	-515.8**	-5.31	-0.91	-0.15	0.04	2.37	3.30	-0.01	-1345.3*	-28.1*	-5.40	-460.2	-213.9	0.016	-0.005	-142	-1666
87226	1.09**	-3.3	92.7	0.47	0.76	0.51	0.02	-0.08	11.85	0.06	305.4	3.3	-3.07	-253.6	404.9	0.104**	-0.111	-10	285
ICMB 841	0.31	43.5	64.3	1.47	0.87	0.95*	-0.03	-2.52	4.41	0.08	419.5	7.4	2.05	618.6	153.7	-0.032	0.183	-3	115
863B	-1.25**	887.3**	556.6**	13.47**	19.09**	0.18	0.66**	-1.30	-90.50**	-0.58**	1047.2	24.2*	10.27**	993.1*	289.6	-0.037	2.567**	364**	547
H 770833-2	0.58**	294.2**	63.9	7.28**	3.06**	-0.96**	-0.19**	4.20*	16.50**	0.05	294.7	4.4	2.83**	436.3**	221.5**	-0.009	-0.363**	144**	2325**
PPM1 301	-0.35**	485.4**	270.4**	8.15**	1.44	1.97**	0.22**	-3.07	-36.20**	-0.18**	181.2	4.4	6.28**	258.1	-94.3	-0.044**	1.2994**	134**	464
RIB 3135-18	-0.23**	-779.5**	-334.3**	-15.43**	-4.50**	-1.00**	-0.02	-1.23	19.70**	0.13**	-475.9*	-8.8**	-9.11**	-694.4**	-127.2	0.052**	-0.936**	-279**	-2790**

** Significant at 0.01 level of probability. * Significant at 0.05 level of probability

lines 87148 and 87170 found to be significantly positive and 87150 and donor parent found to be significantly negative for panicle number. These two lines along with other line 87035 showed significant negative *gca* effects for tiller number also. There was only one line 87148 with significant positive *gca* effects for this same trait. There were no lines found to be significantly positive for traits biomass yield, vegetative growth index, grain harvest index, fresh stover yield and dry stover yield. Anyhow, donor parent had significant positive *gca* for vegetative growth index, grain harvest index and fresh stover yield. Lines 87032, 87035 and 87217 showed significant negative *gca* effects for biomass yield and vegetative growth index. There were three lines (87035, 87104 and 87174) expressed significant negative *gca* effects for grain harvest index. There was only one line 87032 for fresh stover yield and this line along with other line 87035 for dry stover yield showed significant negative *gca* effects. There was only one line 87226 observed with significant positive *gca* for stover dry matter fraction whereas remaining lines did not show any significant effects. For 1000 grain mass, three lines 87081, 87150 and donor parent exhibited significant positive *gca* results and six lines 87016, 87032, 87071, 87104, 87148 and 87174 showed significant negative *gca*. In case of grain number per panicle, line 87150 and donor parent expressed significant positive *gca* effect and lines 87104, 87117 and 87148 showed significant negative *gca*. There were no lines with significant effects for grain number per m² except line 87104, which was significantly negative.

Among testers RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction whereas PPMI 301 showed positive results for most of the observed traits except for flowering time panicle number, tiller number and stover dry matter fraction. Tester H 77/833-2 exhibited positive *gca* effects for all observed traits except panicle length, panicle diameter and 1000 grain mass.

4.2.2.7.2. Early drought stress treatment

4.2.2.7.2(A). Analysis of variance

The analysis of variance for LG7 under early-onset drought stress conditions during summer 2010 are presented in Table 55. Hybrids showed highly significant differences for all

Table 55 : Analyses of variance for testcross hybrids of LG7 in early-onset terminal drought stress conditions

Source of Variation	df	Time to		Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
		75% Flowering (d)	Flowering (d)									
Replication	2	4.64*	9.74**	7.4**	8.58**	46.09**	11.92**	0.64	1.91	2.02	3.26*	
Hybrids	80	157440.6**	6.65**	4.9**	30512188.32**	483.32**	5860.05**	102976.7**	4.91**	3.2**	22734.51**	
Lines (L)	26	3.79**	5.2**	3.44**	3.46**	5.1**	6.53**	8.24**	2.54**	2.09**	2.38**	
Testers (T)	2	52.06**	162.45**	22.25**	200.96**	9.73**	226.66**	148.45**	3.8*	45.8**	36.88**	
L × T	52	3.13**	1.38	2.57**	1.21	1.37	1.57*	1.4	2.45**	1.78**	1.08	
Error	160											

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
Hybrids	80	1.52*	1294.66**	57932697.09**	80278.1**	1.59**	10606590.88**	132812.91**	6.05**	3.67**
Lines (L)	26	1.71*	1.7*	3.37**	1.72*	1.62*	1.11	9.63**	3.22**	1.95**
Testers (T)	2	1.01	0.45	125.07**	29.53**	8.52**	36.46**	231.87**	137.06**	87.7**
L × T	52	1.45*	1.56*	1.14	1.33	1.21	1.23	1.96**	1.47*	1.26
Error	160									

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

characters. Similarly, there were clear significant differences in lines also for all traits except for stover dry matter fraction. As expected, effects of testers were highly significant for all observed characters but not for biomass yield and vegetative growth index. Lines x tester effects were significant for flowering time, panicle length, panicle number biomass yield, vegetative growth index, grain mass and grain number per panicle.

4.2.2.7.2(B). General Combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG7 in early-onset drought stress conditions during summer 2010 are presented in Table 56. For flowering time, the best negative combiners among lines were 87032 and donor parent 863B. Among testers both RIB 3135-18 and PPMI 301 showed negative *gca* effects for flowering time. Lines 87035, 87150 and 87226 found to have significant positive *gca* for flowering time. It was observed that 10 lines (three CSSLs and seven RPHLs) out of 20 flowered later to recurrent parent. The combining ability for grain yield revealed that only one line 87056 and donor parent were significantly positive and five lines 87041, 87130, 87134, 87195 and 87226 were significantly negative. There was one line 87035 along with donor parent found to have significant positive *gca* effects for panicle yield and four lines 87041, 87150, 87195 and 87226 showed significant negative *gca* effects for this trait. In case of panicle harvest index, lines 87056, 87150 and donor parent had significant positive *gca* and lines 87130, 87134, 87174 and 87226 had significant negative *gca*. It was noticed that none of the lines except donor parent showed better *gca* effects than that of recurrent parent for all above traits, grain yield, panicle yield and panicle harvest index. There were four lines 87001, 87035, 87081 and 863B observed to have significant positive *gca* effects whereas three lines 87032, 87134 and 87157 exhibited significant negative *gca* for plant height. Five lines, 87001, 87035, 87081, 87104 and 87150 showed significant positive *gca* effects and seven lines, 87002, 87005, 87157, 87170, 87174, 87188 and 87195 showed significant negative effects for panicle length. In case of panicle diameter, lines 87005 and 863B showed significant positive *gca* effects whereas lines 87071, 87104, 87167 and ICMB 841 showed significant negative *gca* effects. It was noticed that 10 CSSLs out of 14 and 5 RPHLs out of 11 expressed better *gca* results when compared with recurrent parent for this trait, panicle diameter. There were no lines found to be significantly positive whereas 87150 and donor parent found to be significantly negative for panicle number. There were two lines 87081 and 87214 with significant positive *gca* effects and two

Table S6 : Estimates of *gca* effects of lines and testers of LG7 in early-onset terminal drought stress conditions

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle Yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
87001	0.05	132.5	167.4	2.58	4.60*	0.75*	-0.07	8.16	-10.50	-0.15	353.9	6.8	2.00	342.5	118.6	-0.024	0.123	99	748
87002	-0.17	-96.4	-142.0	-2.20	-2.06	-0.69*	-0.03	-7.40	-7.94	0.02	-704.2*	-13.6*	-1.89	-109.7	-345.7*	-0.073	-0.326	-16	-182
87005	-0.40	78.4	33.5	2.80	-3.72	-0.80*	0.13**	6.82	-13.50	-0.16	208.1	4.8	1.89	199.2	106.9	-0.012	0.165	65	619
87016	0.16	40.0	17.9	1.25	-1.17	-0.02	-0.08	2.38	25.28	0.13	107.6	1.8	1.44	139.2	21.9	-0.019	-0.259	-6	678
87032	-0.50*	-63.6	-54.2	-1.09	-9.16**	-0.25	0.01	4.38	14.84	0.02	-585.0	-10.6	-2.22	-417.5	-254.3	-0.064	-0.047	-51	-97
87035	0.49*	136.8	241.2*	2.25	6.60**	1.97**	0.01	1.93	18.61	0.08	683.3*	12.3	1.33	1115.8**	374.2*	-0.060	0.074	38	1023
87041	0.16	-213.6*	-227.2*	-4.86	-1.17	0.09	-0.07	-0.07	-30.39	-0.21	-211.6	-4.5	-2.44	-193.0	-52.4	0.018	0.053	-79	-1421*
87056	0.16	197.5*	151.8	4.91*	2.72	0.31	-0.06	9.27	6.17	-0.08	231.2	4.2	4.11*	212.5	11.4	-0.020	0.455*	35	708
87071	-0.40	59.1	-119.8	4.25	1.05	0.53	-0.09*	-7.84	6.84	0.12	-74.2	-0.6	2.44	-118.6	-22.4	0.012	0.118	17	497
87081	-0.06	143.6	157.4	2.58	8.16**	1.19**	-0.05	-7.29	29.17	0.30**	269.5	5.4	2.33	-113.0	44.3	0.038	0.375	2	517
87104	0.38	0.6	42.6	0.14	1.39	0.97**	-0.10*	-0.07	8.28	0.03	542.0	9.8	-0.78	358.1	431.2*	0.046	-0.169	6	420
87117	-0.40	-58.7	9.2	-1.64	-0.84	0.09	-0.08	4.38	9.84	-0.02	-552.6	-10.3	-2.00	-569.7*	-306.1	-0.050	-0.184	-1	-37
87130	0.16	-243.9**	-99.4	-6.64**	-0.61	0.31	0.01	-0.62	-29.61	-0.18	46.9	0.5	-3.66*	255.8	78.0	-0.008	0.088	-97	-1486**
87134	0.16	-183.5*	42.0	-5.86*	-4.83*	-0.58	0.07	-5.07	21.72	0.20	203.3	3.7	-2.67	-75.3	93.4	0.035	-0.206	-104*	-1005
87148	0.05	-63.5	-64.0	-1.98	-3.61	0.31	0.03	6.71	7.95	-0.04	-480.5	-9.7	-1.67	-257.5	-178.0	0.018	-0.145	-33	-280
87150	0.93**	-5.6	-304.3**	5.02*	2.50	1.53**	-0.02	-24.50**	-50.70**	0.06	-417.5	-8.9	2.22	-259.7	-180.8	-0.010	0.052	111*	-83
87157	-0.40	64.2	0.6	2.36	-4.28*	-1.13**	-0.01	11.16	-7.83	-0.18	-27.5	0.2	2.33	11.4	-96.1	-0.029	-0.090	71	737
87167	-0.06	54.8	123.4	-0.09	1.17	0.53	-0.10*	0.05	0.06	0.00	420.1	8.5	0.11	163.6	228.7	0.032	-0.112	32	450
87170	-0.17	0.0	-16.0	0.25	-2.50	-1.24**	-0.01	13.70*	3.17	-0.14	91.1	2.1	0.44	120.3	39.3	-0.005	-0.045	-14	136
87174	0.38	-149.5	-54.4	-5.08*	-3.95	-1.58**	0.01	-1.29	13.72	0.09	-124.7	-3.1	-1.89	-231.9	-138.3	0.010	-0.445*	-65	-438
87188	0.05	-72.3	18.0	-0.09	-2.61	-0.69*	-0.04	-8.07	16.17	0.20	-401.9	-8.0	-4.22*	73.6	-225.9	-0.039	-0.124	-55	-310
87195	-0.06	-181.5*	-253.4*	-3.75	-1.84	-1.69**	0.02	11.70*	-17.94	-0.25*	-368.8	-7.2	-1.44	-549.7*	-182.9	0.045	0.004	-89	-1160*
87214	-0.28	-147.0	-174.4	-2.20	-1.61	-0.47	0.09	-24.00**	-7.94	0.30**	-173.8	-3.0	-0.89	-400.8	-66.7	0.047	-0.576**	23	-8
87217	0.05	-24.2	52.0	-1.20	1.50	-0.36	0.00	5.71	27.17	0.09	370.1	7.1	-0.33	14.7	250.2	0.051	-0.945**	-51	-280
87226	0.82**	-282.8**	-329.4**	-7.86**	-0.06	0.53	-0.02	1.49	25.84	0.12	54.6	-0.5	-5.22**	-53.0	316.6	0.086*	-0.575**	-179**	-1506**
ICMB 841	-0.28	50.7	74.1	0.36	-1.39	0.09	-0.13**	-1.07	3.72	0.04	-482.6	-9.2	-0.44	-214.2	-313.4	-0.024	0.089	6	184
863B	-0.83**	827.7**	707.5**	15.80**	15.72**	0.31	0.62**	-0.51	-62.10**	-0.38**	1023.2**	22.1**	11.11**	557*	248.2	-0.002	2.653**	336**	1577**
H 77R33-2	0.55**	5.6	-86*	1.44	0.68	-0.96**	-0.18**	-3.80*	21.50**	0.18**	120.4	1.4	0.22	433.5**	170.2**	-0.021	-0.249**	-2	486**
PPM1 301	-0.35**	384.6**	197.5**	11.13**	1.79*	2.03**	0.20**	0.07	-41.40**	-0.24**	-31.0	0.1	6.40**	100.4	-158.5**	-0.063**	1.092**	205**	1473**
RIB 3135-18	-0.19**	-390.2**	-111.5**	-12.58**	-2.46**	-1.07**	-0.01	3.74	19.80**	0.06	-89.4	-1.5	-6.63**	-533.9**	-11.7	0.085**	-0.843**	-203**	-1959**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

lines 87195 and donor parent with significant negative *gca* effects for tiller number. There were no lines found to be significantly positive for traits biomass yield, vegetative growth index and grain harvest index. Anyhow, donor parent had significant positive *gca* for all these traits. Lines 87056 and 87170 showed significant negative *gca* effects for both biomass yield and vegetative growth index. In case of these two traits, it was also observed that 5 CSSLs of 14 and 4 RPHLs of 11 exhibited better *gca* effects than that of recurrent parent. There was only one line 87056, expressed significant negative *gca* effect for grain harvest index. For fresh stover yield, two lines 87167 and donor parent had significant positive *gca* and two lines 87056 and 87195 had significant negative *gca*. For dry stover yield, though, none of the lines showed any significant *gca* effects except 87170 which was significantly negative. It was observed that 12 lines including 7 CSSLs of 14 and 5 RPHLs of 11 exhibited better *gca* results than that of recurrent parent for dry stover yield. There was only one line 87056 observed with significant positive *gca* and 863B observed with significant negative *gca* for stover dry matter fraction. For 1000 grain mass, two lines 87081 and donor parent exhibited significant positive *gca* results and six lines 87002, 87005, 87016, 87148, 87214 and 87226 showed significant negative *gca*. In case of grain number per panicle, only donor parent expressed significant positive *gca* effect and lines 87016, 87134 and 87217 showed significant negative *gca*. There were two lines 87167 and donor parent with significant positive effect and one line 87217 with significant negative effect for grain number per m².

Among testers RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number and stover dry matter fraction whereas PPMI 301 showed positive results for most of the observed traits except for flowering time, panicle number, tiller number, dry stover yield and stover dry matter fraction. Tester H 77/833-2 exhibited positive *gca* effects for all observed traits except panicle length, panicle diameter and 1000 grain mass.

4.2.2.7.3. Late drought stress treatment

4.2.2.7.3(A). Analysis of variance

The analysis of variance for LG7 under late-onset drought stress conditions during summer 2010 is presented in Table 57. Hybrids showed highly significant differences for all

Table 57 : Analyses of variance for testcross hybrids of LG7 in late-onset terminal drought stress conditions

Source of Variation	df	Time to									
		75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Replication	2	1.33	5.76**	13.01**	0.92	15.72**	3.01	5.64**	9.42**	6.51**	0.78
Hybrids	80	324469.53**	10.25**	4.07**	77944918.76**	782.83**	8969.59**	127714.82**	4.66**	2.65**	18352.82**
Lines (L)	26	5.36**	6.67**	2.36**	6.53**	5.22**	3.28**	7**	1.55	2.02**	2**
Testers (T)	2	52.67**	285.59**	28.12**	406.71**	35.66**	156.01**	91.67**	2.98	35.64**	22.87**
L × T	52	3.75**	1.45*	1.43*	1.31	1.37	2.34**	1.01	0.89	1.31	1.34
Error	160										

Source of Variation	df	Biomass		Vegetative		Dry		Stover		Grain	
		Yield (kg/ha)	Index (kg/ha/d)	Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Matter Fraction	1000-Grain Mass (g)	Number/Panicle	Number/m ²
Replication	2	9.76**	9.82**	2.44	25.31**	9.78**	31.81**	1.99	0.93	8.67**	
Hybrids	80	1.01	850.15**	38714747.86**	68913.77**	1.3	14425649.62**	284520.13**	6.89**	4.14**	
Lines (L)	26	1.24	1.32	2.26**	1.74*	1.17	1.67*	20.41**	3.58**	1.25	
Testers (T)	2	2.94	2.21	82.7**	21.82**	10.67**	54.88**	505.64**	166.48**	110.01**	
L × T	52	0.81	0.83	0.84	0.79	0.89	1.29	1.58*	1.44*	1.48*	
Error	160										

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

characters except for biomass yield and dry stover yield. There were clear significant differences in lines for all traits except for biomass yield, vegetative growth index, dry stover yield and grain number per m². As expected, effects of testers were highly significant for all observed agronomic characters but not for biomass yield and vegetative growth index. Significant differences were found due to line x tester effects in case of grain yield, panicle yield, flowering time, panicle length, 1000 grain mass and grain number.

4.2.2.7.3(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG7 in late-onset terminal drought stress conditions, during summer 2010 are presented in Table 58. For flowering time, the best negative combiner among lines was only donor parent 863B. Among testers both RIB 3135-18 and PPMI 301 showed negative *gca* effects for flowering time. Lines 87001, 87016, 87035, 87130, 87150 and 87226 found to have significant positive *gca* for flowering time. The combining ability for grain yield revealed that only one line 87081 and donor parent were significantly positive and two lines 87016 and 87226 were significantly negative. In case of panicle yield, there were no significant *gca* results observed in lines except donor parent which was significantly positive. In case of panicle harvest index, lines 87071, 87081, 87150, 87167 and donor parent had significant positive *gca* and lines 87016, 87104, 87134, 87217 and 87226 had significant negative *gca*. It was noticed that 13 lines showed worse *gca* effects for this trait when compared with recurrent parent. There were three lines 87081, 87104 and 863B observed to have significant positive *gca* effects whereas three lines 87032, 87170 and 87214 exhibited significant negative *gca* for plant height. It was noticed that 14 lines out of 20 expressed better *gca* results when compared with recurrent parent for this trait. Five lines, 87001, 87035, 87081 and 87104 showed significant positive *gca* effects and five lines, 87134, 87157, 87174, 87188 and 87214 showed significant negative effects for panicle length. In case of panicle diameter, lines 87214 and 863B showed significant positive *gca* effects whereas lines 87016, 87035, 87056 and 87117 showed significant negative *gca* effects. For panicle number and tiller number, there was only one line 87134 exhibited significant positive *gca* effect and there were two lines 87001 and donor parent found to be significantly negative. Two lines 87035 and 863B showed significant positive *gca* results and only one line 87002 showed significant negative *gca* results for biomass yield. This line also showed similar results for vegetative growth index unlike donor parent which was

Table 58 : Estimates of *gca* effects of lines and testers of LG7 in late-onset terminal drought stress condi

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle Yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000- Grain Mass (g)	Grain Number/ Panicle	Grain Number/m ²
87001	0.44*	-96.5	-202.4	0.71	2.19	0.83*	0.05	2.06	-34.90*	-0.24*	176.9	2.4	0.82	-95.9	217.0	0.056	-0.120	61	-367
87002	0.11	-145.3	-59.9	-3.74	0.31	-0.28	0.07	-6.61	-19.58	-0.03	166.5	2.9	-0.63	-105.9	63.8	0.025	-0.386**	13	-55
87005	0.00	-110.7	-32.1	-2.74	-3.81	-0.39	0.09	2.73	-4.81	-0.07	-476.0	-9.1	-2.29	-257.0	-235.4	0.007	-0.412**	17	-62
87016	0.44*	-300.1**	-266.1	-4.51*	-3.47	-0.39	-0.18**	9.51	10.42	-0.07	-647.3	-13.5	-4.85	-409.2	-189.7	0.024	-0.445**	-133*	-1002
87032	-0.34	-128.2	23.0	-3.51	-8.14**	-0.05	-0.10	3.51	22.97	0.15	-487.3	-9.1	-1.63	-347.0	-336.9	-0.016	-0.192	-89	-442
87035	0.55**	117.1	93.9	2.15	-1.81	0.72*	-0.12*	3.95	-0.58	-0.07	418.3	7.0	2.60	204.1	161.8	-0.012	0.124	46	442
87041	0.22	41.7	277.9	-1.51	1.42	0.61	-0.07	20.00**	16.86	-0.15	-141.1	-3.2	-2.07	-79.2	-182.0	-0.034	-0.230	5	687
87056	0.00	-7.2	-211.0	3.15	0.42	-0.05	-0.16**	-9.38	-10.25	0.08	-1142.4*	-22.4*	-5.51*	-1108.1**	-370.7	0.078*	-0.155	27	374
87071	-0.23	39.8	-249.9	5.93**	1.64	0.28	-0.01	-2.16	6.75	0.06	-17.2	0.0	4.26	19.7	70.0	0.001	0.002	6	382
87081	-0.12	251*	-1.1	7.59**	7.74**	1.50**	-0.01	-2.05	1.97	0.02	443.9	9.0	4.93	100.8	282.4	0.043	0.526**	90	561
87104	-0.34	-69.3	-139.1	-4.84*	4.30*	0.94**	-0.08	-5.27	3.86	0.08	228.5	5.2	-2.40	437.4	204.6	-0.010	-0.227	-6	41
87117	-0.34	-174.3	-78.9	-3.29	-1.58	0.17	-0.16**	3.28	18.08	0.08	141.9	3.5	-0.63	15.2	58.1	-0.001	-0.142	-104	-652
87130	0.55**	-97.1	-125.2	-0.18	2.53	0.50	0.01	-4.16	-11.70	-0.03	169.5	1.9	1.04	-85.9	131.9	0.048	-0.020	2	-380
87134	-0.34	-133.6	103.1	-5.40**	-3.70	-1.05*8	0.02	-4.72	37.00*	0.29**	422.6	9.1	-1.40	259.7	157.0	-0.010	-0.094	-129*	-909
87148	-0.12	-171.9	-125.2	-3.07	-3.58	-0.05	-0.08	8.40	1.75	-0.10	108.8	2.4	-0.63	-74.8	71.3	0.015	-0.330*	-35	-489
87150	1.21**	50.2	-127.1	4.04*	-2.81	0.50	-0.06	-18.20**	-19.70	0.11	-489.6	-11.7	0.04	254.1	-84.4	-0.054	0.147	63	107
87157	-0.34	101.0	224.7	0.26	-1.58	-1.05**	0.09	10.17	22.97	0.00	477.6	10.1	2.37	204.1	90.0	-0.026	0.059	-3	598
87167	-0.23	208.9	130.9	4.48*	2.86	-0.05	-0.01	8.28	16.75	-0.03	568.5	11.7	3.82	869.7*	274.9	-0.065	0.102	53	1246*
87170	-0.23	109.0	-6.8	3.04	-5.36*	-0.61	0.03	0.28	-3.58	-0.02	-1025.3*	-20.1*	-2.40	-438.1	-461.3*	-0.033	0.135	27	348
87174	-0.23	-63.8	201.4	-3.85	-4.03	-1.05**	0.02	-1.61	27.86	0.18	-30.4	-0.3	-4.07	-62.6	-28.9	0.018	-0.167	-70	-21
87188	-0.12	57.1	106.4	0.26	-0.81	-0.94**	-0.05	-3.49	3.75	0.08	436.7	8.8	1.82	138.6	167.8	0.000	-0.058	-6	264
87195	-0.12	-207.1	-225.9	-3.07	2.31	-0.05	0.03	3.40	-15.92	-0.15	-817.4	-16.0	-3.63	-829.2*	-375.9	0.049	-0.184	-71	-1049
87214	-0.12	-65.0	82.2	-2.29	-4.58*	-0.72*	0.11*	-10.05	-9.81	0.09	404.8	8.2	0.15	366.3	159.9	-0.022	-0.328*	31	243
87217	0.22	-210.7	-134.5	-4.73*	-2.36	-0.16	0.01	-6.61	4.53	0.13	13.7	-0.3	-0.85	-260.3	-14.7	0.049	-0.069	-123*	-1291*
87226	0.44*	-251.6*	-169.8	-5.51**	1.08	0.39	-0.01	-5.38	4.97	0.11	-17.6	-1.4	-1.96	-179.2	-10.9	0.025	-0.665**	-82	-540
ICMB 841	0.00	46.7	-7.4	2.37	3.64	0.61	-0.02	1.95	3.75	-0.01	-249.2	-4.9	-1.51	77.4	-101.0	-0.012	0.136	-9	270
863B	-1.00**	1210.3**	918.6**	18.26**	17.19**	-0.16	0.62**	2.17	-73.40**	-0.48**	1362.7**	29.7**	-8.37**	1385.2*8	281.3	-0.142**	2.993**	416**	1695**
H 77/833-2	0.50**	60.7	-42.8	3.30**	2.93**	-0.84**	-0.16**	-1.32	21.40**	0.15**	236.5	3.6	0.91	407**	250.2**	-0.015	-0.200**	18	964**
PPM1 301	-0.17**	575.3**	292**	11.74**	1.97**	1.76**	0.18**	-2.70	-34.90**	-0.18**	71.9	1.8	7.47**	231.5	-147.3*	-0.077**	1.154**	227**	1512*8
RIB 3135-18	-0.32**	-636**	-249.2**	-15.05**	-4.90**	-0.91**	-0.02	4.03	13.40**	0.02	-308.4	-5.5	-8.37**	-638.5**	-102.9	0.092**	-0.953**	-246**	-2476**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

significantly negative for this trait. There were two lines 87056 and donor parent found to be significantly positive and three lines 87130, 87188 and 87226 found to be significantly negative for grain harvest index. In case of fresh stover yield, two lines 87035 and donor parent had significant positive *gca* and two lines 87117 and 87195 had significant negative *gca*. For dry stover yield, lines 87035 and 87104 were significantly positive and only one line 87002 was significantly negative. There was only one line 87226 observed with significant positive *gca* and remaining lines did not show any significant *gca* results for stover dry matter fraction. For 1000 grain mass, two lines 87056 and 863B had significant positive *gca* and four lines 87174, 87214, 87217 and 87226 showed significant negative *gca*. In case of grain number per panicle, two lines 87150 and donor parent exhibited significant positive *gca* results and two lines 87134 and 87226 showed significant negative *gca* effect. Only donor parent expressed significant positive *gca* effect whereas lines 87041, 87130, 87195 and 87226 showed significant negative *gca* effect for grain number per m².

Among testers RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number and stover dry matter fraction whereas PPMI 301 showed positive results for most of the observed traits except for flowering time, panicle number, tiller number, dry stover yield and stover dry matter fraction. Tester H 77/833-2 exhibited positive *gca* effects for all observed traits except panicle length, panicle diameter and 1000 grain mass.

4.2.2.7.4. Genotype x Environment interaction analysis

The results of analyses of variance and general combining ability for the grain yield and stover yield and its related traits across these three environments (Genotype x Environment interaction) are explained below.

4.2.2.7.4(A). Analyses of variance

Analysis of variance for LG7 across three moisture regimes during summer 2010 is presented in Table 59. Anova did not reveal any significant differences for flowering time due to different moisture treatments, similarly to other traits like plant height, panicle length, panicle

Table S9 : Analyses of variance for testcross hybrids of LG7 over three moisture regimes

Source of Variation	df	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)
Environments (Env)	2	1.62	321.64**	101.82**	171.19**	1.72	3.35	5.46*	1.65	3.5	13.76**
Hybrids	80	12.84**	31.38**	6.04**	47.97**	7.39**	17.67**	17.14**	2.2**	5.95**	3.96**
Lines (L)	26	12.65**	18.57**	5.89**	20.97**	14.46**	11.39**	21.9**	2.94**	5.25**	4.16**
Testers (T)	2	142.64**	948.5**	103.88**	1583.16**	64.81**	479.34**	367.87**	3.18*	107.49**	62.76**
L × T	52	7.94**	2.51**	2.35**	2.42**	1.65**	3.05**	1.27	1.79**	2.4**	1.6**
Env × Lines	52	1.21	1.24	1.11	1.75**	0.96	0.91	1.02	0.9	0.91	1.19
Env × Testers	4	1.71	36.29**	5.32**	20.62**	3.27*	0.92	0.99	3.63**	0.57	3.12*
Env × Hybrids	160	1.21	2.2**	1.38**	2.08**	1.07	1.03	1.17	1.35**	0.91	1.19
Env × L × T	104	1.19	1.36*	1.36*	1.52**	1.04	1.09	1.26	1.48**	0.92	1.13
Error df		480	464	477	464	480	480	480	480	480	480

Source of Variation	df	Biomass Yield (kg/ha)	Vegetative			Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000-Grain Mass (g)	Grain Number/Panicle	Grain Number/m ²
			Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)					
Environments (Env)	2	89.48**	104.36**	113.05**	13.42**	9.56*	3.63	103.99**	277.69**	181.39**
Hybrids	80	5.26**	5.46**	43.05**	8.11**	4.19**	5.19**	43.99**	18.3**	13.46**
Lines (L)	26	5.18**	5.82**	18.91**	4.37**	2.94**	2.55**	38.9**	11.61**	4.08**
Testers (T)	2	60.54**	52.87**	1426.7**	186.51**	59.87**	147.51**	1175**	525.73**	425.46**
L × T	52	3.18**	3.46**	1.9**	3.12**	2.67**	1.04	3.03**	2.12**	2.31**
Env × Lines	52	1.29	1.31	1.57**	1.58**	1.29	1.11	1.42*	1.01	1.29
Env × Testers	4	5.59**	4.62**	21.93**	3.24*	2.26	6.04**	4.01**	15.8**	20.64**
Env × Hybrids	160	1.49**	1.48**	2.27**	1.37**	1.21	1.24*	1.63**	1.48**	1.89**
Env × L × T	104	1.44**	1.45**	1.87**	1.2	1.12	1.12	1.65**	1.16	1.47**
Error df		448	448	435	454	450	473	479	463	463

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

number and stover dry matter fraction. Significant differences were observed for remaining all traits. Hybrids were highly significant for both grain and stover yield traits. In case of environment x hybrid effects, there were clear significant differences for many of the traits except for flowering time, plant height, panicle length, panicle diameter, panicle number, tiller number and dry stover yield. As expected, testers were significantly different for all the observed agronomic characters and similarly, significant differences were observed between lines for all traits. The environments x line interactions were significant for panicle harvest index, grain harvest index, fresh stover yield and 1000 grain mass. The environment x tester effects had significant differences for many traits but non-significant for flowering time, panicle length, panicle diameter, panicle number and dry stover yield. Significant differences were observed for all traits except panicle diameter and stover dry matter fraction due to line x tester effects. The effects of environment x line x tester, were significant for grain yield, panicle yield, panicle harvest index, biomass yield, vegetative growth index, grain harvest index, 1000 grain mass and grain number per m².

4.2.2.7.4(B). General combining Ability (*gca*)

Estimates of *gca* effects of lines and testers for LG7 across three moisture regimes are presented in Table 60. For flowering time, the best negative combiners among lines were 87005, 87032, 87071, 87117, 87157, 87170, 87195 and donor parent 863B. Among testers both RIB 3135-18 and PPMI 301 showed negative *gca* effects for flowering time. Lines 87001, 87016, 87035, 87130, 87150, 87217 and 87226 found to have significant positive *gca* for flowering time. The combining ability for grain yield revealed that four lines 87081, 87157, 87167 and donor parent were significantly positive and 10 lines (87002, 87016, 87117, 87130, 87134, 87148, 87195, 87214, 87217 and 87226 were significantly negative. There were two lines 87157 and donor parent found to have significant positive *gca* effects for panicle yield and five lines 87016, 87071, 87150, 87195 and 87226 showed significant negative *gca* effects for this trait. In case of panicle harvest index, seven lines 87001, 87056, 87071, 87081, 87150, 87167 and donor parent had significant positive *gca* and eleven lines 87002, 87032, 87041, 87117, 87134, 87148, 87174, 87195, 87214, 87217 and 87226 had significant negative *gca*. There were five lines 87001, 87035, 87081, 87104 and 863B observed to have significant positive *gca* effects whereas eight lines 87005, 87016, 87032, 87134, 87157, 87170, 87174 and 87214 exhibited significant negative *gca* for plant height.

Table 60 : Estimates of *gca* effects of lines and testers of LG7 over three moisture regimes

Lines & Testers	Time to 75% Flowering (d)	Grain Yield (kg/ha)	Panicle yield (kg/ha)	Panicle Harvest Index (%)	Plant Height (cm)	Panicle Length (cm)	Panicle Diameter (cm)	Plant Population ('000/ha)	Panicle Number ('000/ha)	Tillers per Plant (Number)	Biomass Yield (kg/ha)	Vegetative Growth Index (kg/ha/d)	Grain Harvest Index (%)	Fresh Stover Yield (kg/ha)	Dry Stover Yield (kg/ha)	Stover Dry Matter Fraction	1000- Grain Mass (g)	Grain Number/ Panicle	Grain Number/ m ²
87001	0.34**	50.8	-5.2	1.95*	4.33**	0.73**	-0.04	4.90	-12.26	-0.13*	67.9	0.5	0.96	89.8	64.9	0.003	0.033	53	309
87002	0.04	-113.6*	-118.5	-2.3*	-0.52	-0.30	0.05	-4.21	-13.71	-0.04	-200.6	-4.3	-1.32*	-223.2	-87.1	0.000	-0.294**	-2	-272
87005	-0.32**	-36.5	14.1	-0.89	-4.96**	-0.81**	0.09**	2.16	-6.82	-0.07	-52.2	-0.2	-0.16	90.4	-54.8	-0.014	-0.174	24	190
87016	0.26*	-128.5*	-152.2*	-1.18	-3.25*	-0.34	-0.18	3.49	21.80*	0.09	-193.8	-4.4	-0.88	-15.7	-38.3	-0.007	-0.423**	-70*	6
87032	-0.43**	-63.4	9.0	-2.14*	-7.59**	-0.04	-0.02	3.27	15.55	0.06	-200.7	-2.9	-0.71	-62.2	-147.8*	-0.016	-0.197*	-47	-64
87035	0.67**	84.1	106.5	1.34	4.26**	1.47**	-0.02	4.64	-4.00	-0.10	189.2	2.0	0.52	346.7**	112.9	-0.029	0.142	46	346
87041	0.12	-72.5	25.0	-2.44*	0.48	0.51*	-0.07*	6.38	-3.00	-0.11	4.7	-0.1	-1.04	76.9	-33.8	-0.016	-0.057	-37	-364
87056	0.04	11.0	-126.3	3.08**	0.85	-0.08	-0.13**	1.20	-9.74	-0.07	-94.7	-1.9	1.56*	-167.3	-20.9	0.017	0.033	44	279
87071	-0.25*	-18.1	-227.8**	3.37**	0.56	0.18	-0.07*	-4.58	-0.56	0.05	-311.7**	-5.7*	1.40*	-283.2*	-74.4	0.009	-0.095	15	255
87081	-0.03	213.4**	109.8	4.37**	7.77**	1.29**	-0.02	-5.36	17.63	0.18**	276.8*	5.5*	2.07**	12.4	169.6*	0.037*	0.407**	22	545
87104	0.04	70.0	139.7	-0.21	3.00*	0.88**	-0.09**	2.38	10.11	0.02	378.1**	7.4**	-0.45	358.0**	219**	0.004	-0.410**	49	1109**
87117	-0.28*	-176.9**	-124.0	-3.30**	-1.55	0.18	-0.12**	2.60	12.44	0.03	-255.2*	-4.6*	-1.82**	-283.0*	-113.8	0.008	-0.224*	-90**	-637*
87130	0.26*	-120.1*	-103.4	-1.44	1.52	0.40	0.00	-2.03	-19.40*	-0.10	-33.0	-1.4	-1.52*	-54.0	79.6	0.021	0.002	-1	-590*
87134	-0.10	-173**	115.5	-5.99**	-4.22**	-0.63**	0.00	-3.06	24.00*	0.18**	110.9	2.4	-3.18**	-157.3	4.8	0.013	-0.110	-113**	-938**
87148	-0.07	-180.3**	-123.9	-3.66**	-2.07	0.18	-0.04	4.42	21.40*	0.08	-246*	-4.7*	-1.98**	-348.9**	-107.8	0.017	-0.305**	-93**	-627*
87150	1.11**	25.6	-218.9**	4.63**	1.04	1.10**	-0.03	-15.80**	-44.60**	-0.06	-365.1**	-8.9**	1.71**	-215.3	-108.5	0.000	0.175	118**	-149
87157	-0.47**	119.5*	180.3*	0.96	-3.29*	-1.18**	0.04	7.70*	11.92	-0.02	67.3	2.4	1.37*	78.2	-103.7	-0.041*	0.009	32	686*
87167	-0.07	108.9*	65.9	2.00*	1.63	0.26	-0.06*	3.01	-0.48	-0.04	213.8	4.3	0.63	273.1*	157.3*	-0.009	-0.011	49	622*
87170	-0.25*	63.3	74.2	0.37	-3.77**	-0.89**	0.04	4.68	11.85	0.02	31.3	1.1	1.03	142.7	-78.2	-0.026	0.041	-30	195
87174	-0.03	-79.0	47.6	-3.33**	-4.88**	-1.63**	0.01	-2.03	14.15	0.11	76.3	1.6	-1.46*	78.9	-31.3	-0.004	-0.378**	-36	223
87188	-0.18	28.3	12.7	1.00	-2.22	-0.85**	-0.04	-3.66	15.55	0.14*	30.9	1.1	-0.22	60.1	10.8	-0.004	-0.036	-42	55
87195	-0.25*	-153.6*	-176*	-2.66**	-0.11	-0.66**	0.05	7.40*	-7.52	-0.14*	-389.9**	-7.1**	-1.08	-496.4**	-215.7**	0.025	-0.006	-88**	-1017*
87214	-0.22	-137.4*	-29.8	-3.07**	-5.36**	-0.70**	0.07*	-16.30**	-8.74	0.19**	-45.6	-0.5	-1.92**	-110.7	-6.1	0.007	-0.338**	1	-215
87217	0.30*	-159.3**	-78.7	-3.38**	-0.59	-0.23	0.02	0.49	11.66	0.07	-119.0	-3.1	-1.65**	-175.7	-2.2	0.033*	-0.186*	-82**	-762*
87226	0.78**	-204.0**	-152.6*	-4.7**	0.59	0.47*	-0.01	-1.32	14.22	0.10	115.6	0.4	-4.03**	-158.9	244.2**	0.066**	-0.456**	-96**	-652*
ICMB 841	0.01	14.2	26.6	0.56	1.04	0.55*	-0.06*	-0.55	3.96	0.04	74.3	1.3	0.68	334.7**	-8.2	-0.028	0.130	-18	-10
863B	-1.03**	1028**	710.5**	17.12**	17.33**	0.11	0.64*	0.12	-75.40**	-0.48**	870.2**	19.9**	11.52**	809.7**	169.4*	-0.066**	2.732**	396**	1477**
H 77/833-2	0.54**	106.1**	-38.7	3.62**	2.22**	-0.92**	-0.18**	-0.28	19.80**	0.12**	157.2**	1.8*	0.94**	415.4**	202.8**	-0.016**	-0.259**	47**	1188**
PPMI 301	-0.29**	494.8**	272.2**	10.60**	1.73*	1.92**	0.20**	-1.90	-37.50**	-0.20**	185.1**	4.3**	7.5**	258.4**	-99.9**	-0.058**	1.176**	193**	1177**
RIB 3135-18	-0.25**	-600.9**	-233.4**	-14.23**	-3.95**	-0.99**	-0.02	2.18	17.70**	0.07**	-342.3**	-6.2**	-8.45**	-673.8**	-102.9**	0.073**	-0.917**	-240**	-2364**

** Significant at 0.01 level of probability; * Significant at 0.05 level of probability

Eight lines (87001, 87035, 87041, 87081, 87104, 87150, 87226 and recurrent parent) showed significant positive *gca* effects and eight lines, 87005, 87134, 87157, 87170, 87174, 87188, 87195 and 87214 showed significant negative effects for panicle length. It was also observed that many lines 13 out of 20 obtained worse *gca* results than that of recurrent parent for this trait. In case of panicle diameter, lines 87005, 87214 and 863B showed significant positive *gca* effects whereas lines 87016, 87041, 87056, 87071, 87104, 87117, 87167 and recurrent parent showed significant negative *gca* effects. It was also noticed that many lines 11 (including 7 CSSLs and 3 RPHLs) out of 25 obtained better *gca* results than that of recurrent parent for this trait. There were three lines 87016, 87134 and 87148 found to be significantly positive and three lines 87130, 87150 and donor parent found to be significantly negative for panicle number. For tiller number, four lines 87081, 87134, 87188 and 87214 showed significant positive *gca* effects and three lines 87001, 87195 and donor parent showed significant negative *gca* effects.

There were three lines 87081 and 87104 found to be significantly positive for traits biomass yield and vegetative growth index whereas five lines 87071, 87117, 87148, 87150 and 87195 were significantly negative for these two traits. For grain harvest index, six lines 87056, 87071, 87081, 87150, 87157 and donor parent exhibited significant *gca* effect and nine lines 87002, 87117, 87130, 87134, 87148, 87174, 87214, 87217 and 87226 had significant negative *gca* effect. In case of fresh stover yield, six lines 87035, 87104 and 87167 including both recurrent parent and donor parent showed significant positive *gca* effects, however donor parent 863B had far better *gca* effects than that of recurrent parent ICMB 841. There were four lines 87071, 87117, 87148 and 87195 with significant negative *gca* for this character. It was also noticed that none of the lines (except donor parent) showed better *gca* effects than that of recurrent parent for both grain harvest index and fresh stover yield. In case of dry stover yield, five lines 87081, 87104, 87167, 87226 and 863B expressed significant favourable results whereas two lines 87032 and 87195 showed significant negative results. There were three lines 87081, 87217 and 87226 with significant positive *gca* values and two lines 87157 along with donor parent had significant negative *gca* values for stover dry matter fraction. It was also observed that 11 lines showed better *gca* effects than that of recurrent parent for this trait but donor parent 863B expressed worse results. For 1000 grain mass, two lines 87081 and donor parent exhibited significant positive *gca* results and ten lines 87002, 87016, 87032, 87104, 87117, 87148, 87174, 87214, 87217 and 87226 showed significant

negative *gca*. In case of grain number per panicle, line 87150 and donor parent expressed significant positive *gca* effect and lines 87016, 87117, 87134, 87148, 87195, 87217 and 87226 showed significant negative *gca*. There were four lines 87104, 87157, 87167 and 863B with significant positive and seven lines 87117, 87130, 87134, 87148, 87195, 87217 and 87226 with significant negative *gca* effect for grain number per m². It was observed that only one CSSL 87081 was found be better line of all 14 CSSLs for grain yield, panicle harvest index, plant height, panicle diameter, grain harvest index, dry stover yield and grain mass.

Among testers RIB 3135-18 showed significant negative *gca* effects for all grain yield and its related traits except for panicle number, tiller number and stover dry matter fraction whereas PPMI 301 showed positive results for most of the observed traits except for flowering time panicle number, tiller number, dry stover yield and stover dry matter fraction. Tester H 77/833-2 exhibited positive *gca* effects for all observed traits except panicle length, panicle diameter stover dry matter fraction and 1000 grain mass.

CHAPTER V

DISCUSSION

5. DISCUSSION

A set of contiguous segmental substitution lines (CSSL) was developed by marker assisted backcross selection. The agronomic performance of these lines were examined across three moisture regimes, which provide a great opportunity to identify both major and minor QTLs, for the fine mapping and gene cloning of agronomic important traits in pearl millet.

5.1. Contiguous segmental substitution lines (CSSLs)

In the present study, marker assisted backcrossing was used to develop a set of contiguous segment substitution lines (CSSLs) in pearl millet. One thousand four hundred and ninety two progenies (derived from BC₃F₃, BC₅F₂ and BC₆F₁ families) expected to provide coverage across most of each of the seven pearl millet linkage groups were selected for genotyping at 74 polymorphic marker loci to develop CSSLs. These markers were selected based on the linkage maps constructed using the mapping population developed from the same parents *i.e.*, (CMB 841B and 863B by Qi *et al.* (2004), Bertin *et al.* (2005) and Senthilvel *et al.* (2008). For all seven linkage groups of pearl millet, a total 124 CSSLs was detected and among them, 56 lines were unique, which were consequently selected to construct a contiguous segmental substitution line population (CSSL population). These CSSLs consist of 1-3 homozygous introgression segments of donor parent 863B in the genetic background of recurrent parent ICMB 841. In this population, few lines have overlapping segments and all lines together cover 60 markers out of 74 used for genotyping and had alleles of donor parent 863B. Most introgression segments were detected by 1-4 marker loci and some are by more than four marker loci. Different introgression frequencies were observed among the seven linkage groups; among 56 unique introgression segments, 14 introgression segments existed on LG5, while only three on LG2. However, the major portion of LG2 was not covered and also three regions on LG1, LG3 and LG5 were not covered at least by overlapping introgression segments. The low introgression ratio of the donor genome may primarily be due to the fact that the size of the population used for genotyping and selection was small. For example, in the present study, only 69 plants for LG2 were selected for genotyping and this might be the reason for the identification of lines with only three introgressions. If more plants had been genotyped, more distinct introgression segments could have been obtained.

Development of incomplete set of CSSLs was reported by Kubo *et al.* (2002) in chromosome substitution series derived from Japonica and *Indica* cross of Rice and also by Tian *et al.* (2006) in construction of introgression lines carrying wild rice, *O.rufipogon* segments in the background of *Indica* cultivar. The latter group reported that only 67.5% of the genome coverage of *O.rufipogon* was achieved which might be due to random selection of lines during MAS.

In the present study, the complete set of CSSLs that cover the pearl millet genome could not be developed, which certainly impacts QTL mapping because some intervals lack segments of donor parent. The reason could be due to limited number of markers used and their uneven distributions across chromosomes. Only 74 markers with an average of approximately 10 per chromosome were used. Some markers were clustered in a small region while no marker was found in some other regions. For example in the present study, in case of LG6, three markers viz *Xicmp3058*, *Xicmp3086* and *Xicmp3050* were clustered at the same region as per the linkage map developed by Senthilvel *et al.*(2008). In case of LG3, the maximum distance between the markers observed was 34.5 cM according to linkage map developed by Qi *et al.* (2004) and Bertin *et al.*, (2005). These could prevent the detection of some introgression segments. Out of 1492 lines genotyped, 298 lines were found to be homozygous recurrent parent type and 1070 lines contained 1-5 heterozygous segments. The reason for obtaining recurrent parent homozygotes might be that some introgression segments of donor parent were completely replaced by recurrent parent during backcrossing. There were many heterozygous individuals generated which needs to be self pollinated further to obtain more number of CSSLs that could cover entire genome of donor parent 863B with its segmental introgressions.

The output of the present study could be taken forward to complete the construction of whole genome introgression lines of donor parent ICMB 863B in the genetic background of recurrent parent ICMB 841 by screening more BC₅F₃ plants and by using more molecular markers. We can improve the set of CSSLs by selfing of plants with heterozygous alleles, for example from BC₅F₂ to BC₅F₃.

CSSL are valuable genetic stocks as they decrease the interaction effects between QTLs anchored in different segments and subsequently divide the QTL into single mendelian factors, resulting in higher precision QTL mapping. Hence, CSSL are ideal novel population for QTL mapping. However, the development of CSSL is time consuming and CSSL in pearl millet had not been developed completely for the entire genome.

5.2. Drought nursery trials

In the present study, testcross hybrids of introgression lines, both donor and recurrent parents and few recurrent parent homozygotes were evaluated under three moisture regimes viz., fully irrigated non-stress control, early-onset moisture stress and late-onset moisture stress environments. In case of early onset terminal drought stress treatment, irrigation was terminated so that drought stress commenced from the mid flowering stage. In the late onset terminal drought stress treatment, irrigation was stopped so that drought stress commenced about one week after the mid flowering stage. Line \times tester analysis was performed after recording various agronomic characters for all three treatments and also across treatments. The characterization of testcross hybrids under managed drought stress environments were also described for QTL mapping in pearl millet by Yadav *et al.* (2002, 2004), and Bidinger *et al.* (2007).

5.2.1. GCA effects of parents and testers

In the present study, it was observed that both the parents and three testers showed consistent results across all drought nursery trials conducted for all seven linkage groups. Donor parent 863B showed better *gca* effects for grain yield and its component traits viz., panicle yield, plant height, panicle diameter, grain mass and grain number. But it had negative *gca* effect for flowering time, panicle length, panicle number and tiller number. Recurrent parent ICMB 841 did not show any significant *gca* effects for flowering time, grain yield and also for its component traits. 863B showed better *gca* effect for fresh stover yield, however it did not show any significant effects for dry stover yield. It was observed that donor parent 863B showed negative *gca* effects for stover dry matter fraction in few trials. The donor parent 863B expressed positive *gca* effects for panicle harvest index, vegetative growth index, biomass yield and grain harvest index. ICMB 841 did not express any significant *gca* results

for stover yield and its related traits. In some instances, it exhibited significant negative *gca* effects for all these traits. These results were observed to be consistent in line \times tester analysis of all three moisture treatments and also across the treatments. The significant positive *gca* effects for grain yield by this wild cultivar might be due to contribution of grain yield components panicle diameter, grain mass and grain number. By its nature, it has superior combining ability for grain filling under drought stress conditions. This *Iniadi* landrace cultivar expressed negative *gca* effect for flowering time in the present study, indicating its early flowering character to escape from terminal drought stress. Selection for early flowering helps the plant in escaping from terminal drought by reducing growth duration (Rattunde *et al.*, 1989). Recurrent parent ICMB 841 did not show any significant *gca* effects for flowering time, grain yield and also for its component traits. In case of stover yield also, it did not express any significant *gca* results. In some instances it exhibited significant negative *gca* effects for all these traits. Anyhow, recurrent parent ICMB 841 showed better *gca* effects than that of donor parent 863B for panicle length, panicle number, tiller number and stover dry matter fraction, though the *gca* effects for these traits were not significant in all instances. Production of large number of tillers provides potential compensation for damage to the main shoot or primary tillers during mid season drought stress, but can increase vulnerability to terminal drought stress (Mahalakshmi and Bidinger, 1985a, 1986; Mahalakshmi *et al.*, 1987; Bidinger *et al.*, 1987a). This might be the reason for ICMB 841, being drought sensitive.

Combining ability analysis of this present study indicated that the best combiner for both grain and stover- related character was donor parent 863B. This parent confers early flowering, which facilitates escape from drought stress situations. The most widely used sources of earliness, low tillering, combined with a thick main panicle and large grain size are the *Iniadi* landrace from West Africa (Andrews and Bramel-cox, 1993; Andrews and Anand Kumar, 1996) and 863B was bred by direct selfing and selection within a sample of this gemplasm.

Among testers, H 77/833-2 showed significantly positive *gca* effects for all observed characters except for panicle length, panicle diameter, 1000 grain mass and stover dry matter fraction. The contribution of this tester for testcross hybrids to increase in grain yield might

be contributed by panicle number and grain number. It can also be inferred that this tester expressed positive *gca* effects for flowering time, so its testcross hybrids could be effected by drought stress treatments because of its late flowering nature. This might mainly effects grain filling stage. The tester PPMI 301 also exhibited significant positive *gca* effects for grain yield and its related traits except for panicle number and tiller number. It showed significant negative *gca* effects for flowering time but in few trials, it expressed negative *gca* effects but not significant. Hence this tester can be considered as good negative combiner for flowering time. These results were in agreement with the earlier report that combining abilities of tester H 77/833-2 were often significantly different from those of PPMI 301(Yadav *et al.*, 2004). This tester expressed negative *gca* effects for dry stover yield and stover dry matter fraction. In case of RIB 3135-18, significant negative *gca* effects were observed for flowering time, which is a better drought escaping character under moisture stress conditions. This tester was the best combiner for early flowering, but it showed negative *gca* effects for grain yield and its component traits except for tiller number and panicle number. This relation between flowering time and grain and stover yield performance was expected where delay in flowering is expected to allow time for greater accumulation of biomass prior crop maturity. In case of stover dry matter fraction this tester expressed positive *gca* effects in all drought trials of this present study.

5.2.2. GCA effects of CSSLs

Analysis of variance and *gca* effects for all CSSLs observed in the present study are discussed for each linkage group of all seven linkage groups of pearl millet here in following paragraphs.

5.2.2.1. Linkage group 1(LG1)

A total 63 testcross hybrids (involving three testers) of 12 homozygous segmental introgression lines and a sampling of 7 recurrent parent homozygotes (to serve as near-isogenic controls) of LG1, which were selected based on marker genotyping of 376 plants at 10 marker loci of LG1 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in three environments *i.e.*,

fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions during summer 2010 at ICRISAT, Patancheru.

Analysis of variance (ANOVA) of drought nursery trials for LG1 across moisture regimes revealed that the effect of moisture environment was highly significant for both grain yield and stover yield but not for time to 75% flowering, plant height, panicle length, panicle diameter, panicle number and stover dry matter fraction. Flowering time was not affected by the stress treatments as expected because effect of drought stress started to appear approximately 10 days after the flowering started. There were clear differences between the lines for grain yield in all three moisture treatments and across environments. Significant differences between lines for stover yield were observed only in the pooled data. The differences between testers were high for both grain and stover yield in all three moisture treatments as well across treatments. But in case of non-stress treatment, neither lines nor testers showed differences for panicle yield, panicle harvest index and tiller number. In this case, the main components for grain yield might be grain mass and grain number. There was no effect due to lines for the characters, biomass yield and vegetative growth index in all three moisture environments. No significant differences were observed due to line \times tester effects for most of the variables in all three environments.

The combining ability analysis for LG1 revealed that CSSL 71080 showed higher values than that of the recurrent parent for grain yield, panicle yield, grain number and grain mass in early-onset, late-onset drought stress conditions and across three moisture conditions. This line expressed almost similar results to that of donor parent for all observed traits except for plant height. This line showed negatively significant *gca* effects for plant height. This line has two introgression segments of donor parent at marker loci *Xpsmp2273*, *Xpsmp2069* and *Xpsms86*. The other introgression lines containing either single or overlapping introgression segment of donor parent alleles at marker loci *Xpsmp2273* and *Xpsmp2069* were 71085, 71134, 71174, 71178, 71268, 71273 and 71281. Even though some of these lines did not show significant positive *gca* effects for grain yield, all these lines showed better results than recurrent parent ICMB 841 and similar to donor parent 863B for most of the observed characters in at least one of the two moisture stress regimes or across the three moisture regimes but not in stress free environment. Two CSSLs (71218 and 71220) having

introgression segments at marker loci *Xicmp3032*, *Xicmp3080*, *Xicmp3017* and *Xpsms58* were found to have better *gca* effects for plant height and panicle length, diameter and number but significant negative *gca* effects for grain number and mass. These two lines showed varied results and 71220 had better grain yield in late-onset stress and across environments. The introgression line, 71074 having only one donor parent allele at marker locus *Xpsms38* was negatively significant for many agronomic traits in all three moisture environments. This allele of 863B might have affected this line and causing poor agronomic performance. CSSL 71118 has large single introgression segment at marker loci *Xicmp3080*, *Xicmp3017* *Xpsms58*, *Xpsms38*, *Xpsms35*, *Xpsms86*, *Xpsmp2273* and *Xpsmp2069*. This line did not show significant positive *gca* effects in any of the observed characters. One or more of these alleles of 863B had deleterious effects on its agronomic performance.

The drought nursery experiments conducted for LG1 demonstrated that out of 12 CSSLs tested, four CSSLs (71080, 71134, 71178 and 71281) under early stress conditions, six CSSLs (71080, 71134, 71178, 71220, 71273 and 71281) under late stress conditions and seven CSSLs (71080, 71085, 71134, 71178, 71220, 71268 and 71273) in across three moisture regimes exhibited better *gca* effects for grain yield than that of recurrent parent ICMB 841. In early-onset stress conditions, out of 21 lines tested, 13 lines (8 CSSLs, 4 RPHLs and donor parent) showed better *gca* results for panicle yield than that of recurrent parent. Out of these 8 CSSLs, 6 CSSLs (71080, 71085, 71134, 71178, 71268 and 71218) share the same 863B introgression segment at *Xpsmp2069* and *Xpsmp2273* but the other two CSSLs had 863B introgression at marker interval *Xpsms58*, *Xicmp3080*, *Xicmp3017* and *Xicmp3032*. Similar results were also observed in late-onset stress conditions and across environments. It was observed that 10 CSSLs (71074, 71080, 71085, 71134, 71174, 71178, 71218, 71220, 71273 and 71281) and three RPHLs in late stress and nine CSSLs (71080, 71085, 71134, 71178, 71218, 71220, 71268 71273 and 71281) and two RPHLs in across moisture treatments showed better *gca* values for panicle yield. However, the same results were not observed in fully irrigated non-stress conditions. The *gca* results in late-onset stress and across environments also revealed better *gca* effects of seven CSSLs out of 12 for plant height and panicle diameter. Interestingly, one recurrent parent homozygous line 71083 unlike recurrent parent ICMB 841 performed better for grain yield, panicle yield and grain number in early-onset stress and across environments. There might be some favourable

alleles of 863B at some markers, which were not covered in this study, could have made this line better for these three traits.

The overall *gca* results for LG1 demonstrated that four CSSLs (71080, 71134, 71178 and 71281) in early-onset drought stress, six CSSLs (71080, 71134, 71178, 71220, 71273 and 71281) in late-onset drought stress and seven CSSLs (71080, 71085, 71134, 71178, 71220, 71268 and 71273) in across three environments were found to be better lines out of 12 CSSLs tested, for grain yield, panicle yield and grain number. All these CSSLs (except 71220) had single/overlapping introgression segment of donor parent at marker loci *Xpsmp2273* and *Xpsmp2069*. These results imply that the introgression lines having donor parent 863B introgression segments at marker loci *Xpsmp2273* and *Xpsmp2069* with genetic background of recurrent parent ICMB 841 are the best lines for grain yield, panicle yield and grain number under both early and late onset stress environments and across environments. Hence it is strongly inferred that there might be a drought tolerance QTL at marker interval *Xpsmp2273* and *Xpsmp2069* for these three traits.

The results obtained in the present study are compared with previously identified QTLs. The grain yield QTL at marker loci *Xpsmp2273* and *Xpsmp2069* observed in the genomic region of LG1 in the present study was also reported earlier by Bidinger *et al.*, (2007) and Yadav *et al.*, (2004). Using the same mapping population and two years of stress environment phenotyping data but with two testers, Yadav *et al.*, 2004 mapped QTLs for grain yield and yield-related traits under terminal stress. They have reported a genomic region associated with flanking markers *Xpsm761* and *Xpsmp2069* for grain yield on LG1 and this genomic region was specifically associated with grain yield in the late stress environment of the year 1999 and in the PPMI tester background only. Bidinger *et al.*, 2007 also identified a grain yield QTL based on 79 skeleton mapped F2-derived F4 progenies from the same mapping population of ICMB 841 x 863B. They reported a grain yield QTL at marker interval of *Xpsmp2069* and *Xpsm756* for LG1 in non-stress control and early-onset stress environment. They also identified a string QTL for grain number and panicle harvest index in the genomic regions of LG1 at marker interval of *Xpsm761* and *Xpsm756* in all three moisture environments and also across environments. With the support of earlier reports, the present results concluded that the genomic region of LG1 at marker loci *Xpsmp2273* and *Xpsmp2069*

could be a strong QTL for grain yield. It is recommended that genotyping these introgression lines with more number of markers could be continued to detect this QTL region more precisely for fine mapping studies.

5.2.2.2. Linkage group 2 (LG2)

A total 48 testcross hybrids (involving three testers) of 10 homozygous segmental introgression lines and a sampling of 4 recurrent parent homozygotes (to serve as near-isogenic controls) of LG2, which were selected based on marker genotyping of 69 plants at 19 marker loci of LG2 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in three environments *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions during summer 2010 at ICRISAT, Patancheru.

Analysis of variance (ANOVA) of drought nursery trials for LG2 across moisture regimes revealed highly significant differences for both grain yield and stover yield related traits but non-significant for flowering time, panicle length, panicle diameter and stover dry matter fraction. Flowering time was not affected by the stress treatments as expected because effect of drought stress was initiated approximately 10 days after the flowering time was started. Hybrids were highly significant for grain yield and fresh stover yield in all three environments and across three environments. There were clear differences between the lines for grain yield in all three moisture treatments as well in across environments. Significant differences between lines for stover yield were observed only in across moisture environments. There were big differences between testers for both grain and stover yield. There was no effect due to lines for the characters, biomass yield and vegetative growth index in early and late drought stress environments.

Introgression line 72001, which had single donor marker allele introgression at *Xpsmp2072*, did not show any significant *gca* effects for all observed traits. Introgression line 72028 showed significant positive *gca* effects for grain yield, panicle harvest index, fresh stover yield and grain number in non-stress control and across environments. This line consisted of

one homozygous and one heterozygous alleles of donor parent 863B at marker loci *Xpsms84* and *Xpsmp2255* respectively. These results indicated that donor alleles at this marker position may be favourable for these traits. Introgression lines 72030, 72038 and 72039 showed varied results even though they had homozygous introgression segment of 863B at marker loci *Xpsmp2255* and *Xpsmp2225*. CSSL 72030 showed significant negative *gca* effects for grain yield, panicle yield and panicle harvest index in across moisture treatments where as CSSL 72039 showed same results under control fully irrigated condition. CSSL 72030 showed significant negative *gca* results under early on-set drought stress and across environments for biomass yield, vegetative growth index, stover yield and grain number and grain mass. Interestingly, it was observed that CSSL 72039 exhibited significant positive *gca* for 1000 grain mass in both the stress conditions and across treatments. These results communicate that there might be putative QTL of donor marker allele's viz., *Xpsmp2255* and *Xpsmp2225* (which was not detected in earlier studies) in this line may be favourable for 1000 grain mass. But, it was yet to be confirmed further as the other two CSSLs having the same introgression did not show this favourable result. Hence it can also be said that there might be some other favourable donor alleles, which are not covered in this present study, for this trait. CSSLs 72054, 72060, 72066, 72067 and 72069 had single introgression segment of donor parent at marker locus *Xpsmp2225*. All these five lines showed significant results here and there for across all observed traits but not specific.

Combining ability analysis for LG2 revealed that 9 CSSLs (72001, 72028, 72030, 72038, 72054, 72060, 72066, 72067 and 72069) and 3 RPHLs (72002, 72044 and 72062) exhibited better *gca* effects for both grain yield and panicle harvest index than that of recurrent parent ICMB 841 under fully irrigated non-stress conditions and across three environments. These lines also expressed better results for panicle yield only in across environments. It was also noticed that 11 lines (nine CSSLs and two RPHLs) out of 14, showed better *gca* effects for plant height and panicle length in across environment. Under fully irrigated non-stress conditions, for plant height, six CSSLs and two RPHLs and for panicle length, nine CSSLs and two RPHLs exhibited better *gca* values when compared with ICMB 841. Seven CSSLs and one RPHL in case of grain mass and all 14 lines tested (including 10 CSSLs and 4 RPHLs) in case of grain number exhibited better *gca* effects when compared with ICMB 841 in the same environment. These better results were also observed in across environments. But these lines showed worse *gca* effects for panicle number and tiller number and stover dry

matter fraction. These results indicated that all these lines reflecting the behaviour of donor parent 863B and there may be a favourable introgression segment from 863B in all these lines. But, interestingly, all these lines had better *gca* effects than those of recurrent parent, showing late flowering behaviour unlike donor parent. Drawing any conclusion from the above observations was problematic as there was no clear pattern of donor parent allele homozygosity associated with the detected differences. Therefore, although these CSSLs were homozygous for few genotyped 863B alleles (on LG2 itself or any other LG), these results do not necessarily suggest that LG2 carries genes contributing substantially to variation in grain yield and related traits. Instead, it appears that some portion of the genome (perhaps in distal regions of LG2) where marker information is not yet available may be contributing to the observed variation in *gca* for this set of CSSLs.

The results obtained in the present study for LG2 are compared with previously identified QTLs on this linkage group for mapping population based on same parents, 863B and ICMB 841. Yadav *et al.*, 2004 reported a QTL associated with drought tolerance of grain yield in early stress environments using two testers, H 77/833-2 and PPMI 301. Bidinger *et al.*, 2007 also observed QTLs for grain yield, grain mass, harvest index and panicle harvest index for the population based on the same parents but using only one tester PPMI 301 in all three moisture environments and across environments. They identified QTL for grain yield, stover yield and harvest index at marker intervals *Xpsmp322* and *Xpsmp2050*. The present study covered few marker loci (*Xpsmp322*, *Xpsmp2072*, *Xpsmp2066*, *Xpsmp2206*, *Xpsmp2201* and *Xpsmp2232*) for identification of CSSLs. But unfortunately, introgression was found only at one marker locus *Xpsmp 2072*. Even though this line did not show significant *gca* effects for grain and its related traits, it expressed better *gca* effects than those of recurrent parent ICMB 841 except for panicle number, tiller number and stover dry matter fraction. CSSLs with introgression segments of the donor parent at other markers were not identified as substitution line development for this portion of LG2 was carried out earlier research program (Sathish Kumar, 2004), and hence not concentrated in the present study.

5.2.2.3. Linkage group 3 (LG3)

A total 45 testcross hybrids (involving three testers) of 10 homozygous segmental introgression lines and a sampling of 3 recurrent parent homozygotes (to serve as near-isogenic controls) of LG3, which were selected based on marker genotyping of 88 plants at 10 marker loci of LG3 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in three environments *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions during summer 2010 at ICRISAT, Patancheru.

Analysis of variance (ANOVA) of drought nursery trials for LG3 across moisture regimes revealed that the effect of moisture environment was highly significant for all the measured variables. There were no significant differences observed for flowering time by all source of variance in all three environments and also across environments. There were clear differences between the lines for grain yield and its related component traits in all three and across environments. It was also noted that big differences among testers for these characters. But in case of non-stress treatment, lines did not show significant differences for panicle yield, panicle harvest index and grain number. In this case, the main contributing trait for grain yield might be grain mass. For fresh stover yield, significant differences were not observed in case of early-onset and late-onset stress environments but significant in non-stress treatment. There were no significant differences between lines for biomass yield and vegetative growth index in all three moisture environments but significant in across three moisture environments. No significant differences were observed due to line x tester effects for most of the variables in all three environments.

General combining analysis for LG3 demonstrated that all 10 CSSLs exhibited late flowering when compared with recurrent parent in all or at least one environment. But CSSL 73052 showed negative *gca* effects for flowering time in all three and across environment, which is a favourable behaviour of donor parent 863B. This CSSL 72052 consisted of homozygous donor introgression segment at marker loci *Xpsmp108* and *Xpsmp2070*. But, this line did not show better *gca* effects for grain yield in any environment. Other CSSL 73079, also having introgression segment from 863B at marker locus *Xpsmp108* and *Xpsms60* showed better *gca*

effects than those of recurrent parent for grain yield, panicle yield, panicle harvest index, plant height, panicle diameter and grain number in fully irrigated control environment but it showed worse results in early on-set stress environment. These results are in contrast with earlier reports of QTL at marker interval of *Xpsmp108-Xpsmp2214* from ICMB 841 on LG3 for grain yield, grain mass and panicle harvest index by Bidinger *et al.*, (2007). But the present study revealed that donor parent 863B allele at marker locus *Xpsmp108* may be associated with a favourable QTL for these traits on LG3.

There was one CSSL 73089 having single marker introgression at locus *Xpsms60* did not show good *gca* effects in all environments with few deviations in late-onset stress environment for dry stover yield, stover dry matter fraction. Introgression line 73014, which had introgression segment at marker loci *Xpsmp2267* and *Xpsmp2070* did not express any significant positive *gca* effects in all environments except for panicle number only in late-onset stress conditions. CSSLs 73023, 73029, 73046 and 73048 consisted of similar two introgression segments from donor parent at marker loci, one at *Xpsmp2267*, *Xpsmp2070*, *Xpsmp2214*, *Xpsmp2249* and *Xpsmp2227* and another at single marker *Xctm10*. CSSL 73048 had one additional donor allele at marker locus *Xpsms60* in first introgression segment. The agronomic performance of these lines was consistent in all three environments. They expressed better *gca* effects for plant height, panicle length, panicle diameter, dry stover yield and stover dry matter fraction, mostly in at least one of the three *gca* analysis of non-stress control, late-onset stress and across treatments. But these lines show worse results than that of recurrent parent ICMB 841 for grain yield, panicle yield, panicle harvest index, panicle number, grain harvest index and grain mass. Any how these lines expressed better *gca* effects for grain number in at least one of the environments other than early on-set drought stress environment. CSSLs 73082 and 73086 showed varied results even though they shared same three introgression segments from 863B at marker loci *Xpsms 60*, *Xpsmp2214*, *Xpsmp2249* and *Xpsmp2227* and *Xctm10*. CSSL 73082 exhibited better *gca* results in non-stress control and across environments for grain and stover- related traits whereas CSSL 73089 showed worse results for these traits except for dry stover yield and stover dry matter fraction in late on-set stress and across environments. All these above results were found to be in contrast to the earlier reports. Yadav *et al.* (2004) reported harvest index and panicle harvest index QTL on LG3 associated with marker interval of *Xpsmp325- Xpsmp2070*. Bidinger *et al.*, 2007 identified QTLs on this LG3 for grain yield, grain mass, harvest index and panicle harvest

index at marker interval *Xpsmp108*, *Xpsmp2070* and *Xpsmp2214*. Both these earlier studies reported that the ICMB 841 alleles at these QTLs on LG3 contributed favourably for these traits. The present study revealed that only one marker *Xpsmp108* as *gca* effects of CSSLs 73052 and 73079 having 863B marker allele at this locus showed better performance, but here, 863B allele contributed favourably unlike in earlier reports. All other lines having donor segment introgression at loci *Xpsmp2070* and *Xpsmp2214* did not perform better. The reason for these results might be due to presence of 863B alleles at these marker loci of present study or few other marker loci, which are not covered in this study, either from donor or recurrent parent, unfavourable to these traits.

The combining ability analysis for LG3 revealed that six CSSLs (73014, 73023, 73029, 73048, 73082 and 73086) in fully irrigated non-stress conditions and all 10 CSSLs and one RPHL (73074) in late-onset drought stress conditions showed better *gca* effects for dry stover yield when compared with recurrent parent. These results revealed that LG3 carries genes contributing to dry stover yield, which may be environmental specific. Nepolean *et al.*, (2006) identified dry stover yield QTL on LG3, provided by 863B at marker interval *Xctm10-Xpsm174* in stress free environment. The present results also revealed that six CSSLs (73023, 73029, 73046, 73048, 73082 and 73086) out of 10 consisting of 863B allele at locus *Xctm10* showed better *gca* effects for dry stover yield and stover dry matter fraction. Hence this region can be confirmed as QTL for this trait. Anyhow, the other 4 CSSLs consisting of 863B allele at other marker loci also expressed better *gca* effects for dry stover yield. Hence, there may be favourable alleles at some other markers that are not covered in this study for this trait.

The combining ability analysis for LG3 demonstrated that nine CSSLs out of 10 and two RPHLs of three exhibited better plant height than that of recurrent parent in fully irrigated non-stress conditions. These CSSLs exhibited better *gca* effects than that of recurrent parent for biomass yield and vegetative growth index also in late-onset drought stress conditions. It was observed that five CSSLs (73023, 73029, 73048, 73079 and 73082) and one RPHL (73074) in fully irrigated non-stress conditions and all 10 CSSLs and one RPHL (73078) in across three environment expressed better grain number per panicle than that of ICMB 841. These results suggest the presence of QTL for plant height, biomass yield, vegetative growth

index and grain number per panicle but genomic region could not be associated to these traits as more number of markers are required for screening. It was also clear that grain and stover-related traits were much more affected in early-onset drought stress conditions when compared with non-stress control and late-onset stress conditions. These results are in concordance with the earlier reports of Bidinger *et al.*, 2007 in QTL detection for grain yield in late-onset stress environment and across environments.

5.2.2.4. Linkage Group 4 (LG4)

A total 90 testcross hybrids (involving three testers) of 20 homozygous segmental introgression lines and a sampling of 8 recurrent parent homozygotes (to serve as near-isogenic controls) of LG4, which were selected based on marker genotyping of 328 plants at 7 marker loci of LG4 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in two environments *i.e.*, fully-irrigated control conditions and terminal drought stress conditions during summer 2009 at ICRISAT, Patancheru.

Analysis of variance (ANOVA) of drought nursery trials for LG4 across moisture regimes revealed that the effect of moisture treatment was highly significant for grain yield and stover yield traits except for flowering time, plant height and panicle number. Hybrids showed significant differences for both grain and stover yield in non-stress, stress as well in across environments. There were clear difference between lines for grain yield and stover yield in across environments but results were significant only for grain yield but not for stover yield in non-stress control and drought stress treatments. There were big differences between testers for all these characters in both environments and across environments. For stover yield related traits, there were no differences observed in case of control and stress environments but in case of across environments, big differences were noticed. No significant differences were observed due to line x tester effects for most of the variables in both environments.

Combining ability analysis for LG4 revealed that many CSSLs except 74411 (did not show any significance) showed better positive *gca* effects for flowering time than those of recurrent

parent in all three *gca* analysis, indicating their late flowering behaviour. There may be unfavourable alleles from any of the two parents for early flowering in these CSSLs but could not be related to any marker loci in this present study as all these lines have donor introgression at varied positions on LG4. CSSL 74015 did not show any significant *gca* effects for grain yield- related traits but it exhibited significant positive *gca* effects for both fresh and dry stover yield in drought stress and across environment. This line consisted of single introgression segment from donor parent 863B at marker loci *Xpsmp305*, *Xpsmp2084* and *Xpsms16*. CSSLs 74535, 75574, 74614 and 74875 did not show any significant *gca* effects for all grain yield- related traits and also did not show consistent results despite consisting of same single marker introgression segment from 863B at marker locus *Xpsmp716*. Anyhow, combining analysis of across moisture regimes revealed that two CSSLs (74614 and 74875) out of these four CSSLs exhibited better *gca* values than those of recurrent parent for panicle length, fresh and dry stover yield. Hence there might be a putative QTL at this position on LG4 for stover yield but cannot be confirmed as this result was not observed in other CSSLs having similar introgression segment at this marker locus and also in control and stress environments. CSSLs 74411, 74449, 74481, 74526, 74539 and 74567 (which consisted of single introgression segment from donor parent at loci *Xpsmp716*, *Xpsmp2076* and *Xpsmp2081*) did not show significant and uniform results for many observed traits even though they exhibited better or worse *gca* effects here and there for few traits. These results were compared to the previous reports QTL on LG4 at marker interval *Xpsm1003d-Xpsm1007c* from ICMB 841 which includes marker *Xpsmp716* for grain yield, grain number and harvest index (Bidingger *et al.*, 2007).

Introgression lines 74567, 74605, 74618 and 74698 which shared similar introgression segments of 863B at two positions *i.e.*, one at markers *Xpsmp716*, *Xpsmp2076* and *Xpsmp2081* and another at marker *Xpsms61* also did not show considerable *gca* effects though, showing significant *gca* effects in very few instances that were not logical. Two lines (74618 and 74698) of these four lines expressed better results for fresh and dry stover yield. Significant *gca* effects were not noticed in case of single marker (*Xpsms16*) introgression segment lines 74614 and 74605, anyhow, these two lines showed better *gca* effects for plant height, panicle length, panicle diameter, and fresh and dry stover yield in one or more moisture treatments and across treatments. There was an enthusiastic notice in this experiment was that 19 lines including 13 CSSLs out of 20 and 6 RPHLs out of 8 expressed

better *gca* results when compared with recurrent parent for both fresh and dry stover yield only in across two environments. This implies that there may be a QTL for these traits on this LG4 at some marker position which were not covered in the present study. Anyhow, there were no earlier reports of stover yield QTL on this LG4 for population based on the same parents, ICMB 841 and 863B. But Yadav *et al.*, 2003 reported stover yield QTL on this LG4 at marker interval of *Xpsm265* and *Xpsm416.1* for population derived from H 77/833-2 × PRLT 2/89-33.

5.2.2.5. Linkage Group 5 (LG5)

Total of 54 testcross hybrids (involving three testers) of 5 homozygous introgression lines, 8 lines heterozygous at one or more LG5 loci along with testcrosses of their donor and recurrent parents and a sampling of 3 recurrent parent homozygotes (to serve as near-isogenic controls) of LG5, which were selected based on marker genotyping of 302 plants at 10 marker loci of LG5 (done to identify CSSLs in the previous objective) were evaluated for drought tolerance in two environments *i.e.*, fully-irrigated control conditions and terminal drought stress conditions during summer 2009 at ICRISAT, Patancheru.

Analysis of variance (ANOVA) of drought nursery trials for LG5 across moisture regimes revealed that the effect between moisture treatments was highly significant for all the measured variables except for flowering time. Hybrids did not show significant differences for both grain yield and dry stover yield in non-stress irrigated environment but these results were significant in drought stress and across environments. There were no significant differences between the lines for grain and stover yield in both non-stress control and stress environments. Anyhow there were significant differences among lines for all observed traits across two moisture regimes. There were big differences among testers for these traits in both environments and across environments. No significant differences were observed due to line x tester effects for most of the variables in both environments but for grain mass, results were significant.

General combining ability results for LG5 revealed that three introgression lines, 75035, 75094 and 75159 expressed early flowering behaviour than that of recurrent parent ICMB 841 in control and across environments. These three lines share a common introgression segment from 863B at loci *Xpsmp2078* and *Xpsms70*. This region may be linked to early flowering nature but there was one line 75064, having introgression at same markers showed late flowering character. Fourteen lines out of 18 expressed better positive *gca* effects for panicle length in fully irrigated control and across environments. None of the lines showed significant positive *gca* effects for grain yield, panicle yield and panicle harvest index. Introgression lines 75035, 75048, 75044 and 75033 (consisting introgression segment from 863B at loci *Xpsms56*, *Xpsmp2078* and *Xpsms70*) showed better *gca* effects for panicle length and stover dry matter fraction in one or both moisture treatments and across treatments, whereas worse *gca* effects for grain yield, panicle yield, grain mass, biomass yield, vegetative growth index and fresh stover yield. Interestingly, line 75048 showed better tiller number and worse panicle diameter than those of recurrent parent. These results could be compared with the reports of Nepolean *et al.*, 2006. They identified three QTLs for grain yield at marker intervals *Xicmp3027-Xbm1RA1d*, *Xbm1RA5a-Xbm 3RA1d* and *Xpsms70-Xpsm732.1* on LG5 under stress free treatment for the mapping population based on the same parents 863B and ICMB 841. They also reported that QTL at marker interval *Xpsms70-Xpsm732.1* had negative additive effect indicating unfavourable contribution of 863B allele to this trait. Introgression lines 75094, 74133, 75159 and 74173, possessing introgression segment of 863B at marker loci *Xpsmp2202*, *Xicmp3027*, *Xicmp3078*, *Xctm25*, *Xpsmp318* and *Xpsms74* also did not show any logical significant results but showed worse *gca* effects for grain yield, panicle yield and panicle harvest index and showed better effects for panicle length and grain mass when compared with the *gca* effects ICMB 841. These results are contrary to the previous report of Nepolean *et al.*, 2006 as they identified a favourable 863B allele at marker position *Xicmp3027* for grain yield and an unfavourable 863B allelic region at marker interval *Xpsmp2064* and *Xpsmp318*.

Single marker introgression segmental lines *i.e.*, 75126 (at locus *Xpsmp 318*), 75134 (at locus *Xpsms74*) and 75180 (at locus *Xctm25*) also did not show any significant positive results for any grain and stover yield- related traits. The other heterozygous introgression segmental lines and homozygous recurrent parent lines also did not show any favourable and consistent results and mostly resembling recurrent parent, ICMB 841. Yadav *et al.*, 2004 reported a QTL

at marker interval *Xpsmp2064* and *Xpsm345* for grain yield, stover yield, harvest index and panicle harvest index and Bidinger *et al.*, identified harvest index QTL at marker interval *Xpsmp2064-Xpsm318*. Unfortunately, in the present study, significant introgression segments of 863B in the genetic back ground of ICMB 841, favourable for either grain yield or stover yield- related traits were not confirmed on LG5. The reason may be that very few number of lines were evaluated and marker coverage for identification CSSLs was also not sufficient for good coverage of unique introgression segments of donor parent in the background of recurrent parent.

5.2.2.6. Linkage Group 6 (LG6)

A total 42 testcross hybrids (involving three testers) of 10 homozygous segmental introgression lines and a sampling of 2 recurrent parent homozygotes (to serve as near-isogenic controls) of LG6, which were selected based on marker genotyping of 102 plants at 9 marker loci of LG6 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in three environments *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions during summer 2010 at ICRISAT, Patancheru.

Analysis of variance (ANOVA) of drought nursery trials for LG6 across moisture regimes revealed that the effect of moisture treatments was highly significant for both grain and stover yield traits except for flowering time, plant height, panicle diameter, panicle number and stover dry matter fraction. There were significant differences between lines for all grain yield and its related traits in all three moisture conditions and across three moisture regimes. But for stover yield related traits, there were no significant differences in case of all three individual moisture environments but in case of across three environments, big differences were noticed. There were big differences between testers for both grain and stover yield traits in all three environments and across environments but results were non-significant for stover yield in fully irrigated non-stress conditions. No significant differences were observed due to line x tester effects for many variables in all three different environments.

General combining ability results for LG6 revealed that lines did not show significant *gca* effects for flowering time except donor parent and 86083 (negative combiners) and 86053 (positive combiner). Even though 86083 was recurrent parent homozygote, it showed early flowering nature like donor parent and it also exhibited better *gca* effects than those of recurrent parent for grain mass in all three moisture treatments and across treatments. There may be a favourable allele from donor parent at some markers (which were not covered in the present study) in this line, contributing for early flowering and better *gca* for grain mass. In case of CSSL 86053, there may be an unfavourable allele from one/both the parents, causing late flowering character. CSSL 86014 line (had introgression at marker loci *Xicmp3002*, *Xicmp3038* and *Xpsms41*) did not show any significant *gca* effects for grain and stover yield-related traits. Anyhow, it showed worse effects for panicle diameter and grain mass. CSSLs 86019, 86025 and 86027, consisted of donor parent introgression segment at marker loci *Xpsmp2270* and *Xpsmp2213* did not show significant *gca* effects for grain and stover yield related traits but they expressed better *gca* values than those of recurrent parent for grain yield, panicle yield, panicle harvest index, fresh and dry stover yield, grain mass and grain number per m² in at least one of three moisture regimes and across environments. All these lines showed significant positive *gca* effects for panicle diameter and despite, two CSSLs 86019 and 86027 showed significant negative *gca* effects for plant height and grain mass, these lines performed better when compared with ICMB 841 for these traits. CSSL 86042 which had single marker introgression at locus *Xicmp3038* did not show significant positive *gca* effects for all observed traits but showed significant negative *gca* effects for plant height, panicle diameter and grain mass in at least two or more environments. It was observed that this line also showed better *gca* effects than those of ICMB 841 for fresh and dry stover yield.

CSSLs 86052, 86053 and 86064 possessing long single 8 marker introgression segment from 863B at markers *Xpsmp2270*, *Xpsmp2213*, *Xicmp3058*, *Xicmp3086*, *Xicmp3050*, *Xpsms59*, *Xicmp3002* and *Xicmp3038* showed significant *gca* effects for varied traits but not consistent even though they had same introgression segment. Anyhow, the agronomic performance of all these lines was better when compared with recurrent parent. Better *gca* effects than those of ICMB 841 for grain yield, panicle yield, panicle harvest index, plant height, panicle diameter, harvest index, fresh and dry stover yield, grain mass and grain number in at least two of these three lines and also in at least one of the three and across moisture regimes. CSSL 86053 was found to be the best CSSL among all CSSLs of LG6 as it expressed better

gca values for all most all grain and stover yield and its related traits. The remaining two CSSLs 86088 and 86096 having introgression of donor parent segment at markers *viz.*, *Xicmp3058*, *Xicmp3086*, *Xicmp3050*, *Xpsms59* and *Xicmp3002* expressed varied results. CSSL 86088 exhibited significant positive results for plant height and panicle length in more than two environments whereas CSSL 86096 showed significant positive *gca* effects for biomass yield, fresh and dry stover yield only in across moisture regimes. Anyhow, both these lines found to have better performance when compared with recurrent parent for grain yield, plant height, panicle number, biomass yield, vegetative growth index, dry stover yield and grain number per m².

Six CSSLs (86019, 86027, 86053, 86064, 86088 and 86096) out of 10 exhibited better *gca* effects for grain yield and grain number per m² than that of recurrent parent in fully irrigated non-stress environment. Four CSSLs (86019, 86027, 86053 and 86096) for panicle yield and four CSSLs (86019, 86053, 86064 and 86096) and one RPHL 86015 for panicle harvest index exhibited better effects when compared with ICMB 841 in the same control treatment. These results indicated that under stress free environment, in common, three lines 86019, 86053 and 86096 expressed better results for all these four traits *viz.*, grain yield, panicle yield, panicle harvest index and grain number per m². It was noticed that six CSSLs (86019, 86025, 86052, 86053, 86064 and 86096) for grain yield and seven CSSLs (86019, 86025, 86027, 86052, 86053, 86064 and 86096) for panicle harvest index exhibited better *gca* effects than that of recurrent parent in across moisture environments. The *gca* results of the same across moisture environments demonstrated that out of 10 CSSLs, six CSSLs (86025, 86027, 86042, 86053, 86064 and 86096) for fresh stover yield and 7 CSSLs (86019, 86027, 86042, 86053, 86064, 86088 and 86096) for dry stover yield exhibited better *gca* effects than that of recurrent parent. Out of 10 CSSLs for LG6 in this study, six CSSLs (86019, 86025, 86027, 86052, 86053 and 86064) having donor parent 863B alleles at marker positions, *Xpsmp2270* and *Xpsmp2213*. As all these lines performed well for grain yield, panicle harvest index and stover yield, the present study suggest that this region *Xpsmp2270* and *Xpsmp2213* from 863B may be favourable for grain yield and panicle harvest index in stress free control and in across three environments and also for stover yield in across treatments. There is another observation that five CSSLs (86052, 86053, 86064, 86088 and 86096) out of 10 had 863B introgression at marker loci *Xicmp3058*, *Xicmp3086*, *Xicmp3050*, *Xpsms59* and *Xicmp3002*, exhibited better *gca* effects for the above four traits in both stress free control and in across

three environments. From the above results, the precise QTL for these traits could not be identified as five CSSLs out of 10 had overlapping introgression segments from donor parent.

All these above results could be comparing with earlier reports of QTLs observed on this LG6 for population based the same parents of this present study. Bidinger *et al.*, (2007) identified QTLs for grain mass in early-inset stress environment and panicle harvest index in all three and across environments at marked interval of *Xpsm588-Xpsm713*, including markers *Xpsmp2270* and *Xpsmp2213*. Yadav *et al.*, (2004) also mapped stover yield and harvest index QTL on this LG6 between markers *Xpsm514* and *Xwg110* in both non-stress and stress and across environments. They also identified QTL for panicle harvest index at marker interval *Xpsm514-Xpsm870*. In the present study, six CSSLs having donor parent 863B alleles at marker positions, *Xpsmp2270* and *Xpsmp2213* exhibited better *gca* effects for grain yield and panicle harvest index, hence this region conferred the presence of QTL for these traits on LG6 which in line with earlier reports. It was also observed that at least four lines out of these six CSSLs exhibited better *gca* values in comparison with ICMB 841 for panicle yield, panicle diameter, grain mass and grain number per m² in one or more moisture treatments. This observation indicated the probability of these markers favourable for all these traits. In case of stover yield, the present research results were in agreement with the previous report that a genomic region at marker interval (*XtstRA6c-Xicmp3002*) on LG6 was favourable for dry stover yield and was provided by 863B in stress free environment (Nepolean *et al.*, 2006).

5.2.2.7. Linkage group 7 (LG7)

A total 81 testcross hybrids (involving three testers) of 14 homozygous segmental introgression lines and a sampling of 11 recurrent parent homozygotes (to serve as near-isogenic controls) of LG7, which were selected based on marker genotyping of 227 plants at 10 marker loci of LG7 (done to identify CSSLs in the previous objective) along with their donor and recurrent parents were evaluated for drought tolerance in three environments *i.e.*, fully-irrigated control conditions, early-onset terminal drought stress conditions, and late-onset terminal drought stress conditions during summer 2010 at ICRISAT, Patancheru.

Analysis of variance (ANOVA) of drought nursery trials for LG7 across moisture regimes revealed that the effect of environment was highly significant for all the measured variables except for flowering time, plant height, panicle length, panicle number and stover dry matter fraction. Hybrids expressed significant differences for both grain and stover yield in all three different environments and across three environments but results were non-significant for dry stover yield and biomass yield in non-stress control and late-onset stress environments. There were significant differences between lines for all grain and stover yield and its related traits in early-onset stress conditions and across moisture regimes. In case of control treatment, lines did not show significant differences for panicle yield, grain number per m², biomass yield, vegetative growth index and fresh and dry stover yield. Significant differences were not observed for biomass yield, vegetative growth index, dry stover yield and grain number per m² under late-onset stress conditions. There were big differences observed between testers for both grain and stover yields in all three moisture environments and across three environments. No significant differences were observed due to line x tester effects for many variables in all three different environments.

General combining ability results for LG7 revealed that lines (three CSSLs and seven RPHLs) out of 20 flowered later to recurrent parent in early-onset drought stress conditions. Nine introgression lines, 87005, 87081, 87167, 87188 and 87195 having introgression segment of 863B at 8 markers *Xpsmp2224*, *Xpsmp2271*, *Xpsmp2074*, *Xpsmp2063*, *Xpsmp2263*, *Xpsmp2203*, *Xicmp3043* and *Xctm8* exhibited varied results despite sharing same introgression segment. It was observed that only one CSSL 87081 was found to be a better line for grain yield, panicle harvest index, plant height, panicle diameter, grain harvest index, dry stover yield and grain mass in across three environments. The remaining lines did not show considerable better *gca* effects and mostly resembling recurrent parent ICMB 841. Two CSSLs 87002 and 87134 (having introgression segment at 7 markers *Xpsmp2271*, *Xpsmp2074*, *Xpsmp2063*, *Xpsmp2263*, *Xpsmp2203*, *Xicmp3043* and *Xctm8*) and one CSSL 87148 (having introgression segment at 6 markers *Xpsmp2074*, *Xpsmp2063*, *Xpsmp2263*, *Xpsmp2203*, *Xicmp3043* and *Xctm8*) three CSSLs 87214, 87217 and 87226 (having introgression at 4 markers *Xpsmp2263*, *Xpsmp2203*, *Xicmp3043* and *Xctm8*) exhibited varied, inconsistent results. All these CSSLs mostly exhibited ICMB 841 characters with reference to grain yield and stover yield-related traits. CSSL 87071 consisting of 863B alleles at marker position *Xicmp3048* and *Xpsmp2224* did not show any favourable *gca* values for all traits

except for panicle harvest index. It expressed significant positive *gca* effect for this trait in late-onset stress and across environments. CSSL 87032 possessing introgression segment from 863B at loci *Xpsmp2271*, *Xpsmp2074* and *Xpsmp2063* also did not show significant positive *gca* effects for all observed traits but interestingly this line showed significant negative *gca* values for flowering time in all treatments and across treatments except in late-onset stress. Hence it was a best combiner line for early flowering to escape from stress in drought conditions.

The combining ability analysis for LG7 demonstrated that 10 CSSLs and 5 RPHLs in early-onset stress conditions and 7 CSSLs and 3 RPHLs in across environments expressed better *gca* results when compared with recurrent parent for panicle diameter. These results suggest that LG7 carries a drought tolerant genomic region contributing for panicle diameter. In early-onset drought stress conditions, the *gca* results revealed that 12 lines including 7 CSSLs (87005, 87081, 87130, 87134, 87167, 87217 and 87226) of 14 and 5 RPHLs of 11 exhibited better *gca* results than that of recurrent parent for dry stover yield. These results suggest that LG7 carries a QTL contributing to drought tolerance for dry stover yield. All these 7 CSSLs have common donor parent allele homozygosity at markers *Xpsmp2263*, *Xpsmp2203*, *Xicmp3043* and *Xctm8*. These results do not necessarily suggest that this region is contributing for dry stover yield because five RPHLs also exhibited better *gca* effects for this trait along with these 7 CSSLs. More marker coverage is required to really associate any genomic region favourable to this trait.

Bidinger *et al.*, (2007) identified a secondary QTL on LG7 with favourable allele from 863B at marker interval *Xpsmp2224*- *Xpsm717* for harvest index and grain yield in all stress environments and across environments. Yadav *et al.*, (2004) also mapped QTL on LG7 related to genomic region between markers *Xpsmp2074* and *Xpsmp2027* from 863B for grain yield, harvest index and panicle harvest index. This QTL was environment- specific and contributed to grain yield only in late on-set stress environment. They also reported stover yield QTL on LG7 at the same genomic region but the favourable alleles were from ICMB 841.

The pearl millet CSSLs developed and initially evaluated in the present study can be used to enhance the accuracy and stability of QTL mapping and provide a platform for research on QTL fine mapping, map-based QTL cloning, and the interaction effects between QTLs. Ultimately, these CSSLs appear to provide an excellent set of germplasm for molecular breeding experiments to improve efficiency of developing new cultivars with good grain yield and stover yield.

CHAPTER VI

SUMMARY

6. SUMMARY

The salient findings of the present study are summarized below:

- A total of 124 segmental introgression lines were identified based on genotyping data of 1492 plants, selected from advanced backcross generations. Among them, 56 unique introgression segments from 863B were identified in ICMB 841 genetic background. Out of 74 loci used for genotyping, 863B alleles were observed at 60 marker loci in at least one line. Out of 124 introgression lines identified, 100 lines each contained only single introgression segment, 22 lines had two introgression segments, and two lines had three introgression segments from 863B.
- ANOVA results of all trials for all seven LGs revealed that significant differences were observed between testcross hybrids for both grain yield and fresh stover yield in all three moisture environments (fully-irrigated control, early-onset terminal drought stress and late-onset terminal drought stress) as well as across these three moisture environments. However, in case of LG5, significant differences were not observed in non-stress control environment for either grain yield or stover yield.
- ANOVAs from across-environments analyses demonstrated that (as expected) differences between moisture treatments were significant and substantial for both grain and straw yields. It was also observed that (as expected) the differences between moisture treatments were non-significant for flowering time between moisture treatments, as the moisture treatment differences were initiated just prior to (early-onset stress) or just after (late-onset stress) flowering. G×E interactions were significant for several observed traits in testcross trials of several of the single-LG introgression line sets studied.
- Seven CSSLs containing an introgression segment of 863B alleles involving marker loci *Xpsmp2273* and *Xpsmp2069* on LG1 showed better general combining ability (*gca*) effects for grain yield, panicle yield and grain number than recurrent parent ICMB 841 in early-onset and late-onset drought stress environments, and across moisture regimes. This suggests the presence of a drought tolerance QTL for the above traits within or close proximity to the marker interval flanked by loci *Xpsmp2273* and *Xpsmp2069*. These results validate a major QTL on LG1 for grain

yield under late-onset stress conditions initially identified by Yadav *et al.* (2004); but, Bidinger *et al.* (2007) identified this QTL in stress-free environments also.

- Nine of 10 CSSLs of LG2 evaluated in this trial, as well as 3 of the 4 recurrent parent allele homozygote controls, exhibited better *gca* effects than that of recurrent parent ICMB 841 for grain yield, panicle yield, panicle harvest index, plant height, panicle length, grain harvest index, grain mass and grain number in the fully-irrigated control environment and across-environments. This result is ambiguous as there was no clear pattern of donor parent allele homozygosity associated with the detected differences. Therefore, although these CSSLs were homozygous for few genotyped 863B alleles (on LG2 itself or any other LG), these results do not necessarily suggest that LG2 carries genes contributing substantially to variation in grain yield and related traits. Instead, it appears that some portion of the genome (perhaps in distal regions of LG2) where marker information was not yet available might be contributing to the observed variation in *gca* for this set of CSSLs.
- In case of LG3, all 10 CSSLs in the late-onset drought stress and 6 CSSLs in fully-irrigated control environments were found to have better *gca* effects for dry stover yield than recurrent parent ICMB 841. It was noticed that CSSLs having introgression segment from 863B at marker position *Xctm10* exhibited better *gca* effects. These results revealed that LG3 carries genes contributing to dry stover yield, and validated a dry stover yield QTL between the marker interval *Xctm10-Xpsm174* initially identified by Nepolean *et al.* (2006) in stress free environments.
- The across-environment *gca* results for introgression lines of LG4 revealed that 19 out of 30 lines tested, including 6 homozygous recurrent parent lines, expressed better *gca* for both fresh and dry stover yield than the recurrent parent. This implies that LG4 carries genes contributing to stover yield, but lack of a consistent pattern prevented identification of the portion(s) of this linkage group that are involved. This may be due to lack of marker coverage in portions of this linkage group distal to its centromeric region, in which case enhanced marker saturation across the length of this linkage group should permit identification of the region(s) concerned.
- General combining ability results of LG5 introgression lines were non-significant for both grain and stover yield in both individual environments (a single terminal stress environment and the fully-irrigated control) and across these two environments.

These results suggest that LG5 of ICMB 841 and 863B does not carry genes contributing substantially to differences in these traits in this trial.

- Out of six lines having donor parent 863B alleles at marker positions, *Xpsmp2270* and *Xpsmp2213* on LG6, four CSSLs in the fully-irrigated control environment and five CSSLs across three environments, exhibited better *gca* effects for grain yield, panicle harvest index. These results validate a QTL on LG6 for panicle harvest index in the marker interval of *Xpsm588-Xpsm713* (which includes markers *Xpsmp2270* and *Xpsmp2213*), initially identified by Bidinger *et al.* (2007) in both stress and non-stress environments as well as across environments.
- Twelve lines including 7 CSSLs and 5 recurrent parent homozygous lines involving LG7 were found to have better *gca* effects than their recurrent parent ICMB 841 for dry stover yield in only the early-onset terminal drought stress environments. These results suggest that LG7 carries a QTL contributing to drought tolerance of dry stover yield.

CHAPTER VII

BIBLIOGRAPHY

7. BIBLIOGRAPHY

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ANNEXURE

Table1: Genotyping results of 367 plants for Linkage Group 1 (LG1)

Locus/ Line number	<i>Xpsms86</i>	<i>Xpsmp2069</i>	<i>Xpsmp2273</i>	<i>Xpsms35</i>	<i>Xpsms38</i>	<i>Xpsms58</i>	<i>Xicmp3080</i>	<i>Xicmp3017</i>	<i>Xicmp3032</i>	<i>Xctm12</i>
71001	B	B	B	B	B	B	B	B	B	B
71002	A	A	H	A	H	H	H	-	H	B
71003	A	A	H	B	H	-	H	H	H	B
71004	B	A	H	H	H	H	H	H	H	B
71005	B	A	H	H	B	-	H	H	H	B
71006	H	A	H	H	H	-	H	H	H	B
71007	H	A	H	H	B	-	H	H	H	B
71008	-	-	B	H	B	-	-	B	B	-
71009	B	B	B	B	B	B	H	H	H	B
71010	A	A	A	H	B	-	B	B	B	B
71011	-	-	B	B	B	-	B	B	B	B
71012	B	A	H	H	H	H	B	B	B	B
71013	-	-	B	B	B	-	B	B	B	B
71014	-	A	B	B	B	-	H	H	H	B
71015	B	-	B	H	A	-	H	H	H	B
71016	-	B	B	B	B	-	H	H	H	B
71017	B	B	-	B	B	H	-	B	B	-
71018	A	B	-	B	B	-	-	B	H	-
71019	-	A	A	H	B	-	H	H	H	B
71020	-	-	-	-	H	H	-	B	-	B
71021	-	-	-	H	B	-	-	-	-	-
71022	-	-	-	-	H	-	-	-	B	-
71023	-	-	H	-	H	-	H	H	H	-
71024	-	B	-	B	B	-	B	B	B	B
71025	-	-	-	-	H	-	-	B	B	-
71026	B	A	H	H	H	-	H	H	H	B
71027	-	A	-	-	H	-	-	-	-	-
71028	-	B	B	B	B	B	B	B	B	B
71029	B	B	B	B	B	-	B	B	B	B
71030	-	B	B	-	-	-	B	B	B	-
71031	-	B	B	-	-	-	B	B	B	-
71032	-	B	B	B	-	-	B	B	B	-
71033	-	B	B	-	B	-	B	B	B	-
71034	B	B	B	B	B	-	B	B	B	B
71035	-	B	-	B	B	-	B	B	-	-
71036	-	B	B	B	B	B	B	B	B	B
71037	-	B	-	B	B	-	-	B	-	-
71038	-	B	-	-	A	-	-	B	-	-
71039	-	B	-	-	B	-	-	B	-	-
71040	-	B	-	-	H	-	-	-	-	-
71041	-	A	B	-	H	-	B	B	B	-

Locus/ Line number	<i>Xpsms86</i>	<i>Xpsmp2069</i>	<i>Xpsmp2273</i>	<i>Xpsms35</i>	<i>Xpsms38</i>	<i>Xpsms58</i>	<i>Xicmp3080</i>	<i>Xicmp3017</i>	<i>Xicmp3032</i>	<i>Xctm12</i>
71042	-	B	B	B	H	-	B	B	B	B
71043	-	B	B	B	B	-	B	B	B	B
71044	-	B	B	B	B	B	B	B	B	B
71045	-	-	B	-	A	-	-	B	B	-
71046	-	-	B	-	H	-	B	B	B	B
71047	-	-	B	-	H	-	-	B	B	-
71048	-	-	-	H	B	-	-	B	-	-
71049	B	-	-	B	A	-	-	B	H	B
71050	B	B	B	B	B	B	B	B	B	B
71051	B	B	B	B	B	B	B	B	H	B
71052	B	-	H	H	B	B	H	B	H	B
71053	-	B	H	H	H	-	H	H	H	B
71054	H	B	H	H	B	H	H	H	H	B
71055	B	-	H	-	B	-	-	B	H	B
71056	-	B	B	B	B	H	B	B	B	B
71057	B	-	H	H	B	B	B	B	B	B
71058	B	-	B	B	B	-	B	B	B	B
71059	-	B	H	B	B	-	B	B	B	B
71060	B	B	H	H	H	B	B	B	H	B
71061	-	-	B	-	B	-	B	B	H	B
71062	H	B	H	H	H	H	H	H	H	B
71063	-	-	H	-	B	-	B	B	B	B
71064	-	-	B	B	B	H	B	B	B	B
71065	-	-	H	B	B	-	B	B	B	B
71066	B	-	B	-	B	-	B	B	B	B
71067	-	-	B	-	B	-	B	B	B	B
71068	-	-	B	-	H	-	B	B	B	B
71069	-	A	H	H	H	-	B	B	B	B
71070	-	B	B	B	B	-	B	B	B	B
71071	B	-	B	-	H	-	B	B	B	B
71072	H	A	H	H	H	H	B	B	B	B
71073	-	-	B	-	B	-	-	B	B	B
71074	-	-	B	B	A	-	B	B	B	B
71075	-	-	B	B	B	-	B	B	B	B
71076	-	-	B	B	B	-	B	B	B	B
71077	-	-	B	-	B	H	B	B	B	B
71078	-	-	B	-	-	-	B	B	B	B
71079	-	-	B	B	B	-	B	B	B	B
71080	A	A	A	B	B	B	B	B	B	B
71081	-	-	B	-	H	-	B	B	B	B
71082	-	A	H	H	B	-	H	H	H	B
71083	-	B	B	B	B	-	B	B	B	B

Locus/ Line number	<i>Xpsms86</i>	<i>Xpsmp2069</i>	<i>Xpsmp2273</i>	<i>Xpsms35</i>	<i>Xpsms38</i>	<i>Xpsms58</i>	<i>Xicmp3080</i>	<i>Xicmp3017</i>	<i>Xicmp3032</i>	<i>Xctm12</i>
71210	H	A	A	A	A	A	A	A	A	B
71211	H	A	H	H	H	H	B	B	B	B
71212	B	B	B	B	B	H	-	H	H	B
71213	B	A	H	H	H	H	H	H	H	B
71214	H	A	H	H	H	H	H	H	H	B
71215	B	B	B	B	B	B	B	B	B	B
71216	B	B	B	B	B	H	H	H	H	B
71217	B	B	B	B	B	H	H	B	H	B
71218	B	B	B	B	B	A	A	A	A	B
71219	B	B	B	B	B	H	A	A	A	B
71220	B	B	B	B	B	A	A	A	A	B
71221	B	B	B	B	B	-	-	B	B	B
71222	B	B	B	B	B	H	A	A	A	B
71223	B	B	B	B	B	H	B	B	B	B
71224	B	B	B	B	B	H	H	H	H	B
71225	B	B	B	B	B	H	H	H	H	B
71226	B	B	B	B	B	B	B	B	B	B
71227	B	B	B	B	B	H	A	H	H	B
71228	B	B	B	B	B	H	A	A	A	B
71229	B	B	B	B	B	H	H	H	H	B
71230	B	B	B	B	B	B	B	B	B	B
71231	B	B	B	B	B	H	B	B	B	B
71232	B	B	B	B	B	B	B	B	B	B
71233	B	B	B	B	B	H	H	H	H	B
71234	B	B	B	B	B	H	H	H	H	B
71235	B	B	B	B	B	B	B	B	B	B
71236	B	B	B	B	-	B	B	B	B	B
71237	B	B	B	B	B	B	B	B	B	B
71238	B	A	H	B	B	B	B	B	B	B
71239	B	B	B	B	B	B	B	B	B	B
71240	B	A	H	B	B	B	B	B	B	B
71241	B	A	H	B	B	B	B	B	B	B
71242	B	B	B	B	B	B	B	B	B	B
71243	B	A	H	B	B	B	B	B	B	B
71244	B	B	B	B	B	B	B	B	B	B
71245	B	A	H	B	B	B	B	B	B	B
71246	B	B	B	B	B	B	B	B	B	B
71247	B	A	A	A	A	H	H	H	H	B
71248	B	A	H	H	H	H	H	H	H	B
71249	B	A	H	H	H	H	A	H	H	B
71250	B	A	H	B	H	H	H	H	H	B
71251	B	A	H	H	H	-	H	H	H	B

Locus/ Line number	<i>Xpsms86</i>	<i>Xpsmp2069</i>	<i>Xpsmp2273</i>	<i>Xpsms35</i>	<i>Xpsms38</i>	<i>Xpsms58</i>	<i>Xicmp3080</i>	<i>Xicmp3017</i>	<i>Xicmp3032</i>	<i>Xctm12</i>
71252	B	A	H	H	H	H	H	H	H	B
71253	B	B	B	B	B	H	H	H	H	B
71254	B	B	B	B	B	B	H	H	H	B
71255	B	A	H	H	H	H	H	H	H	B
71256	B	A	A	A	A	A	H	H	H	B
71257	H	A	H	H	B	H	H	H	H	B
71258	B	A	H	B	B	H	H	H	H	B
71259	B	B	B	B	B	B	B	B	B	B
71260	B	A	H	H	B	H	H	H	H	B
71261	B	A	H	B	B	B	B	B	B	B
71262	H	A	A	A	A	A	H	H	H	B
71263	H	A	H	H	H	A	A	A	A	B
71264	H	A	A	H	H	H	H	H	H	B
71265	B	A	H	H	B	H	H	H	H	B
71266	B	A	H	H	H	H	H	H	H	B
71267	B	B	B	B	B	B	B	B	B	B
71268	B	A	A	B	A	B	B	B	B	B
71269	B	A	H	A	A	B	B	B	B	B
71270	B	A	H	H	H	B	B	B	B	B
71271	B	A	H	H	H	B	B	B	B	B
71272	B	A	H	H	H	B	B	B	B	B
71273	B	A	A	A	A	B	B	B	B	B
71274	B	A	H	H	H	B	B	B	B	B
71275	B	A	H	A	-	-	B	B	B	B
71276	B	A	H	H	H	B	B	B	B	B
71277	B	A	H	H	H	B	B	B	B	B
71278	B	B	H	H	H	B	B	B	B	B
71279	B	A	H	H	H	B	B	B	B	B
71280	B	A	H	H	H	B	B	B	B	B
71281	B	A	A	A	A	B	B	B	B	B
71282	B	A	H	H	H	B	B	B	B	B
71283	-	B	H	H	H	H	H	H	B	B
71284	-	B	B	-	H	H	H	H	H	B
71285	B	B	B	B	B	B	H	B	B	B
71286	B	B	B	B	B	B	B	B	B	B
71287	B	B	H	H	H	H	H	H	H	B
71288	B	B	B	B	B	B	B	B	B	B
71289	B	B	B	B	B	B	B	B	B	B
71290	B	B	H	H	H	-	H	A	H	B
71291	B	B	A	H	H	H	B	-	B	B
71292	B	B	H	H	B	H	H	-	H	B
71293	B	B	H	H	H	B	B	-	-	B

Locus/ Line number	<i>Xpsms86</i>	<i>Xpsmp2069</i>	<i>Xpsmp2273</i>	<i>Xpsms35</i>	<i>Xpsms38</i>	<i>Xpsms58</i>	<i>Xicmp3080</i>	<i>Xicmp3017</i>	<i>Xicmp3032</i>	<i>Xctm12</i>
71294	B	B	H	-	B	H	H	-	H	B
71295	B	B	H	H	H	H	H	-	-	B
71296	B	-	B	-	-	-	-	-	-	B
71297	B	B	B	B	B	B	B	-	B	B
71298	B	B	B	B	B	B	B	-	B	B
71299	B	B	B	B	B	B	B	-	B	B
71300	-	B	B	B	B	B	B	-	B	B
71301	B	B	B	B	B	B	B	-	B	B
71302	-	B	B	B	B	-	B	-	B	B
71303	B	B	B	B	B	B	B	-	B	B
71304	B	B	B	B	B	B	B	-	B	B
71305	B	B	B	B	B	H	B	-	B	B
71306	B	A	H	B	B	B	B	-	B	B
71307	-	A	A	A	A	H	B	-	B	B
71308	B	A	H	H	H	H	B	B	B	B
71309	H	A	H	H	H	H	B	B	B	B
71310	H	A	H	H	H	H	B	B	B	B
71311	-	B	B	B	B	B	B	B	B	B
71312	-	A	H	H	H	-	B	B	B	B
71313	-	B	B	B	B	B	B	-	B	B
71314	B	A	H	H	H	H	B	B	B	B
71315	H	A	H	H	H	H	B	-	B	B
71316	B	A	H	H	H	H	B	H	B	B
71317	H	A	H	H	H	H	B	A	H	B
71318	B	B	B	B	B	B	B	B	B	B
71319	B	A	H	H	H	H	H	-	H	B
71320	B	B	B	B	B	B	B	-	B	B
71321	-	B	B	B	B	B	H	-	H	B
71322	H	A	H	H	H	H	H	-	H	B
71323	B	B	B	B	B	B	B	-	B	B
71324	B	-	B	B	B	B	B	-	B	B
71325	B	B	B	B	B	B	B	B	B	B
71326	B	B	B	B	B	B	B	B	B	B
71327	B	B	B	B	B	B	B	-	B	B
71328	B	B	B	B	B	B	B	B	B	B
71329	B	B	B	B	B	-	B	-	B	B
71330	B	B	B	B	B	B	B	B	B	B
71331	B	B	B	B	B	B	B	-	B	B
71332	B	B	B	B	B	B	B	B	B	B
71333	B	B	B	B	B	B	B	-	B	B
71334	B	B	B	B	B	B	B	B	B	B
71335	B	B	B	B	B	B	B	-	B	B

Table 3: Genotyping results of 88 plants for Linkage Group 3 (LG3)

Locus/ Line number	<i>Xpsmp108</i>	<i>Xpsms60</i>	<i>Xpsmp2267</i>	<i>Xpsmp2070</i>	<i>Xpsmp2214</i>	<i>Xpsmp2249</i>	<i>Xpsmp2227</i>	<i>Xpsms61</i>	<i>Xpsms31</i>	<i>XCTM10</i>
73001	B	B	B	B	B	B	B	B	B	B
73002	B	B	B	B	H	H	H	B	B	A
73003	B	H	A	A	H	A	H	B	B	A
73004	A	H	H	-	H	H	H	B	B	H
73005	B	H	A	A	H	H	H	B	B	H
73006	B	B	A	A	H	B	B	B	B	B
73007	B	H	A	B	H	A	H	B	B	H
73008	B	B	A	A	H	A	H	B	B	A
73009	B	A	H	A	A	A	H	B	B	A
73010	B	H	H	A	H	H	H	B	B	H
73011	B	A	H	A	A	H	A	B	B	A
73012	B	H	H	A	H	H	H	B	B	-
73013	B	H	A	A	H	H	H	B	B	H
73014	B	B	A	A	B	B	B	B	B	B
73015	B	H	H	A	H	H	H	B	B	H
73016	B	H	-	A	H	H	H	B	B	-
73017	B	A	A	-	-	-	-	B	B	H
73018	B	A	H	A	A	A	A	B	B	A
73019	B	B	B	B	B	B	H	B	B	B
73020	B	H	A	A	H	H	H	B	B	H
73021	B	A	A	A	H	H	H	B	B	H
73022	B	A	A	A	H	H	H	B	B	H
73023	B	B	A	A	A	A	A	B	B	A
73024	B	H	A	A	H	H	H	B	B	H
73025	B	B	A	A	H	H	H	B	B	H
73026	B	H	H	A	H	H	H	B	B	H
73027	B	A	A	A	H	H	H	B	B	H
73028	B	H	H	A	H	H	H	B	B	H
73029	B	B	A	A	A	A	A	B	B	A
73030	A	B	H	A	A	A	A	B	B	A
73031	A	B	B	B	H	H	B	B	B	A
73032	A	A	H	A	B	H	H	B	B	-
73033	A	B	H	A	H	B	B	B	B	B
73034	B	B	H	A	A	A	A	B	B	A
73035	B	A	H	A	H	H	H	B	B	H
73036	B	H	H	A	A	A	A	B	B	A
73037	B	B	H	A	H	H	H	B	B	H
73038	B	B	H	A	H	H	H	B	B	H
73039	A	A	H	A	H	H	B	B	B	B
73040	A	B	A	A	H	H	H	B	B	H
73041	A	B	A	A	A	A	H	B	B	A
73042	B	B	A	A	A	A	H	B	B	A

Locus/ Line number	<i>Xpsmp108</i>	<i>Xpsms60</i>	<i>Xpsmp2267</i>	<i>Xpsmp2070</i>	<i>Xpsmp2214</i>	<i>Xpsmp2249</i>	<i>Xpsmp2227</i>	<i>Xpsms61</i>	<i>Xpsms31</i>	<i>XCTM10</i>
73043	B	H	H	A	H	H	H	B	B	H
73044	B	H	H	A	H	H	H	B	B	H
73045	B	A	B	H	H	B	B	B	B	B
73046	B	B	A	A	A	A	A	B	B	A
73047	B	A	H	A	H	H	H	B	B	H
73048	B	A	A	A	A	A	A	B	B	A
73049	B	B	B	B	H	B	B	B	B	B
73050	A	H	H	A	H	H	H	B	B	B
73051	B	H	H	A	H	A	H	B	B	H
73052	A	B	B	A	B	B	B	B	B	B
73053	B	B	B	B	B	B	B	B	H	B
73054	B	A	H	A	H	H	H	B	B	H
73055	B	H	B	B	H	H	B	B	B	B
73056	B	B	-	A	H	H	H	B	B	H
73057	B	H	H	A	H	H	H	B	B	H
73058	B	B	H	A	B	B	B	B	B	B
73059	B	H	B	B	H	H	B	B	B	B
73060	B	A	A	A	A	A	A	B	H	A
73061	B	A	H	A	H	H	H	B	H	H
73062	B	H	A	A	H	H	H	B	H	H
73063	B	A	H	A	H	H	H	B	B	H
73064	B	B	B	B	H	B	B	B	B	B
73065	B	B	B	B	H	B	B	B	B	B
73066	B	H	H	A	H	H	H	B	B	H
73067	B	B	B	B	H	B	H	B	B	B
73068	B	B	B	B	H	B	B	B	H	B
73069	B	H	H	A	H	H	H	B	H	H
73070	B	H	H	A	H	H	H	B	H	H
73071	B	A	A	A	A	A	A	B	H	A
73072	B	H	H	A	B	H	H	B	B	H
73073	B	H	B	B	H	H	B	B	B	B
73074	B	B	B	B	B	B	B	B	B	B
73075	B	B	H	A	H	H	H	B	B	H
73076	B	B	H	A	B	H	H	B	B	H
73077	B	A	B	B	H	H	H	B	B	H
73078	B	B	B	B	B	B	B	B	B	B
73079	A	A	B	B	B	B	B	B	B	B
73080	B	A	B	B	H	H	H	B	B	H
73081	B	A	B	B	-	H	B	B	B	B
73082	B	A	B	B	A	A	A	B	B	A
73083	B	B	B	B	B	B	B	B	B	B
73084	B	A	B	B	H	H	H	B	B	H
73085	B	A	B	B	H	H	H	B	B	H
73086	B	A	B	B	A	A	A	B	B	A
73087	B	A	B	B	B	B	B	B	B	B
73088	B	A	B	-	A	H	H	B	B	H

Table 4: Genotyping results of 328 plants for Linkage Group 4 (LG4)

Locus/ Line number	<i>Xpsmp716</i>	<i>Xpsmp2076</i>	<i>Xpsmp2081</i>	<i>Xpsmp305</i>	<i>Xpsmp2084</i>	<i>Xpsms27</i>	<i>Xpsms16</i>
74001	B	B	B	H	H	H	B
74002	B	B	B	B	B	B	B
74003	B	B	B	H	H	H	B
74004	B	-	B	H	H	H	B
74005	B	B	B	A	H	H	B
74006	B	B	B	B	B	-	B
74007	B	B	B	H	A	H	B
74008	B	B	B	A	H	A	B
74009	B	B	B	B	B	B	B
74010	B	B	B	H	H	H	-
74011	B	-	B	A	A	A	A
74012	B	B	B	B	B	B	B
74013	B	-	B	H	A	H	B
74014	B	B	B	A	A	A	A
74015	B	B	B	A	A	-	A
74016	B	B	B	H	H	H	H
74017	B	B	B	B	B	B	B
74018	B	B	B	B	H	B	H
74019	B	B	B	B	B	B	B
74020	B	B	B	B	B	B	B
74131	B	B	B	B	B	B	B
74132	B	B	B	B	B	B	B
74133	B	B	B	B	B	B	B
74134	B	B	B	H	B	H	B
74135	B	B	B	A	A	A	B
74136	B	B	B	H	H	H	H
74137	B	B	-	B	-	B	B
74138	B	B	B	B	H	H	H
74139	B	B	B	B	H	H	H
74140	B	B	B	B	H	H	H
74158	B	B	B	B	B	B	B
74159	B	B	B	B	B	B	B
74160	B	B	B	B	B	B	B
74161	B	B	B	B	B	B	B
74162	B	B	B	B	B	B	B
74163	B	B	B	B	B	B	B
74164	B	B	B	B	B	B	B
74165	B	B	B	B	B	B	B
74166	B	B	B	B	B	B	B
74167	B	B	B	B	B	B	B
74170	B	B	B	B	B	B	B
74171	B	B	B	B	B	B	B

Locus/ Line number	<i>Xpsmp716</i>	<i>Xpsmp2076</i>	<i>Xpsmp2081</i>	<i>Xpsmp305</i>	<i>Xpsmp2084</i>	<i>Xpsms27</i>	<i>Xpsms16</i>
74172	B	B	B	B	B	B	B
74173	B	B	B	B	B	B	B
74174	B	B	B	B	B	B	B
74175	B	B	B	B	B	B	B
74176	H	H	B	B	B	B	B
74177	H	H	B	B	B	B	B
74178	H	H	H	B	B	B	B
74179	B	B	B	B	B	B	B
74180	H	H	H	B	B	B	B
74181	H	B	B	B	B	B	B
74182	H	H	H	H	B	B	B
74183	B	B	B	B	B	B	B
74184	H	H	H	B	B	B	B
74185	B	B	H	B	B	B	B
74186	B	B	B	B	B	B	B
74377	B	B	B	B	B	B	B
74378	H	H	H	B	B	B	B
74379	H	H	B	B	B	B	B
74380	B	B	B	B	B	H	B
74381	H	H	B	B	B	B	B
74382	A	A	A	B	B	B	B
74383	A	A	A	B	B	B	B
74384	B	B	B	B	B	-	B
74385	H	H	H	B	B	B	B
74386	A	H	H	B	B	B	B
74387	B	B	H	B	B	B	B
74405	H	H	H	B	B	B	B
74406	H	H	H	B	B	B	B
74407	B	B	B	B	B	B	B
74408	B	B	H	B	B	B	B
74409	H	H	H	B	B	B	B
74410	H	H	H	B	B	B	B
74411	A	A	A	B	B	B	B
74412	B	B	B	B	B	B	B
74413	H	H	H	B	B	B	B
74414	H	H	H	B	B	B	B
74415	H	B	H	B	B	B	B
74416	A	A	A	B	B	B	B
74417	H	H	H	B	B	B	B
74418	H	A	A	B	B	B	B
74419	H	H	H	B	B	B	B
74420	H	H	H	B	B	B	B

Locus/ Line number	<i>Xpsmp716</i>	<i>Xpsmp2076</i>	<i>Xpsmp2081</i>	<i>Xpsmp305</i>	<i>Xpsmp2084</i>	<i>Xpsms27</i>	<i>Xpsms16</i>
74437	A	H	H	B	B	B	B
74438	B	H	H	B	B	B	B
74439	H	H	H	B	B	B	B
74440	H	H	H	B	B	B	B
74441	B	B	H	B	B	B	B
74442	H	H	H	B	B	B	B
74443	B	H	H	B	B	B	B
74444	H	H	H	B	B	B	B
74445	H	H	H	B	B	B	B
74446	B	B	H	B	B	B	B
74447	H	H	H	B	B	-	-
74448	A	A	-	B	B	B	B
74449	A	A	-	B	B	B	B
74450	B	H	B	B	B	B	B
74470	A	A	H	B	B	B	B
74471	H	H	H	B	B	B	B
74472	A	A	A	B	B	B	B
74473	H	H	H	B	B	B	B
74474	-	H	B	B	B	B	B
74475	B	B	B	B	B	B	B
74476	B	B	B	B	B	B	B
74477	B	H	H	B	B	B	B
74478	A	A	A	B	B	B	B
74479	H	H	H	B	B	B	B
74480	H	H	H	B	B	B	B
74481	A	A	A	B	B	B	B
74482	A	A	A	B	B	B	B
74483	B	B	B	B	B	B	B
74484	B	B	B	B	B	B	B
74522	A	H	H	B	B	B	B
74523	A	A	A	B	B	B	B
74524	H	H	H	B	B	B	B
74525	H	H	B	B	B	B	B
74526	A	A	A	B	B	B	B
74527	B	H	H	B	B	B	B
74528	H	H	H	B	B	B	B
74529	H	A	A	B	B	B	B
74530	H	H	H	B	B	B	B
74531	H	H	H	B	B	B	B
74532	H	H	H	B	B	B	B
74533	H	H	H	B	B	B	B
74534	A	A	A	B	B	B	B

Locus/ Line number	<i>Xpsmp716</i>	<i>Xpsmp2076</i>	<i>Xpsmp2081</i>	<i>Xpsmp305</i>	<i>Xpsmp2084</i>	<i>Xpsms27</i>	<i>Xpsms16</i>
74535	A	B	B	B	B	B	B
74536	H	H	H	B	B	B	B
74537	A	A	A	B	B	B	B
74538	H	H	H	B	B	B	B
74539	A	A	A	B	B	B	B
74540	H	H	H	B	H	H	B
74541	B	B	B	B	B	B	B
74542	B	B	B	B	B	B	B
74543	A	A	A	B	B	B	B
74544	B	B	B	B	B	B	B
74545	H	H	H	B	B	B	B
74546	H	H	H	B	B	B	B
74547	B	B	B	B	B	B	B
74548	H	H	H	B	B	B	B
74549	A	A	A	B	B	B	B
74550	B	B	B	B	B	B	B
74551	H	H	H	B	B	B	B
74552	H	H	H	B	B	B	B
74553	B	B	B	B	B	B	B
74554	H	H	H	B	B	B	B
74555	H	H	H	B	B	B	A
74556	A	A	A	B	B	B	H
74557	B	B	B	B	B	B	B
74558	H	H	H	B	B	-	B
74559	H	H	H	B	B	B	H
74560	A	H	H	B	B	B	H
74561	A	H	H	B	B	B	H
74562	H	H	H	B	B	B	H
74563	H	H	H	B	B	B	H
74564	H	H	H	B	B	B	H
74565	B	B	B	B	B	B	B
74566	A	B	B	B	B	B	H
74567	A	A	A	B	B	B	-
74568	B	B	B	B	B	B	H
74569	A	A	A	B	B	-	H
74570	A	A	A	B	B	B	A
74571	A	A	A	B	B	B	A
74572	B	B	H	B	B	B	H
74573	B	B	B	B	B	B	B
74574	A	B	B	B	B	B	B
74575	B	B	B	B	B	B	B
74576	A	B	H	B	B	B	B

Locus/ Line number	<i>Xpsmp716</i>	<i>Xpsmp2076</i>	<i>Xpsmp2081</i>	<i>Xpsmp305</i>	<i>Xpsmp2084</i>	<i>Xpsms27</i>	<i>Xpsms16</i>
74577	H	B	B	B	B	B	B
74578	A	B	B	B	B	B	B
74579	H	B	B	B	B	B	B
74580	B	B	H	B	B	B	B
74581	B	B	B	B	B	B	B
74582	A	B	H	B	B	B	B
74583	H	B	B	B	B	B	B
74584	A	B	H	B	B	B	B
74585	A	B	B	B	B	B	B
74586	H	B	H	B	B	B	B
74587	B	B	H	B	B	B	B
74588	H	B	H	B	B	B	B
74589	H	B	H	B	B	B	B
74590	A	B	H	B	B	B	B
74591	B	B	B	B	B	B	B
74592	H	B	H	B	B	B	B
74593	H	B	H	B	B	B	B
74594	H	B	H	B	B	B	B
74595	A	H	H	B	B	B	H
74596	H	H	H	B	B	B	H
74597	H	H	H	B	B	B	H
74598	A	A	A	B	B	B	H
74599	H	B	B	B	B	B	H
74600	H	H	H	B	B	B	A
74601	B	B	B	B	B	B	A
74602	B	H	B	B	B	B	H
74603	B	B	B	B	B	B	H
74604	H	H	H	B	B	B	A
74605	A	A	A	B	B	B	A
74606	A	H	H	B	B	B	H
74607	B	B	B	B	B	B	A
74608	H	H	H	B	B	B	H
74609	B	B	B	B	B	B	H
74610	B	B	B	B	B	B	H
74611	B	B	B	B	B	B	H
74612	A	A	A	B	B	B	H
74613	B	B	B	B	B	B	H
74614	A	B	B	B	B	B	B
74615	H	H	H	B	B	B	A
74616	B	B	B	B	B	B	H
74617	H	H	B	B	B	B	A
74618	A	A	A	B	B	B	A

Locus/ Line number	<i>Xpsmp716</i>	<i>Xpsmp2076</i>	<i>Xpsmp2081</i>	<i>Xpsmp305</i>	<i>Xpsmp2084</i>	<i>Xpsms27</i>	<i>Xpsms16</i>
74619	A	A	A	B	B	B	H
74620	A	A	A	B	B	B	A
74621	H	H	B	B	B	B	B
74622	H	H	H	B	B	B	A
74623	A	A	A	B	B	B	H
74624	H	H	H	B	B	B	B
74625	H	H	H	-	B	B	B
74626	H	H	H	B	B	B	H
74627	B	H	H	B	B	B	A
74628	H	H	H	B	B	B	H
74629	H	H	B	B	B	B	H
74630	B	B	B	B	B	B	H
74631	H	H	H	B	B	B	H
74632	H	H	H	B	B	B	A
74633	B	B	B	-	B	B	A
74634	A	H	H	B	B	B	H
74635	H	H	H	B	B	B	H
74636	H	H	H	B	B	B	H
74669	B	B	B	B	B	B	B
74670	B	B	B	B	B	B	B
74671	B	-	B	B	B	B	B
74672	B	B	B	B	B	B	B
74673	B	B	B	-	B	B	B
74674	B	B	B	B	B	B	B
74675	B	B	B	B	B	B	B
74676	B	B	B	B	B	B	B
74677	B	B	B	B	B	B	B
74678	H	B	B	B	B	B	H
74679	H	H	H	B	B	B	B
74680	B	B	B	B	B	B	H
74681	H	A	A	-	B	B	A
74682	H	H	H	B	B	B	A
74683	A	B	B	B	B	B	H
74684	H	H	H	B	B	B	B
74685	A	A	A	B	B	B	B
74686	H	H	H	B	B	B	A
74687	A	A	A	B	B	B	H
74688	A	A	A	B	B	B	H
74689	A	A	A	-	B	B	A
74690	H	H	H	B	B	B	H
74691	A	A	A	B	B	B	H
74692	B	B	B	B	B	B	B

Locus/ Line number	<i>Xpsmp716</i>	<i>Xpsmp2076</i>	<i>Xpsmp2081</i>	<i>Xpsmp305</i>	<i>Xpsmp2084</i>	<i>Xpsms27</i>	<i>Xpsms16</i>
74693	A	A	-	B	B	B	H
74694	B	B	B	B	B	B	H
74695	H	B	B	B	B	B	H
74696	H	H	H	B	B	B	B
74697	H	H	H	-	B	B	H
74698	A	A	A	B	B	B	A
74699	H	H	H	B	B	B	A
74700	H	H	H	B	B	B	H
74701	A	A	A	B	B	B	H
74702	H	B	H	B	B	B	H
74703	H	H	H	B	B	B	A
74704	H	H	H	B	B	B	A
74705	A	H	H	-	B	B	B
74706	H	-	H	B	B	B	A
74707	H	H	H	B	B	B	H
74708	H	H	H	B	B	B	H
74709	B	B	H	B	B	B	B
74710	B	B	H	B	B	B	H
74711	A	H	H	B	B	B	H
74712	H	H	H	B	B	B	H
74713	A	A	A	-	B	B	H
74714	A	A	A	B	B	B	A
74715	H	H	H	B	B	B	A
74716	H	H	H	B	B	B	H
74717	H	H	H	B	B	B	H
74737	B	B	H	-	B	B	B
74738	B	B	H	B	B	B	H
74739	B	B	H	B	B	B	A
74740	B	B	H	-	B	B	H
74741	B	B	H	B	B	B	B
74742	B	B	H	B	B	B	H
74743	A	A	A	B	B	B	H
74744	B	B	H	B	B	B	H
74745	B	B	H	B	B	B	B
74779	A	A	A	B	B	B	B
74780	H	H	H	B	B	B	B
74781	B	A	A	B	B	B	H
74782	H	H	B	B	B	B	H
74783	H	H	H	B	B	B	B
74784	H	H	H	B	B	B	B
74785	H	H	H	B	B	B	A
74786	H	H	H	B	B	B	A

Locus/ Line number	<i>Xpsmp716</i>	<i>Xpsmp2076</i>	<i>Xpsmp2081</i>	<i>Xpsmp305</i>	<i>Xpsmp2084</i>	<i>Xpsms27</i>	<i>Xpsms16</i>
74787	-	B	B	B	B	B	H
74788	A	H	H	B	B	B	H
74789	H	H	B	B	B	B	A
74790	-	H	H	B	B	B	A
74791	H	H	H	B	B	B	B
74814	H	A	A	H	B	H	H
74815	A	A	H	B	B	H	H
74816	A	A	A	H	B	H	H
74817	-	H	B	B	B	H	H
74818	-	A	-	H	B	H	H
74819	A	A	H	H	B	H	H
74820	A	A	H	B	B	H	H
74821	B	H	H	H	B	H	H
74822	-	A	-	B	B	B	H
74823	A	H	B	H	-	H	H
74824	B	H	B	B	B	H	B
74825	-	H	-	B	B	H	H
74826	B	-	H	B	B	H	H
74827	B	A	H	H	B	H	H
74828	B	A	H	H	B	H	H
74864	H	H	H	H	B	H	H
74865	A	A	-	B	B	B	H
74866	B	H	B	H	-	H	H
74867	B	H	B	H	B	H	H
74868	-	H	B	H	B	H	H
74869	B	H	B	H	B	H	H
74870	B	B	B	B	B	B	B
74871	B	H	H	B	-	B	H
74872	A	H	H	B	-	B	H
74873	A	H	H	B	-	B	H
74874	B	B	B	B	B	B	B
74875	A	B	B	B	B	B	B
74876	H	H	H	B	-	B	H
74877	B	H	B	B	B	B	H

Table 5: Genotyping results of 302 plants for Linkage Group 5 (LG5)

Locus/ Line number	<i>Xpsmp2202</i>	<i>Xicmp3027</i>	<i>Xicmp3078</i>	<i>Xctm25</i>	<i>Xpsmp318</i>	<i>Xpsms74</i>	<i>Xpsms56</i>	<i>Xpsmp2078</i>	<i>Xpsms70</i>	<i>Xpsmp2229</i>
75001	B	B	B	-	B	B	B	B	B	B
75002	B	-	B	B	B	B	B	B	B	B
75003	B	B	-	B	B	B	B	B	B	B
75004	B	B	B	B	B	B	B	B	B	B
75005	B	B	B	B	B	B	B	B	B	B
75006	B	B	B	B	B	B	B	B	B	B
75007	B	B	B	B	B	B	B	B	B	B
75008	B	H	B	B	B	B	B	B	B	H
75009	B	H	B	B	B	B	B	B	B	B
75010	B	B	B	B	B	B	B	B	B	B
75011	A	A	A	A	A	A	A	A	H	B
75012	H	A	A	H	H	H	H	H	H	B
75013	B	B	B	B	-	B	B	B	B	B
75014	H	A	H	H	H	H	H	B	B	B
75015	H	A	H	H	H	H	H	-	H	B
75016	H	A	H	B	H	H	H	H	B	B
75017	H	A	A	H	H	H	H	-	H	B
75018	H	A	H	H	H	H	H	H	H	B
75019	A	A	-	A	A	A	A	-	H	B
75020	H	A	H	H	H	H	H	H	H	B
75021	H	A	A	H	H	H	H	H	H	B
75022	A	A	A	A	A	A	A	A	A	B
75023	-	A	A	A	A	A	A	B	B	-
75024	H	A	H	H	H	H	H	-	H	B
75025	H	A	H	H	H	H	H	H	H	H
75026	A	A	A	A	A	-	A	H	B	H
75027	H	A	H	H	H	H	H	H	H	B
75028	H	A	H	B	H	-	H	B	H	B
75029	H	A	H	H	H	H	H	A	H	B
75030	B	B	B	B	B	B	B	H	H	B
75031	B	B	B	B	B	-	B	B	B	H
75032	B	B	B	B	B	B	B	B	B	B
75033	B	H	B	B	B	B	A	A	A	H
75034	B	B	B	B	B	B	A	A	A	H
75035	B	B	B	B	B	B	A	A	A	H
75036	B	H	B	B	B	H	H	B	B	H
75037	B	B	B	B	B	B	H	B	H	H
75038	B	B	B	B	B	A	H	H	H	H
75039	B	B	B	B	B	B	H	B	B	B
75040	B	B	B	B	B	A	B	H	H	H
75041	B	B	B	B	B	B	H	A	A	H
75042	B	B	B	B	B	B	H	A	A	H
75043	B	B	B	B	B	B	B	H	H	H
75044	B	B	B	B	B	B	B	B	B	B
75045	B	B	B	B	B	B	H	H	H	H

Locus/ Line number	<i>Xpsmp2202</i>	<i>Xicmp3027</i>	<i>Xicmp3078</i>	<i>Xctm25</i>	<i>Xpsmp318</i>	<i>Xpsms74</i>	<i>Xpsms56</i>	<i>Xpsmp2078</i>	<i>Xpsms70</i>	<i>Xpsmp2229</i>
75091	H	A	H	H	H	H	B	H	H	H
75092	H	H	B	B	B	B	B	B	B	B
75093	A	A	A	A	A	A	B	A	A	B
75094	A	A	A	H	A	A	B	A	A	B
75095	H	A	H	H	H	B	B	A	A	H
75096	B	B	B	B	B	B	B	B	B	B
75097	B	H	B	H	B	B	B	B	B	B
75098	B	B	B	A	B	B	B	A	A	B
75099	B	B	B	B	B	B	B	-	A	B
75100	B	B	B	B	B	B	B	H	A	B
75101	H	A	-	H	H	-	-	-	-	B
75102	B	B	B	B	B	B	B	B	B	B
75103	B	B	B	B	B	B	B	H	A	B
75104	B	B	B	H	B	B	B	H	H	B
75105	H	H	H	H	H	H	B	-	A	B
75106	H	H	H	B	H	H	H	A	A	H
75107	H	H	H	H	H	H	A	-	A	B
75108	B	B	B	B	B	B	B	B	B	B
75109	H	H	H	B	H	H	H	H	H	B
75110	H	H	H	H	H	H	H	H	H	B
75111	H	H	H	H	H	H	A	H	H	B
75112	H	H	H	H	H	H	B	B	B	B
75113	H	H	H	H	H	H	B	B	B	B
75114	A	A	A	A	A	A	A	A	A	H
75115	H	H	H	H	H	H	A	A	A	H
75116	H	H	H	B	H	H	H	A	A	B
75117	A	A	A	-	A	A	A	A	A	-
75118	A	A	A	A	A	A	H	H	H	B
75119	H	H	H	B	H	H	A	A	A	H
75120	A	H	A	H	H	H	H	H	H	H
75121	A	H	A	H	H	H	H	H	B	H
75122	B	B	B	B	B	B	B	H	H	B
75123	A	A	A	H	A	A	B	H	H	H
75124	H	H	H	H	H	B	B	H	H	B
75125	A	A	H	H	H	H	B	H	H	B
75126	B	B	B	B	A	B	B	B	B	B
75127	A	A	A	A	A	H	-	H	B	B
75128	B	B	B	B	-	B	B	B	B	B
75129	H	H	H	B	H	H	B	H	B	B
75130	B	B	B	B	B	B	B	B	B	B
75131	H	A	H	H	H	H	B	H	H	B
75132	B	B	B	B	B	B	B	B	B	B
75133	A	A	A	A	A	A	B	H	A	B
75134	B	B	B	B	B	A	B	H	H	B
75135	H	H	H	H	H	H	B	B	H	B

Locus/ Line number	<i>Xpsmp2202</i>	<i>Xicmp3027</i>	<i>Xicmp3078</i>	<i>Xctm25</i>	<i>Xpsmp318</i>	<i>Xpsms74</i>	<i>Xpsms56</i>	<i>Xpsmp2078</i>	<i>Xpsms70</i>	<i>Xpsmp2229</i>
75136	H	H	H	H	H	H	H	B	B	B
75137	H	H	H	H	H	H	A	H	H	B
75138	H	H	H	H	H	A	H	H	H	H
75139	H	H	H	H	H	H	H	H	H	B
75140	B	B	B	B	B	B	H	H	A	H
75141	H	A	H	B	H	H	-	H	H	B
75142	H	A	H	H	H	H	H	H	A	A
75143	A	A	A	-	A	H	A	A	A	B
75144	H	B	H	-	H	H	H	H	A	B
75145	H	H	H	H	H	H	H	A	A	H
75146	B	B	B	B	B	B	B	-	B	B
75147	H	A	H	H	H	H	H	H	A	B
75148	B	B	B	B	B	B	B	H	H	B
75149	H	H	H	H	H	H	H	B	B	B
75150	H	H	H	H	H	H	H	A	A	B
75151	A	B	H	B	H	H	H	H	H	B
75152	B	B	B	B	H	B	H	H	H	B
75153	B	H	B	B	B	B	B	H	H	B
75154	H	H	H	B	H	B	A	H	H	H
75155	A	A	A	H	H	H	H	H	H	B
75156	B	B	B	B	B	B	H	H	H	B
75157	H	B	B	B	B	B	H	H	H	B
75158	B	H	B	B	B	B	B	H	A	B
75159	A	A	A	A	A	A	A	A	A	B
75160	H	H	H	B	H	H	H	H	H	B
75161	A	H	B	H	B	H	H	A	H	H
75162	H	H	H	H	H	H	B	H	H	B
75163	H	H	H	B	H	H	B	B	B	B
75164	B	B	B	B	B	B	B	B	B	B
75165	H	H	H	B	H	H	B	B	B	B
75166	H	H	H	H	H	H	B	B	B	B
75167	H	H	H	H	H	H	B	B	B	B
75168	H	H	H	H	H	H	B	B	B	B
75169	H	H	H	B	B	B	B	B	B	B
75170	H	B	B	B	B	B	B	B	B	B
75171	B	B	B	B	B	B	B	B	B	B
75172	H	H	A	H	H	H	B	B	B	B
75173	A	A	A	A	A	A	B	B	B	B
75174	H	A	H	A	A	A	B	-	B	B
75175	B	B	B	B	B	B	B	B	B	B
75176	H	H	H	H	H	H	B	B	A	B
75177	H	H	H	B	H	H	B	B	B	B
75178	B	B	B	A	B	B	A	A	H	B
75179	B	B	B	H	B	B	A	A	B	B
75180	B	H	B	A	B	B	B	B	B	B

Locus/ Line number	<i>Xpsmp2202</i>	<i>Xicmp3027</i>	<i>Xicmp3078</i>	<i>Xctm25</i>	<i>Xpsmp318</i>	<i>Xpsms74</i>	<i>Xpsms56</i>	<i>Xpsmp2078</i>	<i>Xpsms70</i>	<i>Xpsmp2229</i>
75181	B	B	B	B	B	B	H	H	B	B
75182	B	B	B	B	B	B	B	H	B	B
75183	B	B	B	B	B	B	B	B	B	B
75184	B	H	B	B	B	B	H	B	B	B
75185	B	H	B	B	B	B	H	H	B	B
75186	B	B	B	A	B	B	A	A	B	B
75187	B	B	B	-	B	B	A	A	B	B
75188	B	B	B	B	B	B	B	-	B	B
75189	B	B	B	B	B	B	B	B	B	B
75190	B	B	B	B	B	B	A	A	B	B
75191	B	B	B	A	B	B	H	B	B	H
75192	B	B	B	-	B	B	B	H	B	B
75193	B	H	B	B	B	B	H	B	B	B
75194	H	A	H	H	H	H	H	B	B	B
75195	H	H	B	B	H	H	H	-	H	B
75196	A	H	-	H	H	H	H	H	B	B
75197	A	A	A	H	H	A	B	B	B	B
75198	H	A	-	B	B	B	H	-	H	B
75199	H	A	H	B	H	H	H	H	H	B
75200	B	H	B	B	B	A	B	H	H	-
75201	A	A	A	B	A	A	A	-	H	B
75202	B	B	B	B	B	B	H	H	H	H
75203	H	H	H	H	H	H	H	B	H	B
75204	H	H	H	B	H	H	H	H	H	B
75205	B	B	B	B	B	B	B	H	H	B
75206	B	B	B	B	B	B	B	B	H	B
75207	B	B	B	B	B	B	B	B	B	B
75208	B	H	B	B	B	B	B	B	B	B
75209	B	B	B	B	B	B	B	B	A	B
75210	B	B	B	B	B	B	B	B	H	B
75211	B	B	B	B	B	B	B	B	B	H
75212	B	B	B	B	B	B	B	B	H	H
75213	B	B	B	H	B	B	B	B	H	B
75214	B	B	B	B	B	B	B	B	H	H
75215	B	H	B	B	B	A	B	B	A	B
75216	B	B	B	B	B	H	B	B	B	B
75217	B	B	B	B	B	B	B	B	B	B
75218	B	B	A	B	B	B	B	B	A	B
75219	H	H	H	B	H	H	H	H	A	B
75220	B	B	H	H	B	B	H	A	A	B
75221	A	A	B	A	A	A	B	B	H	B
75222	A	A	H	B	H	H	H	H	H	B
75223	A	-	B	B	A	H	H	B	B	B
75224	H	H	H	B	H	H	H	B	H	B
75225	B	B	B	B	B	H	H	B	A	B

Locus/ Line number	<i>Xpsmp2202</i>	<i>Xicmp3027</i>	<i>Xicmp3078</i>	<i>Xctm25</i>	<i>Xpsmp318</i>	<i>Xpsms74</i>	<i>Xpsms56</i>	<i>Xpsmp2078</i>	<i>Xpsms70</i>	<i>Xpsmp2229</i>
75226	B	B	B	B	B	B	B	H	B	B
75227	A	A	H	B	A	A	A	A	B	H
75228	A	A	B	H	A	A	A	A	H	H
75229	B	B	A	B	B	B	B	H	H	B
75230	H	H	A	-	H	H	H	H	H	B
75231	B	B	A	B	B	B	B	B	A	B
75232	H	H	H	B	H	H	B	H	H	B
75233	A	A	B	B	A	H	H	A	B	H
75234	H	B	B	B	B	B	B	B	A	B
75235	H	H	A	B	H	H	H	H	B	B
75236	B	B	A	B	B	B	B	A	B	H
75237	H	H	B	B	H	H	B	B	B	B
75238	B	B	A	B	B	B	H	H	B	H
75239	H	H	B	B	H	H	H	H	B	H
75240	H	H	H	B	H	H	-	H	H	B
75241	H	H	H	B	H	H	B	H	H	-
75242	B	B	-	B	B	B	B	B	A	B
75243	A	A	B	B	A	A	A	A	H	H
75244	B	B	B	B	B	B	B	H	H	H
75245	H	A	H	B	H	H	A	A	A	-
75246	H	H	A	B	H	H	H	H	H	B
75247	H	H	B	B	H	H	B	B	B	B
75248	B	B	B	B	B	B	B	B	B	B
75249	B	B	H	B	B	B	H	H	-	B
75250	H	H	B	B	H	H	H	A	B	B
75251	H	H	A	B	H	H	H	H	B	B
75252	H	H	B	H	H	H	H	B	B	B
75253	H	H	H	B	H	A	H	B	B	H
75254	H	H	H	B	H	H	B	B	B	B
75255	B	B	B	B	B	B	H	B	B	B
75256	A	A	B	A	H	H	B	B	H	B
75257	B	A	B	-	B	-	-	-	B	B
75258	B	B	H	B	B	B	B	B	A	B
75259	B	B	H	B	B	B	H	B	H	B
75260	H	H	H	B	H	H	H	B	B	B
75261	A	A	H	H	A	H	H	B	B	B
75262	B	B	H	B	B	B	H	B	B	B
75263	B	B	B	B	B	B	B	B	B	B
75264	A	A	A	B	H	H	B	B	B	B
75265	B	H	A	B	H	B	B	B	H	B
75266	H	H	B	B	H	H	H	B	B	B
75267	B	B	B	B	B	B	B	B	B	B
75268	B	B	B	B	B	B	A	A	H	H
75269	B	B	B	B	B	B	B	B	B	B
75270	B	B	B	B	B	B	A	H	H	B

Locus/ Line number	<i>Xpsmp2202</i>	<i>Xicmp3027</i>	<i>Xicmp3078</i>	<i>Xctm25</i>	<i>Xpsmp318</i>	<i>Xpsms74</i>	<i>Xpsms56</i>	<i>Xpsmp2078</i>	<i>Xpsms70</i>	<i>Xpsmp2229</i>
75271	B	H	B	-	B	B	H	B	B	B
75272	B	H	B	B	B	B	A	A	A	B
75273	B	H	B	B	B	B	H	-	A	B
75274	B	B	H	B	B	B	H	A	A	H
75275	B	B	B	B	B	B	H	B	B	B
75276	B	B	B	B	B	B	B	B	B	B
75277	B	B	B	B	B	B	H	H	H	B
75278	B	B	B	B	B	B	H	H	H	B
75279	B	B	B	-	B	B	B	B	H	B
75280	B	H	B	B	B	B	H	B	B	B
75281	B	H	B	B	B	H	H	H	H	H
75282	B	B	B	B	B	B	B	H	H	A
75283	B	B	B	B	B	B	B	H	H	H
75284	B	B	B	H	B	B	A	A	A	H
75285	B	B	B	B	B	B	H	H	H	H
75286	B	H	B	-	H	H	H	B	B	B
75287	B	B	B	-	H	H	B	B	B	B
75288	B	B	B	B	B	B	H	B	H	B
75289	B	B	B	A	A	A	A	B	B	B
75290	B	B	B	B	B	B	B	B	B	B
75291	B	B	B	A	A	A	B	B	B	B
75292	B	B	B	B	B	B	H	B	B	B
75293	B	B	B	H	H	H	H	B	B	B
75294	B	B	B	-	H	A	B	B	B	B
75295	B	B	B	H	H	A	H	B	B	B
75296	B	B	B	B	B	B	B	B	B	B
75297	B	B	B	-	A	A	-	B	B	B
75298	B	B	B	A	A	A	B	B	B	B
75299	B	B	A	B	B	B	B	B	B	H
75300	B	B	B	B	H	H	H	B	B	B
75301	B	B	B	A	A	A	H	B	B	-
75302	B	B	B	H	A	A	-	B	B	B

Locus/ Line number	<i>Xpsmp2270</i>	<i>Xpsmp2213</i>	<i>Xicmp3058</i>	<i>Xicmp3086</i>	<i>Xicmp3050</i>	<i>Xpsms59</i>	<i>Xicmp3002</i>	<i>Xicmp3038</i>	<i>Xpsms41</i>
86085	B	B	B	B	B	H	B	B	B
86086	H	H	H	H	H	H	B	B	B
86087	B	B	B	H	H	H	B	H	B
86088	B	B	A	A	A	A	H	B	B
86089	B	B	B	B	B	H	H	B	B
86090	B	B	A	H	H	H	H	B	B
86091	B	B	H	B	B	H	A	B	B
86092	B	B	B	B	B	B	B	B	B
86093	B	B	H	H	H	H	B	B	B
86094	B	B	H	H	H	B	B	H	B
86095	B	B	H	H	H	H	H	B	B
86096	B	B	A	A	A	A	A	B	B
86097	B	B	A	A	A	H	H	B	B
86098	B	B	H	H	H	H	H	B	B
86099	B	B	H	H	H	H	A	B	B
86100	B	B	A	A	A	A	A	B	B
86101	H	H	B	H	H	H	H	B	B
86102	B	B	A	A	A	A	H	B	B

Table 7: Genotyping results of 227 plants for Linkage Group7 (LG7)

Locus/ Line number	<i>Xicmp3048</i>	<i>Xpsmp2224</i>	<i>Xpsmp2271</i>	<i>Xpsmp2074</i>	<i>Xpsmp2063</i>	<i>Xpsmp2263</i>	<i>Xpsmp2203</i>	<i>Xicmp3043</i>	<i>Xctm8</i>
87001	B	B	-	B	B	B	B	-	B
87002	B	B	A	A	A	A	A	A	A
87003	B	B	H	H	A	A	A	A	A
87004	B	H	A	A	A	A	A	A	A
87005	B	A	-	A	A	A	-	-	A
87006	B	H	A	A	A	A	A	A	A
87007	B	B	B	B	B	B	H	B	B
87008	B	H	H	H	B	H	H	H	B
87009	B	H	A	A	A	A	A	A	A
87010	B	H	B	B	B	B	B	B	B
87011	B	H	H	H	B	H	H	H	B
87012	B	B	B	B	B	B	B	B	B
87013	B	H	-	A	H	H	H	-	B
87014	B	H	A	A	A	A	H	A	A
87015	B	H	H	A	H	H	H	H	B
87016	B	B	B	B	B	B	B	B	B
87017	B	A	H	H	B	H	B	H	B
87018	B	H	H	H	B	H	H	H	B
87019	B	A	A	-	-	H	H	H	B
87020	B	B	B	B	B	H	H	H	B
87021	B	B	H	H	B	H	H	H	B
87022	B	B	H	H	B	A	H	A	A
87023	B	B	B	H	B	H	B	H	B
87024	B	B	H	A	A	A	A	A	A
87025	B	B	H	H	B	H	H	H	B
87026	B	B	-	H	B	H	H	-	B
87027	B	B	H	H	B	H	H	H	B
87028	B	B	H	H	B	H	B	H	B
87029	B	B	-	B	B	B	-	-	B
87030	B	B	H	H	B	H	B	H	B
87031	B	B	H	H	B	H	H	H	B
87032	B	B	A	A	A	B	B	B	B
87033	B	B	-	B	B	H	-	-	B
87034	B	B	-	A	B	H	-	-	-
87035	B	B	B	B	B	-	B	B	B
87036	B	B	-	H	H	H	H	H	B
87037	H	H	H	H	B	H	H	H	B
87038	H	B	H	H	B	H	H	H	B
87039	H	B	B	B	B	H	H	H	B

Locus/ Line number	<i>Xicmp3048</i>	<i>Xpsmp2224</i>	<i>Xpsmp2271</i>	<i>Xpsmp2074</i>	<i>Xpsmp2063</i>	<i>Xpsmp2263</i>	<i>Xpsmp2203</i>	<i>Xicmp3043</i>	<i>Xctm8</i>
87040	B	B	H	H	B	H	A	H	B
87041	B	B	B	B	B	B	B	B	B
87042	B	B	-	H	B	H	H	-	B
87043	B	B	H	A	H	-	H	H	B
87044	H	B	H	A	H	H	H	H	B
87045	B	H	H	H	H	H	H	H	B
87046	A	A	H	A	H	H	H	H	B
87047	H	A	A	A	H	A	A	A	A
87048	B	B	H	H	B	A	H	H	B
87049	A	A	A	A	A	A	A	A	A
87050	H	H	-	A	H	H	H	-	-
87051	H	H	H	A	H	H	H	H	B
87052	H	B	H	A	H	H	H	H	B
87053	H	H	H	A	H	B	B	B	B
87054	H	H	-	A	H	B	-	-	B
87055	H	A	A	A	A	A	B	B	A
87056	B	B	B	B	B	B	B	B	B
87057	H	A	H	-	B	B	B	B	A
87058	H	H	-	H	B	H	B	-	B
87059	H	H	B	B	B	H	B	B	B
87060	A	B	H	A	B	B	B	B	B
87061	H	H	H	H	B	B	B	B	B
87062	H	H	H	H	B	B	B	B	B
87063	H	H	H	H	B	B	B	B	B
87064	H	A	H	A	H	H	H	H	B
87065	A	H	H	A	H	H	A	H	B
87066	H	H	H	A	H	H	H	H	B
87067	H	B	H	A	H	H	H	H	B
87068	H	H	H	H	B	B	B	B	B
87069	A	H	B	B	B	H	H	H	B
87070	A	A	A	A	A	A	A	A	A
87071	A	A	B	B	B	B	B	B	B
87072	H	B	B	B	B	B	B	B	B
87073	A	A	A	H	H	H	H	H	-
87074	B	B	B	B	B	H	A	H	B
87075	H	H	H	A	H	H	H	H	B
87076	B	B	B	B	H	H	H	H	B
87077	H	H	-	H	B	B	B	-	-
87078	H	H	H	H	H	H	H	H	B
87079	B	B	H	A	H	H	H	H	B

Locus/ Line number	<i>Xicmp3048</i>	<i>Xpsmp2224</i>	<i>Xpsmp2271</i>	<i>Xpsmp2074</i>	<i>Xpsmp2063</i>	<i>Xpsmp2263</i>	<i>Xpsmp2203</i>	<i>Xicmp3043</i>	<i>Xctm8</i>
87120	-	B	B	B	B	B	B	B	B
87121	B	B	B	B	B	B	B	B	B
87122	B	B	B	B	B	B	B	B	B
87123	B	B	B	B	B	B	B	B	B
87124	B	B	-	H	B	H	H	H	B
87125	B	B	H	A	A	H	B	H	B
87126	B	B	B	B	B	H	H	H	B
87127	B	B	B	H	H	-	B	B	B
87128	B	B	-	A	H	H	H	-	B
87129	B	B	H	H	H	H	H	H	B
87130	B	B	-	A	-	-	A	-	A
87131	A	B	H	H	B	-	H	H	B
87132	-	-	-	H	B	-	-	-	B
87133	B	B	H	H	B	H	H	H	B
87134	B	B	A	A	A	A	A	A	A
87135	-	B	H	-	-	-	H	H	B
87136	B	B	A	A	A	H	H	H	B
87137	B	B	H	A	H	H	H	H	B
87138	B	B	H	H	B	-	H	B	B
87139	B	B	H	H	B	H	H	-	B
87140	B	B	H	H	A	H	H	H	B
87141	B	B	H	A	A	H	B	H	B
87142	B	B	B	H	B	H	H	H	B
87143	B	H	H	B	B	B	B	B	B
87144	B	B	B	H	H	H	H	H	B
87145	B	B	B	A	H	H	H	H	B
87146	B	B	B	H	B	H	H	H	B
87147	B	B	B	-	-	H	H	H	B
87148	B	B	B	A	A	A	A	A	A
87149	B	B	B	A	A	A	H	H	B
87150	B	B	B	B	B	B	B	B	B
87151	B	B	B	H	B	H	H	H	B
87152	B	B	B	H	H	H	H	H	B
87153	B	B	B	H	H	H	H	H	B
87154	B	B	H	H	B	H	H	H	B
87155	-	B	H	H	B	-	H	H	B
87156	B	B	A	H	B	B	H	B	B
87157	B	B	B	B	B	B	B	B	B
87158	B	B	H	A	B	H	H	H	B
87159	B	B	H	H	H	H	H	H	B

Locus/ Line number	<i>Xicmp3048</i>	<i>Xpsmp2224</i>	<i>Xpsmp2271</i>	<i>Xpsmp2074</i>	<i>Xpsmp2063</i>	<i>Xpsmp2263</i>	<i>Xpsmp2203</i>	<i>Xicmp3043</i>	<i>Xctm8</i>
87160	B	B	H	-	H	H	H	H	B
87161	B	B	B	H	B	H	H	H	B
87162	B	B	H	H	H	A	A	A	A
87163	B	B	H	H	H	H	H	H	B
87164	B	H	B	B	B	B	B	B	B
87165	B	H	A	A	A	A	H	A	B
87166	B	B	H	H	B	H	H	H	B
87167	B	A	A	A	A	A	A	A	A
87168	B	H	H	H	B	A	A	A	A
87169	B	H	H	H	B	A	H	H	B
87170	B	B	B	B	B	B	B	B	B
87171	B	H	A	A	A	A	A	A	-
87172	-	H	H	H	B	H	H	H	B
87173	B	H	B	B	B	H	H	H	B
87174	B	B	B	B	B	B	B	B	B
87175	B	B	B	B	B	A	H	H	B
87176	B	H	H	H	H	H	H	H	B
87177	B	H	H	A	A	A	B	A	A
87178	B	H	H	H	B	H	H	H	B
87179	B	H	A	A	A	A	H	A	-
87180	B	H	-	H	B	H	-	-	B
87181	B	B	H	H	H	H	B	H	B
87182	B	H	H	A	A	A	H	A	A
87183	B	H	B	H	B	H	H	H	B
87184	B	H	H	H	B	H	H	H	B
87185	B	A	H	H	A	H	H	H	B
87186	B	B	H	A	A	A	A	A	A
87187	B	H	H	H	B	B	B	B	B
87188	B	A	A	A	A	A	A	A	A
87189	B	H	H	H	A	A	A	A	A
87190	B	H	A	H	H	H	H	H	-
87191	B	H	H	H	H	H	B	H	B
87192	B	H	A	A	A	B	B	H	-
87193	B	H	A	A	A	B	A	A	A
87194	B	H	H	H	H	B	B	H	A
87195	B	A	A	A	A	B	A	A	A
87196	B	H	H	H	B	B	H	H	B
87197	B	H	A	A	A	B	A	A	A
87198	B	B	H	H	H	H	H	H	B
87199	B	H	A	A	A	B	H	A	A

Locus/ Line number	<i>Xicmp3048</i>	<i>Xpsmp2224</i>	<i>Xpsmp2271</i>	<i>Xpsmp2074</i>	<i>Xpsmp2063</i>	<i>Xpsmp2263</i>	<i>Xpsmp2203</i>	<i>Xicmp3043</i>	<i>Xctm8</i>
87200	B	H	B	B	B	A	A	A	A
87201	B	B	H	H	B	H	B	B	B
87202	B	B	-	H	-	H	-	-	B
87203	B	B	H	H	H	H	H	H	B
87204	B	B	B	B	H	H	H	H	B
87205	B	B	H	H	H	H	H	H	-
87206	B	B	B	H	H	H	H	-	B
87207	B	B	B	H	H	H	A	H	A
87208	B	B	H	-	H	H	B	-	B
87209	B	B	B	B	B	B	B	B	B
87210	-	B	H	H	B	-	B	B	B
87211	B	B	B	B	B	A	H	A	A
87212	B	B	B	B	B	H	H	H	B
87213	B	B	B	-	B	H	H	H	B
87214	B	B	B	B	B	A	A	A	A
87215	B	B	B	B	B	H	H	H	B
87216	B	B	B	B	B	H	H	H	B
87217	B	B	B	B	B	A	A	A	A
87218	-	B	B	B	B	-	H	A	A
87219	B	B	B	B	B	A	H	A	A
87220	B	B	B	B	B	H	H	H	B
87221	B	B	B	B	B	H	H	H	B
87222	B	B	B	B	B	A	A	A	A
87223	B	B	-	B	B	B	H	B	B
87224	B	B	B	B	B	A	H	A	B
87225	B	B	B	B	B	H	H	H	B
87226	B	B	B	B	B	A	A	A	A
87227	B	B	B	B	B	A	H	A	A