GENETIC AND QTL ANALYSES OF SINK SIZE TRAITS IN PEARL MILLET (*Pennisetum glaucum* (L.) R. Br.)

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CERTIFICATE

This is to certify that this thesis entitled **"Genetic and QTL analyses of sink size traits in pearl millet (*Pennisetum glaucum* (L.) R. Br.)"** submitted in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy (Agriculture) in the subject of Plant Breeding and Genetics to the Tamil Nadu Agricultural University, Coimbatore, is a record of bonafide research work carried out by **Mr. V.VENGADESSAN** under my supervision and guidance and that no part of this thesis has been submitted for any other degree, diploma or other similar titles or prizes and that the work has not been published in scientific or popular journal or magazine.

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Abstract

Genetic and QTL analyses of sink size traits in pearl millet

(*Pennisetum glaucum* (L.) R. Br.)

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Present study was carried out to characterize the genetic architecture of three sink size component traits (panicle length, panicle diameter and grain size) through genetic and QTL analyses. The plant materials for genetic analysis consisted of two crosses for the generation means and variance analyses, and one cross for triple test cross (TTC) analysis for each of three traits. The material for QTL analysis consisted of 188 F_2 and their $F_{2:3}$ progeny mapping populations of a cross between the two inbred lines. The plant materials were developed during the 2005-06 and the field experiments were conducted during the 2006 rainy and 2007 summer seasons.

Scaling and joint scaling tests revealed that a simplistic additive-dominance model did not adequately explain the observed variation for all the three traits in both seasons, providing an evidence for the presence of epistasis. The six-parameter model and the TTC analysis revealed significance of both additive and dominance effects for cross 1 of panicle length, panicle diameter and grain size. However, cross 2 of panicle

length and panicle diameter revealed only additive effects and grain size showed the presence of both additive and dominance gene effects. All three types of interactions (additive x additive, additive x dominance and dominance x dominance) were found to be significant in cross 1 for all the traits across seasons using generation means analysis. However, TTC analysis revealed the presence of all types of epistasis for panicle length and panicle diameter. For grain size, it revealed the presence of only additive x dominance and dominance x dominance (j + l) epistasis. In cross 2, additive x additive (i) interaction alone was significant for panicle length and panicle diameter, whereas for grain size, dominance x dominance (l) followed by additive x dominance (j) contributed significantly across seasons. The estimates of broad and narrow-sense heritability were high for all the traits. Correlation coefficient estimates revealed that panicle length, panicle diameter and grain size were positively and significantly associated with grain yield in their respective trait-specific crosses.

The linkage map constructed using 44 markers (SNP, SSR, EST-SSR and STS markers) with 188 $F_{2:3}$ progenies had a total length of 1018.7 cM. The average distance between the marker pairs was 23 cM. QTL analysis performed as composite interval mapping (CIM) identified eight genomic regions for panicle length, one each on LG 1, 2, 4 and 7; and two each on LG 3 and 6. The variation explained by these QTLs ranged from 6.1 to 18.2%. For panicle diameter, five QTLs were found across LG 2, 3, 5, 6 and 7 and the variation explained by these individual QTLs ranged from 6.3 to 30.2%. For grain size also five QTLs were identified across LG 1, 3, 5, 6 and 7 and the individual QTLs explained 6.1 to 21.2% of the observed phenotypic variation across F_2 and $F_{2:3}$ data sets. From the mapped QTLs, one QTL on LG 2 for panicle length, two QTLs each on LG 2 and 3 for panicle diameter and one QTL on LG 3 for grain size are identified as candidate QTLs for marker-assisted selection.

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Date: **V. Vengadessan**

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1. Introduction

Pearl millet is a major cereal crop grown in the semi-arid regions of Asia and Africa. It is cultivated on about 26 million ha in Asia and Africa. Of this, more than 40% of the area is in Asia, where India is a major producer of this crop with about 10 million ha and an average productivity of 870 kg ha^{-1} (Agricultural statistics, 2006). Pearl millet's ability to produce grains with high nutritive value even under hot, dry conditions on infertile soils of low water holding capacity, where other cereal crops fail, makes it a highly desirable crop for farmers in such harsh environments. Further, the high biomass yield of pearl millet is an attribute that increases its value as a fodder crop, with respect to stover and green forage. Pearl millet breeding programme at ICRISAT, Patancheru, mainly focuses on the development of parental lines for high-yielding hybrids. This is a tough remit, considering the harsh environments where it is grown (Harinarayana *et al.,* 1999; Gill and Turton, 2001). Despite this, considerable improvement has already been achieved. The increasing trend in the reduction of pearl millet cropping area would require a further increase in grain yield potential to meet growing demands for pearl millet grain.

Grain yield is a function of total dry matter and harvest index. Therefore, enhancing the total dry matter, harvest index or both can increase grain yield. The harvest index could be increased through improving the sink size capacity. In pearl millet, the poor sink capacity with low harvest index (15 - 20%) is a basic problem of the species itself (Yagya and Bainiwal, 2001), causing this crop to produce low grain yields. Therefore, in pearl millet emphasis needs to be given to increase sink size component traits to achieve further advance in productivity.

In pearl millet, high grain yield potential can be realized more readily through the investment in large panicle and grain size than in large tiller numbers under

favourable environmental conditions for crop growth (Kelly *et al.,* 1996 and van Oosterom *et al.,* 1996). In many correlation studies, traits such as panicle length, panicle diameter and grain size are identified as important sink size components since these traits have shown direct positive correlation to grain yield (Jindla and Gill, 1984; Maman *et al.,* 2004), and hence breeding for these traits would be helpful in developing varieties with higher grain yield and better acceptability among farmers.

Breeding work for the development of new plant types requires variability in germplasm. The ICRISAT Genetic Resources Unit at Patancheru has assembled wild and cultivated pearl millet germplasm from different countries that provides wide variability for panicle length (5 - 114 cm), panicle thickness (13 - 55 mm) and 1000-grain mass (4 - 21 g). Trait-specific gene pools (TSGP) have also been developed at this center for long panicle, thick panicle and large grain size by random mating the selected pearl millet accessions originating from diverse agro-climatic regions with considerable diversity (Rao *et al.,* 1998). These gene pools have yet to be effectively utilized in applied breeding programmes.

Knowledge about genetic factors responsible for the inheritance of sink size characters, for which there is a great genetic variability in the germplasm collections, is essential for any applied breeding programme. Despite five decades of research about the type of gene action and gene effects, there is still debate about the type of gene action predominating for important traits as it varies depending upon the source of genotypes and the evaluation environments. Almost all the previous pearl millet studies have been conducted using parental material not as diverse as those now available with pearl millet research programme at ICRISAT, which were included in the present study.

The genetical studies based on the means and variances of basic generations, is a simple method for estimating the gene effects for a polygeneic trait and has been reviewed in many crop species. The greatest merit of generation means analysis lies in its ability to estimate the epistatic effects (Mather and Jinks, 1982). The possibility of epistasis accounting for a significant proportion of genetic variance of quantitative trait has been investigated extensively in previous studies in crop plants. Amount and type of epistasis can have a major consequence on both the reliability of predictions and the design of breeding programme. Statistically, detection of epistasis using generation means analysis is more reliable and efficient than by the analysis of variance approach (Lamkey and Lee, 1993). However, it has its own limitations and several assumptions. Triple test cross is a powerful method of genetic analysis, which provides unbiased estimates for epistasis. In addition, it also estimates the additive and dominance components of variation with high accuracy when epistasis is absent (Kearsey and Jinks, 1968).

In recent years, quantitative traits loci (QTL) analysis has become a key tool for studying the genetic architecture of complex traits using molecular markers, facilitating estimation of the minimum number of genomic regions that affect a trait, the distribution of gene effects and the relative importance of additive, dominance and epistatic gene actions (Tanksley, 1993; Kearsey and Farquhar, 1998 and Laurie *et al.,* 2004). In pearl millet, genomic positions of QTLs were mapped for disease resistances (Jones *et al.,* 1995; Morgan *et al.,* 1998), abiotic stress tolerances (Howarth *et al.,* 1997; Yadav *et al.,* 1999, 2000, 2002, 2004; Bidinger *et al.,* 2007) and for grain and stover yield and quality components (Hash and Bramel-Cox, 2000; Hash *et al.,* 2001, 2003; Yadav *et al.,* 2003). However, the major determinants of sink capacity traits such as panicle length, panicle diameter and grain size in pearl millet

has been sparingly subjected to QTL analysis. Understanding the nature of QTLs and the magnitude of their effects for sink size traits in pearl millet combined with the knowledge of genetic parameter estimates through conventional genetic analysis will help the plant breeder to determine a breeding strategy to improve these traits in the applied breeding programmes.

From the aforesaid views, the present study was taken up with the following objectives:-

- To estimate the gene effects of sink size traits (panicle length, panicle diameter and grain size) based on generation means and triple test cross analyses using diverse range of parental lines.

- To estimate the components of variances and heritabilities for sink size traits.

- To investigate the association of sink size traits with grain yield and other yield component traits.

- To determine the number and chromosomal locations of loci controlling sink size traits and their genetic effects through QTL analysis using F_2 and $F_{2:3}$ mapping populations.

- To propose a suitable breeding strategy for the improvement of sink size traits in pearl millet.

2. Review of Literature

Grain yield in crops is one of the most important target traits in plant breeding programme. It is largely determined by the relationship between the source and sink capacity. Increasing the sink size capacity is seen as one of the important approaches to increase the grain yield potential (Yoshida, 1972). In pearl millet, the sink size components panicle length, panicle diameter and grain size are the major determinants of grain yield. Selection for sink size component traits to increase the grain yield potential in breeding programmes has been effective to a limited extent because of their compensatory relationships (Grafius *et al*., 1976; Grafius, 1978). Little consideration has so far been given to the genetic mechanism underlying sink size traits in pearl millet. Understanding the genetic components and the magnitude of their effects on sink size component traits in pearl millet will help the plant breeder to determine the selection strategy to improve these traits in applied breeding programmes. Present study was made to characterize the genetic architecture of sink size component traits in pearl millet on the basis of genetic and QTL analyses. Literature pertaining to this objective is reviewed below.

2.1. Architecture of grain yield components

Pearl millet is an important dual-purpose, staple crop in the crop-livestock production systems of the arid and semi-arid zones. Severe drought stress is a regular feature in this environment, but its timing and intensity are unpredictable (Sharma and Pareek, 1993; van Oosterom *et al*., 1996). In areas where the crop is likely to experience mid-season drought stress, minimizing the risk of a crop failure is more important than yield potential *per se*, and farmers preferentially grow landraces that produce many but small productive panicles (Kelley *et al.,* 1996; van Oosterom *et al*., 1996;

Dhamotharan *et al*., 1997; Bhatnagar *et al*., 1998; Christinck, 2002). By contrast, in areas under favourable conditions for crop growth, where drought stress is less of a problem, low-tillering cultivars with large panicles and large grain size have been bred and are widely adopted (Kelley *et al*., 1996; van Oosterom *et al*., 1996).

Grain yield in pearl millet is highly correlated with grain number per panicle (Bidinger and Raju, 2000a). Final grain number in cereals is predominantly determined by the fraction of surviving florets, rather than the maximum number of floret primordia initiated (Miralles *et al*., 1998). Hence, grain number is determined during a brief period around anthesis (Saini and Westgate, 2000), when the success or failure of individual developing florets is dependent upon the growth of the non-grain part (structural panicle mass, SPM - representing panicle size) of the reproductive organ (Kirby, 1988). Strong correlations between SPM and final grain number have been reported in wheat (Fischer, 1993; Miralles *et al*., 1998). The SPM represents the amount of resources the plant has allocated as a sink for subsequent post-flowering reproductive growth, and can thus be considered a measure of potential grain yield. The efficiency with which the potential sink size is realised is determined during grain filling, when the SPM has been fixed. Panicle size (length and diameter) in pearl millet is a major determinant of SPM, which, in turn, controls the final number of florets per panicle.

A study conducted by van Oosterom *et al.* (2006) in pearl millet revealed that panicle number and size (SPM per panicle) were positively correlated with grain yield under optimal conditions. Therefore, genotypic differences in grain yield under optimum conditions are predominantly a consequence of differences in dry matter partitioning, rather than resource capture. Further, the reduced ability of high-tillering, small-panicled landraces to realize their potential sink size (grain yield per unit SPM)

was associated positively with a lower grain number and a lower individual grain mass per unit SPM.

Correlation studies showed that high tillering landraces with small panicles tended to flower earlier than low tillering large panicle types. These associations accounted for the low stover mass of the high tillering, small-panicled landraces, and the later-flowering landraces tended to have a higher stover biomass, an association commonly observed under optimum growing conditions (Bidinger *et al*., 1994).

Low-tillering, and large grain sizes are particularly relevant to the *Iniadi* landrace from the adjoining Togo – Ghana – Benin – Burkina Faso region of western Africa. Breeding materials derived from this source are presently being widely used in the ICRISAT and national breeding programmes in India and Western and Southern Africa (Andrews and Kumar, 1996). This type of material was reported to be more sensitive to variation in plant population than the traditional high tillering cultivar, and thus may be less adapted to production systems where plant population densities are low, either by design or due to stand loss caused by natural factors.

Bidinger and Raju (2000a) reported that the average panicle productivity increased in a curvilinear fashion in both the low-tillering and high-tillering types under low population densities. However, the low-tillering types were much more responsive to the increasing environmental resources than the high tillering types. The low-tillering types demonstrated a greater ability to adjust panicle productivity through both grain numbers per panicle and individual grain mass than the high tillering types. Similarly, van Oosterom *et al.* (2003) observed that the high-tillering landraces had no obvious advantage under the drought conditions than the low-tillering large-seeded cultivars that have been bred for high yield potential through a greater investment of assimilates into panicle mass and individual grain mass.

Grain size is the other component that has large influence on sink size. Individual grain mass in cereals is determined early in the grain filling period by endosperm cell division and its number and size (Jenner *et al*., 1991). Pearl millet grain is about one-third the size of sorghum and the individual grain mass varies from less than 5 mg to more than 15 mg (Murthy *et al*., 1967a). Pearl millet grain has, on an average, 75% endosperm, 17% germ and 8% bran (Abdelrahman *et al*., 1984). The proportion of germ in pearl millet is thus about twice that of sorghum, which is a factor contributing to higher nutritive value of pearl millet grain.

Small individual grain mass and its correlate of a large grain number are important adaptive features of pearl millet to the arid climates in which it evolved, allowing short grain filling periods, rapid seed germination, and large seed numbers to compensate for high rates of failure in seedling establishment (deWet *et al*., 1992). Large individual grain mass, in contrast, is a useful characteristic in managed agricultural environments. Large grain mass is advantageous in crop establishment, conferring improved rates of seedling emergence and plant stands (Lawan *et al*., 1985), faster initial seedling growth and faster early crop growth (Chhina and Phul, 1982).

In addition, large grain size (mass) improves processing quality of the grain; increasing the ease of decortication and improving flour yield with both hand pounding and commercial milling methods (Rooney and McDonough, 1987). A large grain size can bring a higher market price (Phul and Athwal, 1969) and is often found to be a preferred characteristic in pearl millet cultivars in farmer surveys (Choudhary *et al.,* 1997).

2.2. Genetic studies The choice of plant breeding methodology for upgrading the yield potential largely depends on the availability of reliable information on the nature and magnitude of gene effects present in the population. Fisher (1918) developed the model to partition the total genetic variance in a population into components due to additive, dominance and epistatic variance. Cockerham (1954) and Kempthorne (1954) partitioned the total digenic epistatic variance into additive x additive, additive x dominance and dominance x dominance variance components by showing how these epistatic components of variance are involved in the covariance between relatives. Since the pioneering work of Jinks (1954), which implicated epistasis in the expression of heterosis, attempts have been made to classify digenic interaction into primary categories, namely duplicate and complementary epistasis (Mather and Jinks, 1982). Knowledge on these genetic components and their relative magnitude in controlling various traits has led to the proposal of many breeding methods that capitalize on different types of gene action, including recurrent selection for general combining ability and inbred *per se* selection (additive effects), recurrent selection for specific combining ability (dominance effects), and reciprocal recurrent selection (both additive and dominance effects).

Various experimental designs have been proposed for the estimation of the genetic components. In general, the study of gene effects has been approached in two ways. One approach is based on the analysis of generation means while the other utilizes the genetic variances. Mather (1949) presented several generation mean comparisons for the estimation of additive and dominance effects. The primary function of generation means analysis is to obtain information on additive, dominance and epistatic gene effects from crosses derived from two lines. Several models have since been developed based on generation means for the estimation of genetic effects

(Anderson and Kempthorne, 1954; Hayman, 1958; Van der Veen, 1959; Gardner and Eberhart, 1966). Genetic variance analysis characterizes the predominant types of genetic variance into additive and non-additive (including dominance and non-allelic interactions) in populations, an activity leading to the development and analysis of mating designs, including diallel, line x testers, and the North Carolina mating designs (Hallauer and Miranda, 1988).

Present study aims to measure the genetic components of sink size component traits of pearl millet using two complementary biometrical designs (generation means and triple test cross analysis). The generation means analysis estimates the net genetic effects on the mean phenotypic values of the generations (Kearsey and Pooni, 1996). The triple-test-cross (TTC) design, analyses the genetic variance components (Kearsey and Jinks, 1968; Kearsey, 1980). These two approaches (means analysis and variance analysis) are complementary because they measure different aspects of underlying gene action and interaction (Jinks, 1979; Fenster *et al.,* 1997). As the generation means analysis measures net genetic effects on means while the TTC measures variance components, their parameters are not correlated. Thus, when the effects of increasing and decreasing dominant alleles are spread evenly between the two populations, the generation means analysis will show a zero net dominance (i.e. ambidirectional dominance), whereas a TTC analysis may detect significant dominance variance (because the variance is unaffected by the net direction of dominance). Alternatively, when dominance is directional rather than ambidirectional, the generation means analysis has the advantage of showing the direction of the dominance, which cannot be inferred from the variance analysis. Similarly, the generation means analysis may detect epistatic interactions near fixation, while the variance resulting from the same alleles may be very small. Either analysis taken

alone may produce an ambiguous indication of the genetic architecture, but together provide a clearer picture on the genetic architecture of the trait.

2.2.1. Genetic components of sink size traits in pearl millet

Literatures related to the study of genetic components for sink size traits panicle length, panicle diameter and grain size in pearl millet using generation means and variances approaches are reviewed below.

2.2.1.1. Panicle length

The most important transmission criterion of a trait from one generation to the next and for predicting the short-term response to selection is the narrow sense heritability, the ratio of additive variance to phenotypic variance. Hence, additive gene action is a crucial assumption of most models in quantitative genetics and breeding. This gene action indicates that the effect of an allele, or more precisely, of an allelic substitution, will be the same regardless of the genetic background in which it takes place. For a single locus case it indicates an absence of dominance and for two or more loci it refers to the lack of epistasis (Holland, 2001).

Several studies reported the importance of additive gene effects in pearl millet. Singh and Sagar (2001) studied the gene effects of panicle length in pearl millet under both irrigated and rainfed environments and reported significant additive nature of gene effect under rainfed condition using six-parameter model. The importance of additive gene effect for panicle length was emphasized by Singh *et al.* (2000) using joint scaling test. Using both scaling and joint scaling tests, Joshi and Desale (1996) also revealed predominance of additive gene effect for this trait in all the six crosses studied. Findings of Mangath *et al.* (1994) revealed panicle length to be governed mainly by additive gene effects in six generations of the two crosses studied,

involving short and medium panicle type parental lines. The inheritance of panicle length studied through six-generation means analysis by Shinde and Patil (1987) also revealed that additive gene effects were more important than dominance and epistatic effects for this trait. Similar studies conducted by Singh and Singh (1972) and Singh *et al.* (1972) showed significant additive gene effects for panicle length. These reports were in agreement with the results of variance component approaches for determination of gene action of panicle length such as diallel analysis conducted by Izge *et al.* (2007), line x tester analysis by Girgla *et al.* (1985) and North Carolina Design II analysis by Sandhu and Phul (1984).

Most researchers discount the contribution dominance plays in the genetic architecture of traits as it is only additive genetic variance that predicts the short-term response to selection (Lynch, 1994). Fuerst *et al*. (1997) reported that dominance genetic variation has been ignored in genetic evaluation systems and in many breeding programmes due to computational complexity and inaccurate estimations. Dominance variance arises due to an average difference between two alleles of a heterozygote, this results from the fact that heterozygotes are not exactly intermediate between the monozygotes.

Significant role of dominance gene action in the inheritance of grain yield component traits has also been reported in pearl millet. Singh and Sagar (2001) reported the predominance of dominance gene action for panicle length in both irrigated and rainfed environment of the two crosses studied through generation means. Similarly, Sheoran *et al*. (2000) also revealed that the magnitude of dominance gene effect was significant over additive gene effect in a cross across two different locations using joint scaling test proposed by Cavalli (1952).

The experiment involving six generations of six crosses conducted by Gandhi *et al.* (1999) also showed significant dominant gene effects in five crosses for this trait. However, higher influence of partial dominance for this trait was reported by Gill *et al.* (1968). Jain *et al.* (1961) also observed partial dominance for panicle diameter in a diallel cross.

Contrary to the above reports on the role of independent nature of gene action for the inheritance of panicle length, Ramamoorthy and Das (1994) and Desale (1993) observed the role of both additive and dominance gene effects for this trait using generation means analysis.

The estimates of additive and dominance variance may allow the predictions of genetic advance for a particular trait (Gallais, 1993). However, it is also important to consider the proportion of between locus interactions (epistasis) for any trait. Amount and type of epistasis in crop species can have a major consequence on both the reliability of predictions and design of breeding programmes. Epistasis is the interaction of alleles at different loci. The value of an allele or genotype at one locus depends on the genotype at other epistatically interacting loci, complicating the picture of gene action.

Presence of significant epistatic interactions for panicle length in pearl millet has also been emphasized in many studies. Additive x additive gene interaction for panicle length was noted in a cross studied by Singh *et al.* (2000) through the six-parameter model. Similarly, Gandhi *et al.* (1999) and Shinde and Patil (1987) observed additive x additive interaction with duplicate epistasis for panicle length in various crosses through the six-parameter model proposed by Hayman (1958). However, Ramamoorthy and Das (1994) reported dominance x dominance with duplicate epistasis for this trait using generation means of various crosses studied.

Complementary type of epistasis was also reported by Sheoran *et al.* (2000) for panicle length. Ramamoorthi (1996) also observed similar type of epistasis for this trait. The significant contribution of additive x dominance interaction for panicle length was revealed by Singh and Singh (1972) using the generation means.

The presence of non-allelic interaction for panicle length was reported through 8 x 8 diallel analysis of dwarf pearl millet by Mukherji *et al.* (1981). Murthy *et al.* (1967b) also observed the major role of non-allelic interaction for panicle length from a line x tester analysis.

2.2.1.2. Panicle diameter

The generation means of parents (P_1 and P_2), their F_1 and F_2 and back cross populations (BC_1 and BC_2) of two crosses studied by Mangath *et al.* (1994) revealed additive nature of the inheritance for panicle diameter. The importance of additive gene effects for panicle diameter was also reported by Singh *et al.* (1972) in six generations of two crosses studied using Hayman's (1958) approach. Similar highly significant additive effects for this trait was also reported by Ramamoorthi (1996) using the method suggested by Mather and Jinks (1972). Adequacy of additive-dominance model for the inheritance of panicle diameter has been emphasized by Girgla *et al.* (1985) using line x tester analysis. Further, this study reported significant additive effects for this trait. Additive effect for this trait has also been reported by Mukherji *et al.* (1981) in a diallel analysis involving a set of 8 dwarf pearl millet genotypes. Bains (1971) also reported preponderance of additive genetic variance for this trait through diallel analysis. Since the effect of selection depends on the amount of additive genetic variance and not on the genetic variance in general, Sprague (1967) opined that in any selection scheme, additive variance would be important when the commercial product retains high degree of heterozygosity and

heterogeneity, hence population improvements methods like recurrent selection for general combining ability would best exploit this type of gene effects.

Dominance gene effects for this trait have also been reported in various studies. Experiments conducted in two environments by Sheoran *et al.* (2000) revealed higher magnitude of dominance gene effects for panicle diameter in both the environments using joint scaling test of Cavalli (1952). The estimates of dominance gene effects were also found to be significant and greater than additive gene effects in a study conducted by Gandhi *et al.* (1999) and Singh and Singh (1972) through Hayman's (1958) six-parameter model. In a study conducted by Singh *et al.* (1999), dominance gene effect was found to be contributing maximum to the inheritance of panicle diameter. Analysis of six generation means by Joshi and Desale (1996) using both scaling and joint scaling test also revealed preponderance of dominance component for panicle diameter. However, partial dominance for panicle diameter was reported by Jindla (1981), Gupta and Nanda (1968) and Jain *et al.* (1961) and over dominance for this trait was observed by Mukherji *et al*. (1981), Singh *et al.* (1979) and Nanda and Phul (1974).

Contrary to the above reports, the importance of both additive and dominance gene action for this trait has also been reported. Observations made by Singh *et al.* (2000) revealed that the three parameter model was adequate to detect the genetic difference for panicle diameter and reported the presence of both additive and dominance gene effects for this trait. Absence of epistasis for panicle diameter through generation means has also been reported by Sagar and Singh (1996) indicating the adequacy of additive - dominance model. Importance of both additive and dominance gene effects for inheritance of panicle diameter was also reported by Desale (1993). Tyagi *et al.* (1975) also reported the presence of both additive and

non-additive gene actions for panicle diameter using generation means analysis.

Various studies have also suggested the epistatic effects for panicle diameter. Complementary type of epistasis was observed by Sheoran *et al.* (2000) in a six generation experiment conducted in both rainfed and irrigated environment. Similar observations has been made by Singh *et al.* (1972) for this trait through Hayman's (1958) approach. Conversely, Gandhi *et al*. (1999) and Ramamoorthi (1996) reported the presence of duplicate dominant interaction for panicle diameter in six-generation means of a cross. The contribution of additive x dominance interaction was found to be highly significant for panicle diameter by Singh and Singh (1972) from six generation studied according to Hayman's (1958) approach. Inheritance of non-additive nature of gene action through variance analysis was accentuated by Hepziba *et al*. (1996), Balakrishnan and Das (1996), Gill *et al.* (1978) and Singh and Murthy (1973).

2.2.1.3. Grain size

Additive gene effects for 500-grain weight was observed by Singh *et al.* (2000) based on least square estimate of Cavalli (1952). The results of seven generations of a cross studied by Phul and Athwal (1969) revealed that a major portion of the variation in the inheritance of grain size (weight of 1000-grain mass) was due to additive gene effects. Inheritance of 1000-grain weight was reported to be due to additive genetic variance in a study by Izge *et al.* (2007) using a diallel analysis. Yadav *et al.* (1981) also reported additive genetic variance for the expression of 500-grain weight under two environments from a line x tester analysis. In the past, erosion of epistatic variance was considered as prime cause of increase in additive genetic variance in a number of empirical studies (Bryant *et al.,* 1986; Lopez-Fanjul and Villaurede, 1989). However, considerations have now placed a greater importance on the dominance effects acting through changes in gene frequencies as the cause (Willis and Orr, 1993).

The role of dominance gene action for 1000-grain weight has also been reported in various studies. Sheoran *et al*. (2000) showed that 1000-grain weight was predominantly under dominance gene effect than additive gene effects in a study involving six basic generations of a cross studied under two environments (irrigated

and rainfed). The gene action for 1000-grain weight was observed to be predominantly dominant type in the six generations of four crosses studied by Shinde and Patil (1987). However, Shinde *et al.* (1984) reported overdominance for this trait through generation means analysis.

On the contrary, genetic analysis of six basic generations of the three crosses of pearl millet studied by Gotmare and Govila (1999) indicated both additive as well as non-additive gene actions for 500-grain mass. Similarly, Phul and Athwal (1969) reported both additive and non-additive gene action for 250-grain weight. Presence of both additive and dominance gene effects for 1000-grain mass were also reported by Chand *et al.* (1973) in various crosses.

Non-additive gene effects for the inheritance of grain weight were reported by Aher (1993) and Desale (1993). Vaidya *et al.* (1983) also found non-additive gene action though line x tester analysis for grain size, measured from weight of 400-grains. Gupta and Nanda (1968) reported partial dominance for 1000-grain using diallel analysis involving six pearl millet lines.

Sheoran *et al.* (2000) revealed additive x additive interaction to be highly significant for 1000-grain weight in the crosses studied using generation means in two environments. Griffing (1960) and Cockerham (1984) have shown that the additive epistatic variance, which contributes to the initial response to selection in an outbred population declines with the continued random mating if the recombinant fraction is greater than zero. Additive x dominance interaction for 500-grain mass was also observed for two crosses of six generations studied by Singh *et al.* (2000). Gill *et al.* (1974) also reported similar findings for test grain weight. Presence of both additive x dominance and dominance x dominance type of gene actions for 1000-grain weight

was observed by Chand *et al.* (1973) in various crosses through generation means analysis.

Interactions of additive x dominance type and the presence of complementary epistasis for grain size were indicated from studies made on six generations by Phul and Athwal (1969). However, Singh *et al.* (1972) reported duplicate epistasis for this trait by analyzing six generations of a cross using Hayman's (1958) method. Joshi and Desale (1996) reported non-allelic interactions to be predominant for 1000-grain mass from the six crosses analyzed through generation means.

In spite of extensive analysis carried out there are still limitations that exist in applications. The following limitations were observed in most of the studies: (i) No test of adequacy of additive-dominance model has been made and it is assumed that non-allelic genes neither interact nor are linked. (ii) The estimation of parameters is correlated as unweighted analysis has been performed. In order to overcome these limitations, Virk (1988) suggested a weighted analysis using sufficient numbers of generation that will provide a chi-square (χ^2) test for the adequacy of the model and also mentioned that the most efficient means of estimation of genetic component will be provided by triple test cross analysis.

The other important factors for the accurate estimation of genetic components are the type and size of population under the study. According to Burr and Burr (1991) the choice of the population type for genetic studies has important consequences concerning the efficiency and utility of the genetic information. Hanson (1959) and Liu (1998) demonstrated that a given population size affects the power to estimate as well as the accuracy of the recombination frequency.

Most of the earlier studies on generation means were made in crosses between less divergent elite lines which indicates that genes with decreasing and increasing

effects may be equally shared by both the parental strains (dispersed), so the estimate may be biased because the mean expression of parental and back cross generation depends upon the distribution of genes with increasing and deceasing effects between the parental strains. Mean expression thus changes with distribution of genes. Therefore, selecting the lines showing extreme divergence, one parental strain that bears all the genes with increasing effects and other line possessing all the genes with decreasing effects (associated) may precisely estimate the genetic effects.

In spite of several genetic studies, specific information relating to the determination of ideal population size in a given generation needed to establish accurate genetic information have as yet been inconclusive, particularly for estimating the epistatic effects. Epistasis, as a phenomenon amenable to statistical analysis of segregating trait loci, can be difficult to detect. Consider the simple case of an inbred line with backcross population of 100 individuals, where two alleles are segregating at each locus. On average, only 25 lines will display the phenotype induced by a one-way epistatic interaction between these two loci. If the phenotype was wholly determined by this interaction, then such sample size might be satisfactory. In practice, however, complex traits are influenced by many genetic and non-genetic factors, so a starting sample of 100 is usually too small for obtaining robust and compelling epistatic interactions. For example, in a backcross, every additional gene contributing to an interaction effect could potentially reduce the number of lines with epistatic phenotype by 50% (Frankel and Schork, 1996). However, for the backcrosses and F_2 populations, Ferreira *et al*. (2006) reported a total of 200 as being sufficient from a simulation study with population sizes of 50, 100, 150, 200, 500 and 1000 individuals. In pearl millet, few studies on generation mean analysis have adopted the population size of more than 200 individuals for segregating populations.

The optimization of population size is extremely important for optimizing time and costs associated with genetic analysis allowing breeding programs to obtain improved lines in selection process with the maximum efficiency. Keeping these views, present study utilizes the highly contrasting parental lines and also aims to optimize the population size required for the precise genetic estimates of pearl millet sink size component traits.

Though genetic variance approach like diallel and line x tester are commonly employed to study the genetic components, Baker (1978) pointed out that these approaches have not been more informative, probably because of the assumptions of independently distributed genes and the absence of epistasis in the analysis, which are frequently not true. The other disadvantage noted is that the analysis is performed on univariate data. Diallel variance approach has also been frequently used in pearl millet genetic analysis of various characters. However, Virk (1988) reported that the estimates obtained from diallel cross in pearl millet suffer from the following limitations: (a) the estimates are biased by the presence of non-allelic interactions (b) the standard errors are approximately computed (c) the estimate are correlated (d) the environmental components of Vr and Wr have not been accounted and (e) the formulae of full-diallel cross have been mostly used even for half-diallel crosses.

In general, Lamkey and Lee (1993) reported that studies estimating epistatic effect using analysis of variance approach reported non-significant epistatic variance, whereas generation means approach reported significant effects. Though, statistically there is more power to detect epistasis using generation means analysis than by using the analysis of variance approach, the inability to detect epistasis with both the methods cannot be taken as an evidence for the absence of epistasis because of the canceling of epistatic effects among the loci. To test the presence of epistasis, various

other powerful methods are needed such as triple test cross analysis, widely used to test the presence of epistatic variation.

2.2.2 Triple test cross analysis

Triple test cross (TTC), a multiple mating design which is an extension of the NCD III design of Comstock and Robinson (1952) proposed by Kearsey and Jinks (1968), is the best design currently available for detecting the presence of non-allelic interactions, and for estimating the additive and dominance effects with equal efficiency when epistasis is absent (Pooni *et al.,* 1980). It can be applied to any population regardless of its gene and genotypic frequencies or its degree of inbreeding (Jinks *et al*., 1969; Perkins and Jinks, 1970). In pearl millet, only few studies has been attempted to estimate the genetic components using TTC analysis, which are reviewed here along with the studies on the relative efficiency of TTC over generation means and other variance approaches.

Simplified triple test cross of 18 pearl millet lines studied by Singh *et al.* (1990a) showed significant epistatic, additive and dominance components of genetic variation for various traits studied. The additive component was noted as more important than the dominance component for panicle length and panicle diameter.

Singh *et al.* (1991) applied TTC for the genetic analysis of physiological traits in pearl millet. Significant epistasis was observed for various traits studied and additive and dominance components of genetic variance were found to be significant for all traits except peduncle length.

Kearsey *et al.* (2003) examined the genetic control of 22 quantitative traits, in basic generations (parents, F_1's, F_2's, backcrosses), recombinant inbred lines (RILs) and a triple test cross (TTC) of *Arabidopsis thaliana*. The study revealed a good agreement between the basic generations, RILs and the TTC families. QTL analyses

were also consistent with the biometrical approach in many respects. However, significant inconsistencies were also reported.

The results of a study comparing the efficiency of TTC and generation means analysis of faba bean crosses in their ability to detect genetic components by Bakheit *et al.* (2002) revealed the relative importance of additive and dominance components in both the approaches for most of the traits studied. However, larger magnitude of additive effects compared to dominance effects were observed in the triple test cross analysis than the additive effects in the 'six-population' analysis. Hence, it was reported that the triple test cross analysis is expected to provide more reliable estimate of additive and dominance components even if epistasis is present as compared to model fitting analysis. Kearsey *et al.* (1987) also reported a complete agreement between the findings of triple test cross and generation means analyses conducted to study the inheritance of heading date and dry matter production in *Lolium perenne*. Similar conclusions were also reported by Chaudhary (1997) in *Vigna angularis*, Pooni *et al*. (1978) in *Nicotiana* and Chahal and Singh (1974) in *Gossypium*.

Nanda *et al.* (1990) reported TTC and model fitting (generation means) analysis to be in agreement with each other in the detection of genetic components of various quantitative traits while studying the 120 TTC families and six basic generations arising from two bread wheat crosses. Similar results were reported by Thomas and Tapsell (1983) and Tapsell and Thomas (1983) while studying the genetical and environmental control of quantitative traits in five crosses of spring barley using both TTC and model fitting analysis.

Singh *et al.* (1986) compared the relative efficiency of diallel, partial diallel and TTC designs for understanding the genetic architecture of quantitative traits in spring wheat. The result revealed that in the absence of epistasis, all the three designs

gave similar genetic information. However, TTC appeared useful in deriving genetic information about the presence of epistasis and magnitude of additive and dominance components of genetic variation.

The relative efficiency of generation means and North Carolina design III (TTC is an extension of NCD III design) was compared by Kearsey (1980) and the considerable advantages of the NCD III design was emphasized in the study. It was shown that in the six-generation design, generations should ideally be replicated in proportion to the total variation of each generation while in the NCD III, one should sample a minimum of only 20 F_2 plants.

Ponni and Jinks (1976) compared the efficiency and optimal size of TTC and two of its associate designs (Jinks *et al.,* 1969; Jinks and Perkins, 1970) for their theoretical and practical efficiency in detecting epistatic variation in *Nicotiana rustica.* The results showed that the theoretical efficiencies do not differ much among the designs and the optimal sizes required by them to detect non-allelic interactions significantly are largely impractical except when the dominance and heritability are high and the degree of association is 50% or more. All the tests require much smaller experiment to detect duplicate epistasis than complementary epistasis. It was suggested to plan an experiment for the detection of complementary epistasis as this will be adequate to also detect duplicate epistasis, if present.

2.2.3. Heritability estimates for sink size traits

Heritability (degree of genetic determination) is an expression of the extent to which the genotype of an individual determines its phenotype. The phenotype of an individual is the sum of effects of its genotype, environment in which it grows, and genotype x environment interactions. Heritability in broad-sense reflects all possible

genetic contributions to a population's phenotypic variance, and it includes gene effects due to allelic variation (additive variance), dominance variation or which act epistatically. Broad-sense heritability is of little practical importance to plant breeders. Narrow-sense heritability often simply referred to as heritability, is an expression of the reliability with which phenotypic value guides to the breeding value. Narrow-sense heritability is the breeder's best estimate of breeding value and represents the portion of phenotypic variation, which behaves as if it arose from additive effects.

The literatures reviewed here are corresponds to estimate of heritability in broad-sense. Lakshmana and Guggari (2001) studied 32 white grain pearl millet genotypes and reported high heritability for panicle length. High heritability estimates for this trait was also reported by Hepziba *et al.* (1993), Vyas and Srikant (1986) and Shinde *et al.* (1984). Singh *et al.* (1979) showed that selection for yield per plant should be based on panicle length, which showed high heritability. Madhava *et al.* (1971) also reported high estimates of heritability for this trait.

High heritability for both panicle length and panicle diameter was reported by Varu *et al.* (2005). Rama *et al.* (1986) reported high estimates of heritability for panicle diameter.

For 1000-grain weight, Borkhataria *et al.* (2005) reported high heritability estimates. Solanki *et al.* (2002) also revealed high heritability for this trait in three pearl millet composites (EC 87, EC 91 and HHVBC). On the contrary, Sachan and Singh (2001) observed moderate heritability for 1000-grain weight. Pathak and Ahmad (1988) reported high heritability estimate for 100-grain weight and 1000-grain weight, respectively. Kunjir and Patil (1986) reported high heritability for 500-grain weight. Chaudhary *et al.* (1980) analysed the six basic generations of three crosses

derived from four diverse pearl millet inbreds and found that heritability estimate for 1000-grain weight was more than 60%.

High heritability estimates for all three sink component traits panicle length, panicle diameter and 100-grain weight were reported by Gupta *et al.* (1988) while evaluating seventy-two S_3 progeny lines and 144 S_4 progeny lines under two spacing, 60 cm and 30 cm between rows.

2.2.4. Correlation studies

The improvement of one character by selection frequently causes simultaneous changes in other characters. The effect is the result of correlations between characters, which may be genetic or environmental in nature. Genetic correlations arise from pleiotropy, from linkages between loci controlling the characters or from random genetic drift. According to Falconer (1989), pleiotropy is the chief cause of genetic correlations, while Mather and Jinks (1982) have argued that linkage is the more likely explanation. If the correlation is heritable, *i.e.* additive, then the correlated response may be predicted from an early generation. The response is measured by the change in the mean of the distribution of lines for the correlated character compared with the distribution of lines derived without any selection. Literature on correlation studies for grain yield and its component traits in pearl millet is reviewed here.

Positive significant association of sink size component traits panicle length, panicle diameter and test grain weight with grain yield was reported by Salunke *et al.* (2006), Unnikrishnan *et al.* (2004), Kumar *et al.* (2002), Anarase and Ugale (2001), Poongodi and Palaniswamy (1995), and Mukherji *et al.* (1981).

Vidyadhar *et al.* (2007) showed significant positive correlation of both panicle length and panicle diameter with grain yield. Similar positive associations were also reported by Thangasamy and Gomathinayagam (2003) and Satish (2002). It was

suggested that selection should be based on panicle length and panicle diameter for the development of high-yielding lines in pearl millet. Positive significant association of panicle length and test grain weight was reported by Chikurte *et al.* (2003) and Yoshida *et al.* (1999). However, under terminal water stress conditions, Patil and Jadeja (2006) reported that grain yield per plant was negatively correlated with panicle length and panicle diameter but positively and significantly associated with grain weight.

Borkhataria *et al*. (2005) reported highly significant and positive correlation for panicle diameter, 1000-grain weight with grain yield in 22 hybrids of pearl millet, indicating the major role of these traits in controlling grain yield. Similar positive association was also reported by Kulkarni *et al.* (2000) and Harer and Karad (1999). Conversely, Borole and Patil (1991) reported negative association of panicle diameter and 500-grain weight with grain yield. However, the association was positive with panicle length.

Varu *et al.* (2005) and Yadav *et al.* (2001) emphasized that selection for panicle length may result in grain yield improvements in pearl millet due to their high significant positive correlations with grain yield. Navale e*t al.* (1999) reported that panicle diameter had highest positive correlation with grain yield. Singh and Sabharwal (2003) and Singh and Govila (1989) reported a significant and positive association of 1000-grain weight with grain yield. Navale *et al.* (2000) also showed that 500-grain weight was positively correlated with grain yield.

Positive and significant correlation of panicle length with plant height; panicle diameter with plant height and 1000-grain weight was reported by Poongodi and Palaniswamy (1995). Positive and significant correlation between panicle diameter and 500-grain weight was also observed by Borole and Patil (1991). However, Kunjir

and Patil (1986) reported negative correlation of panicle length, panicle diameter and 500-grain weight with tillers per plant.

From the literature cited above on the correlation of yield component traits with grain yield, it could be inferred that panicle length, panicle diameter and grain size are very important target traits to realize potential grain yield in pearl millet.

2.3. QTL studies

2.3.1. QTL analysis overview

Characters exhibiting continuous variation are termed quantitative traits. Quantitative traits show continuous phenotypic variation in a population resulting from the combined allelic effects of many genes and environmental conditions and their interactions (Falconer and Mackay, 1996). In crop plants, most traits of agricultural and economical significance exhibit quantitative inheritance. These include grain and biomass yield, plant maturity, disease resistance and stress tolerance. The genetic loci, which control quantitative traits, are referred to as QTLs (quantitative trait loci). QTLs are identified by statistical analysis of complex traits that are typically affected by more than one gene and often by environment. The earliest documented experiments on linkage analysis between quantitative effects and marker genotypes have been reported by Sax (1923) who noted that seed size in bean, a complex trait, was associated with seed coat color, a simple, monogenically-controlled trait. New interest was generated when studies with maize and tomatoes demonstrated that some markers explained much of the phenotypic variance of complex characters (Tanksley, 1993). As a consequence, vigorous research on QTL mapping for quantitative traits was initiated in many crop species (Lee, 1996). With the development of comprehensive DNA marker maps (Tanksley *et al.,* 1992; Causse *et al.,* 1994), it is now possible to search for QTL throughout the genomes of most species. Genetic

linkage maps assist in understanding the inheritance of complex traits and to improve them through marker-assisted breeding. In addition to marker-assisted selection (MAS), flanking markers of a trait on a genetic map can be used to determine the position on a physical map to clone and characterize genes controlling trait variation (Lagudah *et al*., 2001).

2.3.2. Types of molecular markers

The investigation of genomic structure and gene dynamics is facilitated by the use of polymorphic molecular markers in coding and non-coding regions to detect sequence variation between individuals. The usefulness of a particular marker system in providing linkage information is dependent upon the degree of polymorphism and the mode of inheritance of alleles (presence/absence or co-dominance). The types of molecular marker techniques used are either based on hybridization or polymerase chain reaction (PCR) methods. Hybridization-based techniques detect restriction fragment length polymorphisms (RFLP) and Diversity Array Technology (DArT^{TM}) polymorphisms. Whereas random amplified polymorphic DNA (RAPD), sequence tagged sites (STS), amplified fragment length polymorphisms (AFLP) and microsatellites or simple sequence repeats (SSR) are detected by using the polymerase chain reaction (PCR), as is the more recently developed marker system single nucleotide polymorphism (SNP), which can maximally detect and exploit variation between any two individuals of a given species.

2.3.2.1. RFLP, AFLP and STS markers

The development of restriction fragment length polymorphism (RFLP) markers revolutionized plant genome mapping, offering a new source of virtually unlimited co-dominant markers with extensive genome coverage (Beckmann and Soller, 1983).

Among the various molecular markers developed, RFLPs were developed first and initially used for human genome mapping (Botstein *et al*., 1980). Later, they were adopted for plant genome mapping (Weber and Helentjaris, 1989).

In pearl millet, RFLP markers were utilized in the development of first genetic map (Liu *et al.*, 1994). RFLP-based maps of pearl millet were used to map QTL for resistance to downy mildew (Jones *et al.,* 1995, 2002); rust and pyricularia leaf blast (Morgan *et al.,* 1998); tolerance to drought (Yadav *et al.,* 2002, 2004; Bidinger *et al.,* 2007); components of grain and stover yield and quality (Hash *et al.,* 2001, 2003; Yadav *et al.,* 2003) and for characteristics involved in domestication (Poncet *et al*., 2000, 2002). RFLP markers also allowed improving the understanding of complex relationships between pearl millet genome and those of other cultivated graminaceous species (Devos and Gale, 1997; Devos *et al.*, 2000). RFLP maps have also been developed for a number of species like maize (Helentjaris *et al.,* 1986, Helentjaris 1987), tomato (Bernatzky and Tanksley 1986, Helentjaris *et al.,* 1986, Zamir and Tanksley 1988), lettuce (Landry *et al.,* 1987), rice (McCouch *et al.,* 1988), pepper (Tanksley *et al.,* 1988), Arabidopsis (Chang *et al.,* 1988), rye (Börner and Korzun, 1998; Korzun *et al.,* 2001), barley (Graner *et al.,* 1991; Heun *et al.,* 1991) and peanut (Halward *et al.,* 1992).

Amplified fragment length polymorphisms (AFLPs) produced a source of PCR-based markers with the potential to rapidly saturate genetic maps. In pearl millet, AFLP markers have been used for the saturation of maps (Hash and Bramel-Cox, 2000). Faure *et al.* (2002) fine-mapped a downy mildew resistance gene in pearl millet using AFLP markers. The high efficiency, reproducibility and reliability of AFLP have been mentioned in a number of recent publications, leading to recommendation of their use for the analysis of genetic linkage and gene mapping.

AFLP markers have been used to construct maps for barley (Becker *et al.*, 1995; Waugh *et al.,* 1997; Qi *et al.,* 1998), sugar beet (Schondelmaier *et al.,* 1996), soybean (Keim *et al.,* 1997), petunia (Gerats *et al.,* 1995), rice (Maheswaran *et al.,* 1997), wheat (Lotti *et al.,* 1998) and tomato (Haanstra *et al.,* 1999).

Although the RFLP and AFLP techniques are powerful and reliable in identifying markers closely linked to genes of interest, they had some disadvantages for use in MAS and map-based cloning (Rafalski and Tingey, 1993). Limitations to the large-scale, locus-specific application of RFLPs and AFLPs include the intensity of labour involved and the high costs. Hence, conversion of these markers into sequence-specific polymerase chain reaction (PCR) markers is required for screening large breeding populations at low costs (Dussle *et al*., 2002). The conversion of linked markers to sequence tagged sites (STS) overcomes problems associated with RFLPs and AFLPs. STS is a short, unique sequence that identifies a specific locus and can be amplified by PCR. They are obtained by sequencing terminal regions of genomic fragments and cDNAs expressing RFLP. Since they are based on a specific sequence, STS markers more reliably detect the same locus. There are several instances where RFLP and AFLP markers have been converted to STS markers for use in genetic mapping (Paran and Michelmore, 1993; Schachermayr *et al.,* 1997; Feuillet *et al.,* 1995; Dedryver *et al.,* 1996; Talbert *et al.,* 1996; Blair and McCouch, 1997; Huang *et al.,* 1997; Paltridge *et al.,* 1998; Toojinda *et al.,* 1998; Mohler *et al.,* 2001). This marker system can hence be best utilized in pearl millet for both mapping studies and MAS, provided polymorphism detected is adequate (Hash and Bramel-Cox, 2000).

2.3.2.2. Microsatellites

Simple sequence repeats (SSR) also known as microsatellites, are a PCR-based marker system that remains the choice for most marker-assisted breeding applications

(Qi *et al*., 2004). Microsatellites are abundant in eukaryotic genomes. They are present in both coding and non-coding regions, and are usually characterized by high levels of polymorphism (Tautz 1989; Akkaya *et al.,* 1992; Morgante and Olivieri 1993; Wu and Tanksley 1993; Roder *et al.,* 1995; Bryan *et al.,* 1997; Zane *et al.,* 2002). Their abundance, co-dominant nature and high level of variability make them a suitable assay for detecting variations in a given population (Lagercrantz *et al*., 1993; Schug *et al*., 1997; Harr *et al*., 1998)

The flanking sequences of SSRs are often unique, allowing primers to be designed that result in sequence tagged microsatellite (STMS) markers representing a single locus. The majority of the allelic variation of SSRs are thought to arise as a result of slip strand mis-pairing (SSM). SSM involves denaturing and displacement of strands of DNA duplex followed by mis-pairing of complementary bases at the site of an existing tandem repeat. When followed by replication or repair, this can lead to insertions or deletions of one or several of the short repeat units (Levinson and Gutman, 1987). Unequal crossing over can also generate tandem duplications in DNA, as well as insertion or deletion events in the sequence that flank the SSR region (Grimaldi and Crouau-Ray, 1997). All of these mechanisms can potentially generate allelic variation, providing an important source of highly polymorphic markers. This variation is detected by PCR amplification using primers complimentary to the flanking sequences. SSRs as a marker system have been shown to be highly reproducible between laboratories (Jones *et al*., 1997).

The first report of microsatellites in plants was made by Condit and Hubbel (1991), suggesting their abundance in plant systems. Later, Akkaya *et al*. (1992) reported SSRs polymorphisms in soybean, which opened a new source of PCR-based molecular markers for other plant genomes. It has been demonstrated that

microsatellites are highly informative and locus-specific markers in many species (Song *et al*., 2002; Röder *et al*., 1998).

In pearl millet, bacterial artificial chromosome (BAC) library was initially utilized for the development of SSR markers (Allouis *et al.*, 2001 and Qi *et al.*, 2001). Utilizing SSRs as additional new sets of markers, Qi *et al.* (2004) presented an integrated genetic map in pearl millet. Further, SSRs based on pearl millet expressed sequence tags (ESTs) have also been developed (Senthilvel *et al.,* 2004). These markers provide the base for genomic and comparative analyses of pearl millet and application of MAS in breeding programmes.

Although microsatellites are reported to be highly informative and reproducible, co-dominant and locus specific (Powell *et al*., 1996), they have also several drawbacks, including the high cost and length of time required for their development. These are caused by the need to be isolated *de novo* from most species being examined for the first time (Zane *et al*., 2002). Although the initial cost may be significant, once developed the cost of implementing these markers is greatly reduced. In addition, they are easily transferable between laboratories as the sequence information can be distributed, allowing other research groups to generate their own primers.

2.3.2.3. Single Nucleotide Polymorphism

The primary candidate for the next (and perhaps final) generation of molecular marker is the single nucleotide polymorphism (SNP). A SNP consists of a single base difference between two individuals within a given segment of DNA (Koebner and Summers, 2003). SNPs and insertion-deletions, which are the basis of most differences between alleles, provide an essentially inexhaustible source of polymorphic markers for use in the high resolution genetic mapping of traits, and for

association studies that are based on candidate genes or possibly whole genomes (Rafalski, 2002). SNP is the most common class of polymorphism within different plant, animal and microbe species. In maize (*Zea mays*), it is reported that the frequency of nucleotide change among varieties is high, at around one polymorphism per 31 bp in non-coding regions and one polymorphism per 124 bp in coding regions. Indels are also frequent in non-coding regions (one per 85 bp), but rare in coding regions (Ching *et al*., 2002). SNP frequencies in more conserved crop species, such as highly self-pollinated groundnut and chickpea may be much lower (Bryan *et al*., 1999).

In pearl millet, SNP markers based on single-strand conformational polymorphism were developed using annoted rice genomic sequence tags to identify the intron-exon borders in millet expressed sequence tags (Bertin *et al*., 2005). The rates of polymorphism were low but useable, with a mean PIC of 0.49 relative to 0.72 for SSRs when tested on an eight genotype panel of pearl millet inbred lines (Bertin *et al.,* 2005). However, the SSCP-SNP marker system has lower development costs than simple sequence repeats (SSRs), because much of the work is done *in silico*, and markers have similar deployment costs and throughput potential. The major advantage of the system is in comparative applications. Syntenic information can be used to target SSCP-SNP markers to specific chromosomal regions or, conversely, SSCP-SNP markers can be used to unravel detailed syntenic relationships in specific parts of the genome. Feltus *et al*. (2006) used a similar approach to develop conserved intron spanning primer (CISP) markers that detect syntenic polymorphic regions across monocots. These primers were designed from conserved introns flanking conserved (but polymorphic) exons - based upon *in silico* comparisons of sorghum

EST sequences with their rice genome sequence homologues. Polymorphic CISP markers include both SNPs and indels.

The detection of single nucleotide polymorphisms (SNP) can be achieved by a number of techniques such as electrophoretic separation of PCR products, MALDI-ToF mass spectroscopy and microarray (Langridge *et al.,* 2001; Gupta *et al.*, 2001). However, a dominant SNP detection technology has not emerged so far. Direct sequencing of DNA segments from several individuals is the most direct way to identify SNP polymorphisms (Gaut and Clegg, 1993). However, the cost involved for direct sequencing has been a major limitation. As the prices of DNA sequencing and SNP assays drop, the argument for SNPs will become more compelling. A recent report states that the average cost of SNP genotyping has been reduced from approximately US$ 1.00 to US$ 0.10 per assay, but that a further reduction to US$ 0.01 per assay will be required before wide-scale usage of the technology becomes feasible (Roses, 2002). A rapid and simple method to reliably identify and score SNPs in DNA samples was recently developed by employing DHPLC technology (Oefner and Underhill, 1998; Bäümler *et al*., 2003a). Schwarz *et al*. (2003) reported that the cost-effective and high-throughput DHPLC genotyping technique is particularly suitable for routine diagnosis of SNPs in homologous HMW glutenin gene sequences. This technique showed it's superiority, even compared to CAPS marker analysis. Automated sample feeding allows analysis of up to 200 isolates per day (Bäumler *et al*., 2003b).

Koebner and Summers (2003) mentioned the merits of SNPs as: (i) they offer the potential for a high density of markers; the relevance of this to MAS is that it should be possible to find an informative marker in the right region in any segregating situation, even if the probability of finding polymorphism at any one SNP locus is

low; and (ii) SNP output is of the binary type and this presents an easier target for automated data interpretation than length-based outputs that are typical of sequence tagged microsatellites (STMS or SSR). In addition such data need not be generated by electrophoresis, giving the potential for simpler and cheaper analytical platforms.

2.3.3. Mapping populations

Populations used for mapping are usually derived from F_1 hybrids between two lines (either homozygous or heterozygous), which show allelic differences for selected markers. Genetic maps of plants are constructed based on several different kinds of populations (Paterson, 2002), with each population structure having unique strengths and weaknesses. Four types of population are commonly used for map construction and mapping experiment, they are F_2 population, back cross population (BC), doubled haploid (DH) population, and recombinant inbred lines (RILs). Most genetic mapping populations in plants have been derived from crosses between largely homozygous parents. The present study employs both an F_2 population and the plant version of the daughter design made by replacing the phenotypic value of an F_2 plant by the mean of its F_3 progeny, called the $F_{2:3}$ progeny population (Austin and Lee, 1996; Fisch *et al.,*1996).

The F_2 mapping populations can be quickly developed and they harbor all possible combinations of parental alleles (Lander *et al.*, 1987). However, each F_2 individual has a different genotype and no replication or experimental design can be employed to effectively control environmental influence. So for the inheritance of quantitative traits with low heritability the precision of QTL mapping is relatively low. To solve these problems, one can take family means as the unit of phenotypic measurement (Mather and Jinks, 1982; Paterson, 1997; Lynch and Walsh, 1998; Zhang *et al.,* 2003; Zhang and Xu, 2004). This is an $F_{2:3}$ design in plant genetics

(Austin and Lee, 1996; Cockerham and Zeng, 1996; Fisch *et al.,* 1996; Jiang and Zeng, 1997; Chapman *et al.,* 2003; Zhang and Xu, 2004; Kao, 2006) and daughter or grand daughter design in animal genetics (Weller *et al.,* 1990). These designs are frequently used in mapping QTLs in both the plant and animal kingdoms. This is because the family mean-based heritability can be significantly increased by increasing the number of replicates used in phenotyping the progeny. In the data analysis, the method for the $F_{2:3}$ design is adopted by simply replacing the F_2 phenotype by the average value of the $F_{2:3}$ progeny (Zhang *et al.,* 2003). A major disadvantage of the F_2 population is that the data of marker genotypes cannot be repeatedly used.

In pearl millet, several $F_{2:3}$ and $F_{2:4}$ mapping populations have been developed from diverse inbred lines of Asian, American and African origin (Hash *et al*., 2002). Some of the widely utilized mapping populations for linkage map construction and QTL mapping studies in pearl millet are detailed here.

A population consisting of 133 F_2 individuals was developed from a cross of LGD-1-B-10 and ICMP 85410 by Liu *et al.* (1994). LGD-1-B-10 is a downy mildew-susceptible, extra-early, inbred segregant of (B 70 × Tift 756)-1-4-5 based on germplasm from Togo and India. ICMP 85410 is a downy mildew-resistant, dwarf, restorer, inbred segregant of (ICP 165 × ICP 220)-64 based on germplasm from Uganda, Mali and Nigeria. This population was utilized for construction of the initial RFLP-based genetic map of pearl millet (Liu *et al.,* 1994), and for QTL mapping of downy mildew resistance (Jones *et al.,* 1995). It was subsequently used by Devos *et al.* (2000) for comparative mapping of pearl millet with foxtail millet and rice.

Poncet *et al.* (2000) developed a population consisting of 250 F_2 individuals from a cultivated × wild F_1 hybrid. The wild parent, *Pennisetum glaucum* ssp.

monodii, form *mollissimum*, referred to as 'Molli', is a S_4 line generated from a natural wild population collected near Gao in Mali. The cultivated parent, *P. glaucum* ssp. *glaucum* cv. 'Souna', is an early-flowering landrace from Mali where sympatry with wild forms still occurs. This population was used to understand the genetic control of domestication traits in pearl millet.

Yadav *et al.* (2002) developed a population consisting of 150 F_2 individuals from a cross of two early-maturing inbred lines, H 77/833-2 and PRLT 2/89-33. H 77/833-2 (H77) is the male parent of a number of thermotolerant, extra-early, high-tillering and high-yielding pearl millet hybrids including HHB 67 (843A × H 77/833-2), which is widely cultivated in north-western India. PRLT 2/89-33 (PRLT) is an inbred line derived from the ICRISAT's Bold-Seeded Early Composite (BSEC). BSEC is an elite breeding population based predominantly on *Iniadi* landrace germplasm from West Africa. QTLs associated with drought tolerance were identified using this population (Yadav *et al.,* 2002), along with genotype × environment interactions for grain and stover yield components in non-stress conditions (Yadav *et al.,* 2003).

A population consisting of 168 F_2 individuals was developed from a cultivated × wild F_1 hybrid by Poncet *et al.* (2002), which was used to identify QTLs affecting domestication traits. The wild parent was an individual from a population of the wild subspecies, *P. glaucum* ssp. *monodii*. The cultivated parent, Thiotande, was an S_2 inbred line selected from a Senegalese cultivar, which is cultivated during the off-season, *i.e.* in a situation where no gene flow between the wild forms and this domesticated line occurs.

For identification of genomic regions associated with drought tolerance Yadav *et al.* (2004) developed a population consisting of 151 F_2 individuals from a cross of

two agronomically elite inbred seed parents, ICMB 841 and 863B. The two parents are known to produce hybrids that distinctly differ in their response to post-flowering drought stress. Parent 863B was bred from *Iniadi* landrace material from Togo and was selected for this study based on its combination of agronomic eliteness and superior combining ability for grain filling under terminal drought stress conditions. Parent ICMB 841 (Singh *et al.,* 1990b) is the maintainer of the female parent of several high yielding hybrids (Govila *et al.,* 1997), but it lacks tolerance to terminal drought stress.

2.3.4. Linkage maps

Construction of a genetic linkage map is based on observed recombination between marker loci in the experimental cross. Segregating families, *e.g.* F_2 populations or BC progenies, DHs populations or RILs lines, are commonly used. Genetic map distances are based on recombination fractions between loci. Several computer packages are presently available for genetic linkage mapping but the most widely used are MAPMAKER/EXP (Lander *et al.,* 1987), JoinMap (Stam, 1993), GMENDEL (Echt *et al.,* 1992) and Map Manager QTX (Manly *et al.,* 2001).

Markers are assigned to linkage groups using the odds ratios, which refers to the ratio of the probability that two loci are linked with a given recombination value over a probability that the two are not linked. This ratio is called a logarithm of odds (LOD) value or LOD score (Risch, 1992; Stam, 1993). The critical LOD score used to establish linkage groups is 3.0. A LOD value of 3.0 between two markers indicates that linkage is 1000 times (*i.e.,* 10^3) more likely than no linkage (Stam, 1993). Higher critical LOD values will result in greater numbers of fragmented linkage groups, each with a small number of markers, while lower critical LOD values will tend to create fewer linkage groups each with large numbers of markers per group. Two markers are

placed in distinct linkage groups if they are not linked to any member of the other group. At any stage in the calculation, there is a group of markers, which have been assigned to a linkage group and a group of free markers that have not yet been assigned. Various options (*e.g.,* changing the parameters of analyses, excluding loci or individuals, generation of additional marker data for linkage groups with small numbers of markers, etc.) can be tested until researchers establish satisfactory linkage groups. Ideally one would like to arrive at a number of linkage groups that is the same as the haploid chromosome number of the species under study. For calculating map distances and determining locus order, the researchers need to specify several parameters, including a recombination threshold value and mapping function. The Haldane (1919) or Kosambi (1944) mapping functions are commonly used for converting the recombination fractions to map units or centiMorgans (cM). The Haldane mapping function takes into account the occurrence of multiple crossovers but the Kosambi mapping function accounts also for interference, which is the phenomenon of one cross-over inhibiting the formation of another in its neighborhood (Ott, 1985).

Vision *et al.* (2000) proposed two experimental phases in the construction of a high-density genetic map. The first is to construct a high-confidence framework and the second is to add new markers to this framework. This two-phased strategy allows many markers to be placed on a well-measured map with a minimum of genotyping and avoids the loss in map resolution that would result from arbitrarily shrinking mapping population size.

In pearl millet, the first molecular marker-based genetic map was generated by Liu *et al.* (1994) using 181 RFLP markers. The total length of this map, which comprised seven linkage groups, was 303 cM (Kosambi function) and the average

map distance between loci was about 2 cM, This initial map was transferred to several additional crosses (Busso *et al*., 1995; Liu *et al.*, 1996) in studies of sex specific recombination rates. Further, this map has been used for high saturation marker genotyping using AFLP marker, additional homologus probes from pearl millet and heterologous probes from other grasses. This work has been extended to the total pearl millet genetic linkage map length to approximately 600 cM (Devos and Gale, 1997).

Devos *et al.* (2000) developed a linkage map consisting of 242 markers including RFLP and SSR markers covering 473 cM (Haldane function) across seven linkage groups with an average distance between loci of about 1.95 cM. This linkage map was derived from genotyping a mapping population of 133 F_2 individuals of a cross involving LGD-1-B-10 and ICMP 85410, originally used by Liu et al. (1994) to generate the base map for this species.

Poncet *et al.* (2000) constructed a linkage map of 32 RFLP markers comprising seven linkage groups and covering 171.6 cM (Haldane function) in a population of 250 F_2 individuals developed from single F_1 plant of a cultivated × wild pearl millet hybrid.

Poncet *et al.* (2002) used a 168 individual F_2 population developed from a cultivated × wild hybrid to construct a linkage map of 22 RFLP markers comprising seven linkage groups and covering 176.9 cM (Haldane function).

A linkage map consisting of 50 RFLP markers comprising seven linkage groups and covering 352 cM (Haldane function) with an average map distance between loci of about 7 cM was developed by Yadav *et al.* (2002) using 150 F_2 individuals derived from a cross of two early-maturing inbred lines, H 77/833-2 and PRLT 2/89-33.

Azhaguvel *et al.* (2003) developed a skeleton genetic linkage map of 562 cM (Haldane function), comprising the seven expected pearl millet linkage groups. This map was constructed for an $F_{2:3}$ mapping population of the cross IP 18293 × Tift $238D_1$ using 33 homologous RFLP markers and three morphological markers.

Yadav *et al.* (2004) developed a linkage map based on the cross of two agronomically elite inbred, ICMB 841 and 863B. This map consisted of 91 markers covering 476 cM (Haldane function) across seven linkage groups with an average map distance between loci of about 5.2 cM.

Qi *et al.* (2004) presented a consensus genetic map based on mapping data from four pearl millet F_2 populations, comprising 65 SSR, 220 homologous RFLP and 133 heterologous RFLP markers. These maps and markers provide the base for the QTL and comparative analysis of pearl millet and applications of MAS in breeding programmes.

2.3.5. QTL analysis methods

The principle of QTL mapping is to associate the phenotypically evaluated trait(s) with segregating molecular marker alleles using statistical tools. The map locations of QTLs can then be estimated by means of highly associated markers. QTL analysis can lead to the elucidation of QTL parameters in terms of number, position, effects and interactions between them. Association of morphological markers with quantitative traits in plants was observed early on (Sax, 1923; Everson and Schaller, 1955) and the first steps towards mapping of QTLs or polygenes were taken based on the scarce markers available (Thoday, 1961). Currently, relatively complete genetical maps exist for many crop species and algorithms have been developed for QTL mapping in a wide range of pedigrees (Paterson, 1995).

A number of methods for mapping QTLs and estimating their effects have

been suggested and investigated (Edwards et al., 1987; Haley and Knott, 1992; Jiang and Zeng, 1995; Lander and Botstein, 1989; Jansen and Stam, 1994; Utz and Melchinger, 1994; Zeng, 1994). The most commonly used methods for QTL mapping are based on the maximum-likelihood method. From simple to more complicated, four approaches are commonly used – single marker analysis (SMA), interval mapping (IM), composite interval mapping (CIM) and multiple interval mapping (MIM).

The simplest method is based on single marker analysis (SMA), where the differences between the phenotypic means of the marker classes are compared using F-statistics, linear regression or nonparametric tests (Sax, 1923, Edwards *et al.*, 1987; Soller *et al.,* 1976). SMA is the least informative of the analyses, because recombination (r), as well as the additive (a) and the dominant (d) effects of a QTL may be confounded. SMA often fails to give reliable estimates of numbers and positions of QTL and the magnitude of their effects (McMillan and Robertson, 1974, Lander and Botstein, 1989).

Thoday (1961) introduced interval mapping (IM), and mathematical treatment of this method was presented by Lander and Botstein (1989). IM uses pairs of observable flanking markers to construct intervals within which to search for QTLs along the chromosomes. Factors that weaken interval mapping include the effects of additional QTLs in any single interval that contribute to the sampling variance. Further, if two QTLs are linked, their combined effects will cause biased estimates. The method of composite interval mapping (CIM) was proposed as solution to these limitations of simple interval mapping (SIM) (Jansen and Stam, 1994; Utz and Melchinger, 1994; Zeng, 1994). CIM performs the analysis in the usual way, except that the variance from other QTLs is accounted for by including partial regression

coefficients from markers ("cofactors") in other regions of the genome. CIM gives more power and precision than SIM because the effects of other QTLs are not present as residual variance. CIM removes the bias that can be caused by QTLs that are linked to the position being tested.

Multiple interval mapping (MIM) tends to be more powerful than either SMA or CIM. This method uses multiple marker intervals simultaneously to fit various putative QTLs directly into the model for mapping QTLs (Kao *et al.,* 1999). MIM leads to more accurate QTL position and QTL effect estimates (Mayer, 2005). MIM is appropriate for the identification and estimation of genetic architecture parameters, including the number, genomic positions, effects and interactions of significant QTLs and their contribution to the genetic variance.

The computer programs such as MAPMAKER/QTL (Lander *et al.,* 1987; Whitehead Institute), QTL Cartographer (Basten *et al.,* 1994, 1997), PLABQTL (Utz and Melchinger, 1996), Map Manager (Manly, 1997), QGene (Nelson, 1997) all have been used for performing QTL analysis.

2.3.6. QTL studies in pearl millet

Until 1990, there were no DNA-based pearl millet molecular markers and no mapping population for the development of linkage maps. At present, hundreds of pearl millet markers have been created (Liu *et al.* 1994; Allouis *et al.,* 2001; Qi *et al.,* 2001, 2004; Budak *et al.,* 2003; Senthilvel *et al.,* 2004; Bertin *et al.,* 2005; and Mariac *et al.*, 2006), several mapping populations have been developed from diverse inbred lines of Asian and American origin (Hash *et al*., 2001) and detailed marker-based genetic linkage maps were produced (Liu *et al*., 1994, 1996; Devos *et al.,* 2000 and Qi *et al.,* 2004). Using these maps, genomic positions of QTLs were mapped for disease resistances (Jones *et al.,* 1995; Morgan *et al*., 1998), abiotic stress tolerances

(Howarth *et al.,* 1997; Yadav *et al.,* 1999, 2000, 2002, 2004; Bidinger *et al*., 2007), grain and stover yield and quality components (Hash and Bramel-Cox, 2000; Hash *et al.*, 2001, 2003; Yadav *et al*., 2003).

As a result of these findings, marker-assisted backcrossing (MABC) has been successfully utilized to improve downy mildew resistance of the male parental line of pearl millet hybrid HHB 67. However, for the identification of genomic regions for sink size component traits, much remains to be done. The present study aims at dissecting pearl millet sink size traits to improve the understanding for gene expressions affecting these components of grain yield. Details of QTLs identified in earlier studies for sink size and their component traits in pearl millet are summarized in Table 1 and are briefly described here. QTLs controlling panicle length were reported on LG 1, 2 and 7 (Poncet *et al*., 2000), LG 7 (Poncet *et al.,* 2002), and on LG 4 (Nepolean*,* 2002). The variation explained by these QTLs ranged from 8.6 to 35.9%.

Six QTLs associated with panicle diameter were reported on LG 5 and 7 (Poncet *et al.,* 2000); LG 6 and 7 (Poncet *et al.*, 2002), and two QTLs on LG 4 (Nepolean, 2002), of which a QTL on LG 7 explained the maximum variation (62%).

For 1000-grain weight, Yadav *et al.* (2002), Nepolean (2002) and Bidinger *et al.* (2007) reported a total of nine QTLs across six linkage groups (LG 1, 2, 3, 4, 6 and 7). The QTL located on LG 1 explained highest amount of phenotypic variation.

Poncet *et al.* (2000) and Poncet *et al.* (2002) identified a total of four QTLs contributing to panicle weight, these QTLs were mapped on LG 2, 5, 6 and 6. The variation explained by QTL on LG 2 was maximum (60.9%).

Nepolean (2002) identified three QTLs for single panicle grain weight on LG 4, 6 and 7 explaining a total variation of 54.7% of observed variation.

Yadav *et al.* (2004) reported six QTLs for panicle harvest index on LG 1, 2, 3, 5, 6 and 7. Bidinger *et al.* (2007) also reported four QTLs on LG 1, 2, 3 and 4 for this trait. The variation explained by these QTLs ranged between 13.8% and 72%.

A total of 12 QTLs for plant height have been reported across five linkage groups LG 1, 4, 5, 6 and 7 (Poncet *et al.*, 2000, 2002; Nepolean, 2002; Azaguvel *et al.,* 2003). These QTLs individually explained from 8.2 to 34.7% of observed variation for this trait in the various mapping populations studied.

For productive tillers, a total of eighteen QTLs were identified across LG 1, 2, 4, 6, 7 (Poncet *et al.*, 2000, 2002; Nepolean, 2002 and Yadav *et al.,* 2002, 2003). The variation explained by these individual QTLs ranged between 9.2% and 31.6%.

2.3.7. Conclusions from QTL mapping studies

In the traditional models of quantitative genetics, simplifying assumptions were made about equality and strict additivity of gene effects (Falconer and Mackay, 1996). From the results of the QTL mapping experiments it has become clear that such assumptions are incorrect. In many mapping experiments, a relatively small number of QTLs account for very large portions of phenotypic variance, with increasing numbers of genes accounting for progressively smaller portions of variance, until the significance threshold is reached (Paterson, 1995). The number of QTLs located for particular traits in individual studies varies from one to sixteen, usually being below five (Kearsey and Farquhar, 1998). The proportion of phenotypic variation explained by each QTL and all QTLs together depends on the heritability of the trait as well as on the portion of revealed QTLs. QTLs are usually spread over all chromosomes, but clusters of QTLs in certain chromosomal regions have been observed as well. Differences occur in QTL incidence when quantitative traits are scored in many environments or during many years.

The actual number of loci and their effects on a quantitative trait can be difficult to determine. QTL mapping can underestimate the number of QTLs and their individual effects are often over-estimated (Barton and Keightley, 2002). QTLs can be undetected because of lack of recombinants if they are closely linked to each other and have opposite effects, or if the threshold limit for detection of QTLs is too small. They can also be overlooked when closely linked QTLs, with effects in the same direction, appear as a single QTL with a large effect. The effects of a statistically significant QTL are often overestimated particularly when the mapping population size is small (Barton and Keightley, 2002). The detection of QTLs depends on the quality of the genetic map, in particular the resolution of the map is determined by the number of markers, ensuring that marker order is correct.

3. Materials and Methods

The plant materials and the population for genetic and QTL mapping studies on sink size traits were selected and developed in the Pearl Millet Breeding Unit, and the laboratory work was carried out in the M.S. Swaminathan Applied Genomics Laboratory at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh. The materials and methods utilized in this study are detailed here.

3.1. Plant materials

Pearl millet germplasm collections at ICRISAT has accessions with widest range for panicle length, panicle diameter and grain size. Over the period, pearl millet improvement programme at ICRISAT has utilized these germplasm accessions for the development of trait-specific breeding lines. In the present study, trait-specific breeding lines representing large contrasts for panicle length, panicle diameter and grain size were utilized to develop the basic genetic populations.

3.1.1. Plant material selection

To generate genetic populations for studying the genetic control of sink size traits, three groups of parental inbred lines were selected. The first group included two pairs of contrasting inbred lines for panicle length with diverse pedigree having similar flowering period. These were selected from the advance trait-specific breeding lines during 2005 rainy season. In a similar way, two pairs of contrasting lines for each of panicle diameter and grain size were also selected from their trait-specific breeding lines to form the second and third groups, respectively.

For identifying QTLs for sink size traits, two inbred lines showing substantial variation for the sink size traits and also for molecular marker were selected for

mapping population development. The inbred line used as female parent had small grain size (5 g) and the parent used as male had large grain size (13 g). These lines also had substantial contrast for panicle length and panicle diameter. The pedigree of selected parental lines and their pair-wise cross combination in each group are presented in Table 2 and 3.

3.1.2. Development of genetic and mapping populations

The cross combinations subjected to generation means, triple test cross (TTC) and QTL studies are presented in Table 3.

For developing the basic genetic populations for generation means analysis, the selected parental lines in each group were sown in 4 m four-row plots in a crossing block to generate F_1s during post-rainy season of 2005-06. Selfing and crossing between the selected contrasting lines was carried out during January and February 2006. The crosses were made between lines with low trait value as seed parent and lines with high trait value as pollen parent in all the trait-specific groups. Number of plants selfed and crossed in each group was ensured to get enough parental and F_1 seeds for generating F_2 and backcross populations and for further field evaluations. Crossed F_1s and selfed panicles were harvested separately. Panicles were threshed after proper drying to the optimum moisture content. For generating F_2 and back cross populations, the parental lines and their F_1's of each group were planted under greenhouse conditions. Parental lines were sown in 5 pots and their F_1's in 10 pots at two staggered sowings with a week interval during April 2006. Three plants were maintained in each pot. In each trait-specific group, the F_1's were selfed to generate the F_2 seeds and also backcrossed with their female and male parent to generate BC_1 and BC_2 population, respectively during May-June 2006. Backcrossed and selfed panicles were harvested, dried and threshed. Thus, six generation (P_1, P_2,

F_1, F_2, BC_1 and BC_2) were obtained in all the three trait-specific groups for generation means evaluation trial.

For generating TTC families, a single cross from each trait-specific group was selected based on the maximum contrast between the parental lines (Table 3). The parental lines and the F_1s of selected cross from each trait-specific group were sown in two-row plots at three staggered sowing with one-week interval to synchronize with the flowering period of the F_2 population (planted in 20 rows) along with the six-generation evaluation trial during 2006 rainy season. Sixty individual F_2 plants were tagged from the six-generation population trial of selected cross of each trait-specific group. The tillers of tagged F_2 plants were selfed to collect pollen. Pollens collected from individual F_2 plants were used for crossing to their respective parents (P_1 and P_2) and F_1 to produce three types of families L_{1i} (P_1 x F_{2i}), L_{2i} (P_2 x F_{2i}), and L_{3i} (F_1 x F_{2i}) in each trait-specific group. Crossed panicles were harvested, dried and threshed.

For identifying QTLs for sink size traits, two inbred lines showing substantial variation for the sink size traits and also for molecular marker were subjected for mapping population development (Table 3). The F_1 seeds were produced by making a plant-to-plant cross during the post-rainy season of 2005-06. F_1 seeds were sown in pots under greenhouse conditions during April 2006 and F_2 seeds generated. The selfed panicles were harvested from individual F_1 plants and threshed separately. The F_2 seeds from a single F_1 plant were sown during 2006 rainy season for phenotypic observation on sink size and agronomic traits, and the resulting F_2 populations were also utilized to generate F_2-derived F_3 ($F_{2:3}$) progenies. A set of 188 $F_{2:3}$ progenies were derived from the F_2 population, which were subjected to both genotyping and phenotypic observations (Figure 1).

3.2. Field experiments

The trials were conducted on ICRISAT farm, Patancheru. Generation means trials were evaluated during the 2006 rainy season and 2007 summer season. In both the seasons, six generations (P_1, P_2, F_1, F_2, BC_1 and BC_1) of the two crosses from each trait-specific group were planted in a randomized complete block design in three blocks. In each block, parents and their F_1s, backcrosses (BC_1s and BC_2s) and F_2s were raised in 2, 6 and 20 row plots, respectively.

For triple test cross trial, the TTC families of each trait-specific group were evaluated along with the generation means trial planted as one experiment during 2007 summer season in a randomized complete block design with three replications. It consisted of 180 TTC families (60 each of L_{1i}, L_{2i} and L_{3i}) planted in single-row plots.

For QTL mapping experiment, F_2 and their $F_{2:3}$ mapping populations were evaluated during the 2006 rainy season and 2007 summer season, respectively. During the rainy season of 2006, F_2 seeds from a single F_1 panicle were sown in 20-row plot along with parents for phenotypic observations on single plant basis. In 2007 summer season, 188 $F_{2:3}$ progenies were evaluated for phenotypic observations on sink size and agronomic traits. For this, each of 188 $F_{2:3}$ progenies and their parental lines were raised in single-row plot in a randomized complete block – α design with three replications. The parental lines were repeated 10 times in each replication.

For all the trials, the rows were 4 m long and 60 cm apart, and the seeds were hand dibbled at a spacing of 20 cm in each row. Seeds were treated with fungicide before sowing to protect from soil borne pathogens. Standard cultural practices were followed to raise a successful crop. The experiments were protected from insect and pest by spraying appropriate chemicals.

3.3. Phenotypic observations

For generation means analysis, observations were recorded on 20 individual plants each in parents and their F_1s, 100 plants each in backcrosses (BC_1 and BC_2) and 350 plants in F_2 population from each block for a cross. For the TTC analysis, data were recorded on 10 competitive plants from each of the 180 TTC families in each replication. For both the analyses, phenotypic observations on panicle length, panicle diameter and grain size were recorded in their respective trait-specific crosses.

For correlation analysis, observations were recorded on 100 F_2 plants for days to 50% flowering, plant height, productive tiller number, panicle length, panicle diameter, grain size and grain yield in the generation means trial during 2007 summer season.

In the F_2 mapping population, observations were recorded for panicle length, panicle diameter, grain size, plant height and productive tiller number on 188 individual F_2 plants and 20 plants each in the parents during 2006 rainy season. In $F_{2:3}$ mapping population, observations were recorded for panicle length, panicle diameter, grain size, panicle weight, panicle grain weight, plant height and productive tiller number on 10 random plants in each of 188 $F_{2:3}$ progenies.

The observed traits are described here.

3.3.1. Days to 50% flowering

Number of days taken from sowing to the emergence of stigma in individual plants was recorded. This trait was observed in the F_2 population of all the crosses of generation means trials for correlation analysis.

3.3.2. Plant height (cm)

Plant height was measured in centimeter from the ground level to the tip of the highest panicle. This trait was observed in the F_2 population of all the crosses of

generation means trial for correlation analysis and also in the F_2 and $F_{2:3}$ mapping populations.

3.3.3. Productive tiller number

Total number of productive tillers (tillers with grain bearing panicles) of individual plants was counted and recorded. This trait was observed in the F_2 population of all the crosses of generation means trial for correlation analysis and also from the F_2 and $F_{2:3}$ mapping populations.

3.3.4. Panicle length (cm)

Length of the panicle was measured in centimeter from its base to its tip at maturity. The panicle of the main tiller of the plant concerned was used for this measurement. This trait was observed in all the six basic generations of panicle length group crosses, F_2 population of panicle diameter and grain size group crosses and in F_2 and $F_{2:3}$ mapping populations.

3.3.5. Panicle diameter (mm)

At maturity, the diameter of the panicle was measured in millimeter on the thickest portion of the panicle using vernier caliper. This trait was observed in all the six basic generations of panicle diameter group crosses, F_2 population of panicle length and grain size group crosses and in F_2 and $F_{2:3}$ mapping populations.

3.3.6. Grain size (1000-grain weight)

As an index of grain size, weight of 1,000 grains taken from the open-pollinated panicles of each entry was measured in grams. This trait was observed in all the six basic generations of grain size group crosses, F_2 population of panicle length and panicle diameter group crosses and in F_2 and $F_{2:3}$ mapping populations.

3.3.7. Panicle weight (g)

Panicle weight in grams was determined from the weight of individual main stem panicles. This trait was observed in $F_{2:3}$ mapping population.

3.3.8. Panicle grain weight (g)

Grains obtained from the individual open-pollinated main stem panicles were weighed in grams. This trait was observed in $F_{2:3}$ mapping population.

3.3.9. Panicle harvest index (%)

Derived as an index from the ratio of panicle grain weight to total panicle weight of a particular panicle. This trait was observed in $F_{2:3}$ mapping population.

3.3.10. Grain yield per plant (g)

Grains obtained from open-pollinated panicles of the individual plant were weighed and recorded in grams. This trait was observed in the F_2 population of all the crosses of generation means trials for correlation analysis.

3.4. Laboratory procedures

3.4.1. DNA extraction and purification

Around 30 selfed seeds from each of 188 $F_{2:3}$ progenies and both parents were grown in pots under greenhouse conditions. Bulk DNA was obtained from approximately 30 mg representative per $F_{2:3}$ progeny and parental lines by using CTAB method (Mace *et al.,* 2003) with slight modifications. DNA was further purified by RNase digestion followed by extraction with phenol:chloroform:iso-amyl alcohol (25:24:1) and ethanol precipitation as described by Mace *et al.* (2003). The reagents required for DNA extraction are listed in Appendix 1 and the adopted procedure of 96-well plate mini DNA extraction is described here.

A) Preparation

1. Two chrome-plated grinding balls (4 mm in diameter), pre-chilled at –20°C for about 30 minutes, were dispersed by an automatic ball disperser to 12-x 8- well polypropylene strip extraction tubes with strip caps (Marsh Biomarket, USA) that were kept on ice.
2. 3% CTAB buffer was pre-heated at 65°C in water bath (Precision Scientific Model: Shaking Water Bath 50) before starting DNA extraction.
3. Leaf stips of six-inches long were collected from 25–30 one week-old seedlings of parents or $F_{2:3}$ progeny and cut into small pieces (approximately 30 mg), which were then transferred to an extraction tube fitted in a box. This was repeated for all 188 $F_{2:3}$ progenies and parental lines, to fill two 96-well boxes.

B) Grinding and extraction

1. 450 µl of pre-heated 3% CTAB buffer was added to each extraction tube containing leaf sample, and tightly capped with polyethylene strip caps.
2. Grinding was carried out using a Sigma Geno-Grinder (Spex Certiprep, USA) at 500 strokes/minute for 2 minutes.
3. Grinding was repeated until the colour of the solution became pale green and leaf strip pieces were sufficiently macerated. After the first round of grinding, the boxes were checked for leakage by taking them out from the Geno-Grinder and were shaken for proper mixing of leaf tissues with buffer.
4. After grinding, the box with the tubes was fixed in a locking device and incubated at 65°C in a water bath for 10 minutes with occasional manual shaking.

C) Solvent extraction

1. 450 µl of chloroform:iso-amyl alcohol (24:1) mixture was added to each tube, tubes were inverted twice for proper mixing and the samples were centrifuged at

6200 rpm for 10 minutes (Sigma laboratory centrifuge model 4K15C with QIAGEN rotor model NR09100: 2 x 1120 g Sw).

2. After centrifugation, the aqueous layer (approximately 300 µl) was transferred to a fresh tube (Marsh Biomarket).

D) Initial DNA precipitation

1. To each tube containing the aqueous layer, 0.7 volume (approximately 210 µl) of cold (kept at –20°C) isopropanol was added. The solution was carefully mixed and the tubes were kept at –20°C for 10 minutes.
2. The samples were centrifuged (same centrifuge as earlier) at 6200 rpm for 15 minutes.
3. The supernatant was decanted under the fume hood and pellets were allowed to air dry for about 30 minutes.

E) RNase A treatment

1. In order to remove co-isolated RNA, pellets were dissolved into 200 µl of low salt TE buffer ($T_1E_{0.1}$) and 3 µl of RNase A.
2. The solution was incubated at 37°C for 30 minutes or overnight at room temperature.

F) Solvent extraction

1. After incubation, 200 µl of phenol:chloroform:isoamyl alcohol (25:24:1) was added to each tube, mixed and centrifuged (same centrifuge as earlier) at 5000 rpm for 10 minutes.
2. The aqueous layer in each tube was transferred to a fresh tube (Marsh Biomarket) and 200 µl of chloroform:isoamyl alcohol (24:1) was added to each tube, mixed and centrifuged at 5000 rpm for 10 minutes.
3. The aqueous layer was transferred to fresh tube (Marsh Biomaket).

G) DNA Precipitation

1. 15 µl (approximately 1/10th volume) of 3 M sodium acetate (pH 5.2) and 300 µl (2 volumes) of absolute ethanol (kept at –20°C) were added to each of the tubes and the mixture was subsequently incubated in a freezer (–20°C) for 5 minutes.
2. Following the incubation at –20°C, the tubes were centrifuged (same centrifuge as earlier) at 6200 rpm for 15 minutes.

H) Ethanol wash

1. After centrifugation, the supernatant was carefully decanted from each tube in order to ensure that the pellet remained inside the tube.
2. Subsequently, 200 µl of 70% ethanol was added to each of the tubes and this was followed by centrifugation (same as earlier) at 5000 rpm for 5 minutes.

I) Final re-suspension

1. The supernatant was carefully decanted and the pellet was allowed to air dry for one hour.
2. Dried pellets were re-suspended in 100 µl of $T_{10}E_1$ buffer and kept overnight at room temperature to dissolve completely.
3. The resuspended DNA samples were stored at 4°C.

3.4.2. Quantification of DNA concentration and quality check

The quality of DNA in each sample was checked using 0.8% agarose gels, stained with ethidium bromide. 1 µl of DNA sample was mixed with 1 µl of orange dye and 3 µl of distilled water, and loaded in each well of the agarose gel (Appendix 2). The gel was run at 100 V for 5 minutes, after which the quality of DNA was checked under ultra-violet transilluminator. A smear of DNA indicated poor quality whereas a clear band indicated good quality DNA. Samples of poor quality DNA were re-extracted.

The DNA quantity present in each experimental sample was assessed using a fluorescence spectrophotometer (Spectrafluor plus, Tecan, Switzerland) by staining DNA with picogreen^{TM} (1/200 dilution) (Juro supply Gmbh, Switzerland). Based on the Relative Fluorescence Units (RFU) values and using a calibration graph. DNA concentration of each experimental sample was calculated (DNA concentration = – 2.78273 + 0.002019*RFU). The DNA concentration of each experimental sample was then normalized at 2.5 ng/µl to produce working samples be used in PCR reactions.

3.4.3. Marker polymorphism

A total of 331 primer pairs, which includes 96 markers of each SNP, SSR, EST-SSR, and 43 STS markers, were initially assayed on two parental lines to detect polymorphism. This resulted in identification of 109 polymorphic markers. A final set of 44 primer pairs were chosen based on their expected marker locations across the genome, banding pattern (at least 2bp differences in allele size between parents) and consistent amplification, to allow reliable genotyping using PAGE and/or capillary electrophoresis of the mapping population.

The selected polymorphic primer pairs with sequence information of both forward and reverse primers are listed in Table 4.

3.4.4. Polymerase chain reaction (PCR)

PCR reactions were conducted in 96 and 384-well plates in a GeneAmp PCR system PE 9700 (Applied Biosystem, USA) DNA thermocycler. The PCR were performed in volumes of 5 µl using three different reaction protocols (Table 5). A touchdown PCR program was used to amplify the DNA fragments. Reaction conditions were as follows.

Initial denaturation for 15 minutes at 94°C (to minimize primer dimer formation and to activate the *Taq* polymerase), subsequently 10 cycles of denaturation for 10 seconds at 94°C, annealing at 61°C to 52°C for 20 seconds, the annealing temperature for each cycle is reduced by 1°C, and extension at 72°C for 30 seconds. This was followed by a 20 minutes extension at 72°C to ensure amplification of equal lengths of both DNA strands (Smith *et al.,* 1995).

Table 5. Polymerase chain reaction components

Reaction	Primer (2 pM)	$MgCl_2$ (10 mM)	dNTPs (2 mM)	DNA (2.5ng/µl)	*Taq* (0.5 U/µl)	Buffer (10X)	DDW (µl)
1	0.50	1.00	0.25	1.00	0.20	0.50	1.55
2	1.00	1.00	0.38	0.50	0.20	0.50	1.43
3	0.50	0.75	0.50	0.50	0.25	0.50	2.00

3.4.5. Non-denaturing PAGE (Polyacrylamide Gel Electrophoresis)

The PCR products of EST-SSR and STS markers were separated on 8% non-denaturing PAGE (Plate 1a and 1b). The materials required were sequencing gel apparatus (glass plates, spacers, casting apparatus) and combs (68 or 100 wells). The reagents required are detailed in Appendix 3 and the protocol used for PAGE gel preparations is as follows.

A. For 8% gel (plate size 38 × 30 cm) 75 ml of gel solution will be sufficient.

   a. 10X TBE buffer 7.5 ml

   b. V/V acrylamide/bisacrylamide (29:1) 20.0 ml

   c. Distilled water 47.5 ml

   Mix these ingredients in 200 ml Erlenmayer flask.

B. Vigorously mix the solution and add 450 µl APS, swirl the flask and then immediately add 100 µl TEMED and mix. Pour the solution using a syringe (100 ml) that fits into the slot between the glass plates, and insert the comb (upside down, in order to form wells). Allow the acrylamide solution to polymerize for 30 to 60 minutes. Gels can be stored overnight as long as the plate ends are wrapped in pre-wetted tissue paper (1X TBE) and covered with plastic film.

C. After polymerization, set up the gel for running. Remove the comb. Fill the lower tank with 0.5X TBE buffer (approximately 250-300 ml) and the back plate and upper reservoir too (approximately 400 ml). Ensure that wells are covered with the buffer. Clean the wells by aspirating and dispensing 0.5X TBE buffer on the wells using a Pasteur pipette to remove small fragments of gel and tiny bubbles from the top of the wells. Insert the comb back on top of the wells [comb tips should rest on the well (<1 mm deep), don't force it into the well].

D. Pre-run the gel to warm it, for at least 10 minutes at 5 V/cm (400 V, 9 W, depending on width of the gel).

E. Make up the samples for loading with 5X loading buffer to give a final concentration of 1X (for instance 2 µl sample + 2 µl water + 1 µl 5X loading buffer). Mix properly and load between 3-5 µl (depending on size of the slots) onto each well of the gel.

F. Run the gel at approximately 5 V/cm (400 V, 9 W, depending on width of the gel). Higher voltage causes the gel to overheat and result in un-even run of the samples. Run the gel until the desired resolution has been reached (front of the tracking dye should reach a few cm above the end of the gel)

G. After the run, carefully pull apart the plates, so that the gel is attached to the front plate and start silver staining.

3.4.6. Single-strand conformational polymorphism (SSCP) gel electrophoresis

The PCR products of SSCP-SNP markers were denatured at 94^{o}C for 5 minutes and cooled to 4^{o}C immediately. The denatured products were separated on 300 x 380 x 0.4 mm SSCP gels using mutation detection enhancement (MDE) gel solution (Martins-Lopes *et al.,* 2001; Bertin *et al.,* 2005). This method uses the same equipment and laboratory techniques as PAGE, except that SSCP uses an MDE gel matrix rather than the polyacrylamide gels. The gel mix consisted of 7.5 ml of 10X TBE buffer, 20 ml of MDE gel solution and 47.5 ml distilled deionised water, and polymerized by adding 100 µl TEMED and 450 µl APS. Fragment were electrophoresed for 16 h at a constant power of 8 W at room temperature and then silver-stained (Plate 1c).

DNA separation of SSCP gels was based on both size and conformation. As the conformation was not known in advance, the precise relative gel positions of individual SSCP-SNP could not be predicted. Nevertheless, the mobility of the typical two-band SSCP profile for single-copy amplification was consistent over runs and, once assessed was used for post-hoc classification of individual phenotypes.

3.4.7. Silver Staining

After running the PAGE or MDE gel, electrophoresed DNA fragments were visualized using a modified silver staining procedure (Bassam, 1991). The reagents required are listed in Appendix 4 and the following steps were adopted for silver staining:

A. Rinse the gel in 2 liters of distilled water for 5 minutes (with gentle shaking of the container holding the gel).
B. Soak the gel in 2 liters of 0.1% CTAB for 20 minutes (with gentle shaking of the container holding the gel).

C. Incubate the gel in 2 liters of 0.3% ammonia for 15 minutes (with gentle shaking of the container holding the gel).

D. Place the gel in silver nitrate solution (2 liters) for 15 minutes (with gentle shaking of the container holding the gel).

E. Place the gel in de-ionized water for a few seconds.

F. Place the gel in sodium carbonate solution (developer, 2 liters) and gently shake until the bands become visible.

G. Rinse the gel in water (2 liters) for 1 minute to stop the staining.

H. Place the gel in glycerol solution (fixative, 2 liters).

I. Dry the gel (overnight for scanning or 30 minutes for first reading).

After silver staining of the gel, it was placed on a bench-viewer. The size (in base pairs) of the parental alleles for each SSR or SSCP-SNP were estimated based on their migration relative to the 100 bp DNA ladder (fragments ranging from 100 bp to 1000 bp), and presence or absence of parental alleles in each of the $F_{2:3}$ progenies were scored. The dried gel was removed (after scoring) from the front glass plate by soaking in concentrated NaOH solution for a few hours (or overnight).

3.4.8. Genotyping using capillary electrophoresis

The PCR products amplified using fluorescence-labeled primers genomic SSRs were separated by capillary electrophoresis using an ABI Prism 3700 automatic DNA sequencer (Applied Biosystems Inc.). This has the ability to detect size differences of less than 2 bp using a fluorescence-based detection system, thus dispensing with the need for radioactivity or laborious manual polyacrylamide gel techniques.

For this purpose, forward primers were labeled with 6-FAM™ (Blue), VIC™ (Green), NED™ (Yellow) or PET™ (Red) fluorophores (Applied Biosystems). PCR products of 4 primer pairs labeled with different dyes or same-flourophore-labeled

primers with non-overlapping amplicons (in terms of size) were pooled (post-PCR). The products of different flourophore–labeled primers were pooled in different proportion (1.0 µl of 6-FAM–labeled product, 0.8 µl of VIC-labeled product, 1.4 µl of NED–labeled product, and 1.0 µl of PET-labeled product). The pooled PCR products were then mixed with 0.25 µl of GeneScan 500™ LIZ® internal size standard (Applied Biosystems) and 7.0 µl of Hi-Di™ Formamide (Applied Biosystems). The final volume was made up to 12 µl with sterile double-distilled water. DNA fragments were denatured for 5 minutes at 95°C (Perkin Elmer 9700, Applied Biosystem) and cooled immediately on ice.

3.4.8.1. Fragment size fractionation

The PCR products with denatured DNA were electrophoresed and the capillary run was performed using the "Genscan2 POP6 Default" run module and "G5" filter-set. The analysis module used was "GS500 analysis". The fragments were separated in a 50 cm capillary array using POP6 (Performance Optimized Polymer, Applied Biosystems) as separation matrix.

3.4.8.2. Data Processing

The GenScan 3.1 software (Applied Biosystems) was used to size the peak pattern in relation to the internal size standard, GeneScan 500™ LIZ®. The principle behind this is that standards are run in the same lane or capillary injection as the samples, which contain fragment of unknown sizes labeled with different flourophores. Genscan® analysis software automatically calculates the size of unknown DNA fragments by generating a calibration sizing curve based upon the migration times of the known fragments in the standard. The unknown fragments are mapped on to the curve and the sample data is converted from migration times to fragments size. Genotyper 3.7

(Applied Biosystems) was used for allele calling. The peaks were displayed with base pair values and height (amplitude) in a chromatogram (Plate 1d). The height of the chromatogram peaks (representing the alleles) obtained through capillary electrophoresis is directionally proportionate to the signal strength, which in turn is determined by the amount of amplified product in the sample.

3.4.9. Scoring of amplified bands

The banding pattern of each of amplified PCR products of various marker systems were scored as follows:

A = Homozygote for the allele for female parent at a locus.

B = Homozygote for the allele for male parent at a locus.

H = Heterozygote carrying the alleles from both parents.

- = Missing data for the individual at a locus.

After scoring individual progenies, the data set was assembled in Microsoft Excel spreadsheet in a format suitable for linkage analysis by MAPMAKER (*i.e.* rows = genotype score at a given locus; columns = individual $F_{2:3}$ progenies).

3.5. Statistical analysis

3. 5.1. Genetic analysis

3.5.1.1. Generation means analysis

The analysis of variance was performed on non-segregating generations (P_1, P_2 and F_1) for the crosses evaluated during 2006 rainy season and 2007 summer season, separately to assess the variation between blocks. The 'F' value was tested at 2, 57 degrees of freedom. In the case of non-significance of mean squares between blocks, the observed data from each block were pooled for further analysis.

As results of analysis of variance showed non-significant variation between the blocks for all the traits, the observed data for each trait from each block were pooled, and subjected to generation means analysis as three sets of different population size.

(i) Set 1 consisted of three individual blocks (B_1, B_2 and B_3) with a population size of 20, 100 and 350 plants for parents and their F_1's, backcrosses and F_2 population, respectively.

(ii) Set 2 consisted of three two-way block combinations [*i.e.* block 1 and 2 (B_{12}), block 1 and 3 (B_{13}), and block 2 and 3 (B_{23})] with a population size of 40, 200 and 700 plants for parents and their F_1's, backcrosses and F_2 population, respectively.

(iii) Set 3 consisted of observations pooled across all the three blocks (B_{123}) with a population size of 60, 300 and 1050 plants for parents and their F_1's, backcrosses and F_2 population, respectively.

The main objective of this exercise was to examine the optimum population size required for accurate estimation of gene effects, based on the consistency of the estimates within and between the sets.

3.5.1.1.1. Scaling test

The basic generations data obtained were first used for a scaling test to examine the adequacy of a simple additive-dominance model. The scaling test for A, B and C scales were calculated as per the method suggested by Mather (1949).

$$A = 2\overline{B}_1 - \overline{P}_1 - \overline{F}_1$$
$$B = 2\overline{B}_2 - \overline{P}_2 - \overline{F}_1$$
$$C = 4\overline{F}_2 - 2\overline{F}_1 - \overline{P}_1 - \overline{P}_2$$

Where, $\overline{P}_1$, $\overline{P}_2$, $\overline{F}_1$, $\overline{F}_2$, $\overline{B}_1$ and $\overline{B}_2$ are the means of P_1, P_2, F_1, F_2, BC_1 and BC_2 generations, respectively.

Variance of the scales were calculated as:

$$V_A = 4V\bar{B}_1 + V\bar{P}_1 + V\bar{F}_1$$
$$V_B = 4V\bar{B}_2 + V\bar{P}_2 + V\bar{F}_1$$
$$V_C = 16V\bar{F}_2 + 4V\bar{F}_1 + V\bar{P}_1 + V\bar{P}_2$$

Where, V_A, V_B and V_C are variance of the respective scales A, B and C; $V\bar{P}_1, V\bar{P}_2, V\bar{F}_1, V\bar{F}_2, V\bar{B}_1$ and $V\bar{B}_2$ are variances of mean of P_1, P_2, F_1, F_2, BC_1 and BC_2 generations, respectively, which were calculated by dividing the variances of these generations with the respective number of plants observed.

Standard errors for A, B and C scales were calculated by estimating the square root of respective variances.

$$\text{S.E. of } A = \sqrt{V_A}$$
$$\text{S.E. of } B = \sqrt{V_B}$$
$$\text{S.E. of } C = \sqrt{V_C}$$

To test the deviation of scales from the hypothetical value of zero, 't' test was applied as:

$$\text{t for } A = A \Big/ \sqrt{V_A}$$
$$\text{t for } B = B \Big/ \sqrt{V_B}$$
$$\text{t for } C = C \Big/ \sqrt{V_C}$$

The observed 't' values were compared with the tabular t values at n–1 degrees of freedom, where n is the number of plants used in deriving the variances of all the generations involved.

3.5.1.1.2. Joint scaling test

Joint scaling test of Cavalli (1952) was performed to estimate the three-parameter model consisting of mid-parental value (m), dominance (h) and additive (d) gene effects following the least square method proposed by (Mather and Jinks, 1971).

Adequacy of three-parameter model was tested using chi-square test for goodness of fit at 3 (n–3) degrees of freedom, where n is the number of generation from which the three parameters were estimated.

3.5.1.1.3. Six-parameter model

Equations formulated by Hayman (1958) were utilized to obtain six parameters the average effect (m), additive effect (d), dominance effect (h), additive x additive interaction (i), additive x dominance (j) interaction and dominance x dominance (l) interaction for the traits where a simple additive-dominance model was inadequate to explain the observed variation.

$$m = \bar{F}_2$$

$$(d) = \bar{B}_1 - \bar{B}_2$$

$$(h) = 2\bar{B}_1 + 2\bar{B}_2 - 4\bar{F}_2 + \bar{F}_1 - \frac{1}{2}\bar{P}_1 - \frac{1}{2}\bar{P}_2$$

$$(i) = 2\bar{B}_1 + 2\bar{B}_2 - 4\bar{F}_2$$

$$(j) = \bar{B}_1 - \frac{1}{2}\bar{P}_1 - \bar{B}_2 + \frac{1}{2}\bar{P}_2$$

$$(l) = \bar{P}_1 + \bar{P}_2 + 2\bar{F}_1 + 4\bar{F}_2 - 4\bar{B}_1 - 4\bar{B}_2$$

Variances of the estimates of these parameters were obtained as linear combinations of variance of means of generations used to compute these six parameters.

$$V_m = V\bar{F}_2$$

$$V_{(d)} = V\bar{B}_1 + V\bar{B}_2$$

$$V_{(h)} = 4V\bar{B}_1 + 4V\bar{B}_2 + 16\bar{F}_2 + \bar{F}_1 + \frac{1}{4}\bar{P}_1 + \frac{1}{4}\bar{P}_2$$

$$V_{(i)} = 4V\bar{B}_1 + 4V\bar{B}_2 + 16V\bar{F}_2$$

$$V_{(j)} = V\bar{B}_1 + \frac{1}{4}V\bar{P}_1 + V\bar{B}_2 + \frac{1}{4}V\bar{P}_2$$

$$V_{(l)} = V\bar{P}_1 + V\bar{P}_2 + 4V\bar{F}_1 + 16V\bar{F}_2 + 16V\bar{B}_1 + 16\bar{B}_2$$

Standard error was then obtained by taking the square root of the respective variances and significant departure of the estimate of the parameters from zero was tested applying 't' test with the tabular t values at n–1 degrees of freedom, where n is the number of plants used in deriving the variances of all the generations involved.

3.5.1.2. Triple test cross analysis

'Triple Test Cross' (TTC) design, which is a simple extension of design III of Comstock and Robinson (1952) proposed by Kearsey and Jinks (1968) provides not only an unambiguous test for epistasis, but also provides an efficient estimate of additive and dominance components in the absence of epistasis. In the present study, triple test cross analysis has been carried out using the TTC model proposed by Kearsey and Jinks (1968).

3.5.1.2.1. Test for epistasis

The detection of epistasis was performed according to Kearsey and Jinks (1968). The test of significance of the difference [($L_{1i} + L_{2i} - 2L_{3i}$) where, i = F_2 individuals] provides information about the presence or absence of epistasis. Therefore, $L_{1i} + L_{2i} - 2L_{3i}$ for each line (F_2 individuals) and each replication was first computed and then tested.

The total epistasis for 'n' (n = 60) degree of freedom was calculated as uncorrected genotype (F_2 individuals) sums of square based on the total of these components over the replications.

$$\text{Total epistasis} = \frac{\sum_{i=1}^{60} (\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})^2}{n}$$

The total epistasis was partitioned into two components. The correction factor (c.f) measures mainly the epistasis of additive x additive (i) type with one degree of freedom.

$$\text{[i] epistasis (c.f)} = \frac{\left[\sum_{i=1}^{60} (\overline{L}_{1i} + \overline{L}_{2i} - 2\overline{L}_{3i})\right]^2}{n}$$

The corrected genotypes sum of squares is a measure of the combined additive x dominance and dominance x dominance (j + l) epistasis with n – 1 degrees of freedom.

$$\text{[j+l] epistasis} = \frac{\sum_{i=1}^{60} (\overline{L}_{1i} + \overline{L}_{2i} - 2\overline{L}_{3i})^2}{n} - \frac{\left[\sum_{i=1}^{60} (\overline{L}_{1i} + \overline{L}_{2i} - 2\overline{L}_{3i})\right]^2}{n}$$

The sum of squares associated with the interaction of total epistasis with blocks (*i.e.* Total epistasis x block interaction) was calculated as the difference between uncorrected total sum of squares and sum of squares of total epistasis with n(r–1) degrees of freedom. The (i) type of epistasis x block interaction sum of square was calculated as the difference between uncorrected replication sum of squares and sum of squares of (i) type epistasis with (r–1) degrees of freedom. The (j + l) type of epistasis x block interaction sum of squares was calculated as the difference between line sum of squares and sum of squares of (j + l) type epistasis with (n–1)(r–1) degrees of freedom. Where, n is the number of F_2 individuals and r is the number of replication.

Each of three types of epistasis can be tested against their respective interaction with blocks. However before testing individual epistasis, the homogeneity of the interaction was first tested as proposed by Singh and Choudhary (1999). As there were only two variances (i x block and (j + l) x block) homogeneity was tested using 'F' test.

F (2, 118) = Mean square of 'i' x block interaction / Mean square of (j + l) x block interaction

When the interactions with blocks were non-significant, then 'i' and (j + l) type of epistasis were tested against the total epistasis x block interaction.

3.5.1.2.2 Additive-dominance model

On the assumption of no epistasis, an additive-dominance model was fitted to the observed data as outlined by Kearsey and Jinks (1968).

3.5.1.2.2.1. Estimation of additive variance component (D)

The sum of $L_{1i} + L_{2i}$ for each F_2 genotype was calculated replication wise and subjected to analysis of variance as:

Source of variation	**df**	**MS**	**Expected**
Replication	r–1	MS_r	
Genotype sum ($L_{1i} + L_{2i}$)	n–1	MS_s	$\sigma^2e + 2r\,\sigma^2s$
Error	(n–1)(r–1)	Ms_e	σ^2e

Where, r = replications; n = number of F_2 plants used in producing TTCs; MS_r, MS_s, Ms_e = mean squares of replications, genotypes (sums) and error, respectively; σ^2e and σ^2s = expected mean square of error and genotypes (sums).

The observed mean squares were substituted into the equations as follows:

$\sigma^2s = (MS_s - MS_e)/2r$

$\sigma^2s = (1/8)D$

$D = 8\,(MS_s - MS_e)/2r$

3.5.1.2.2.2. Estimation of dominance component (H)

The difference in $L_{1i} - L_{2i}$ for each F_2 genotype was calculated replication wise and subjected to analysis of variance as:

Source of variation	df	MS	Expected
Replication	r–1	MS_r	
Genotype sum ($L_{1i} - L_{2i}$)	n–1	MS_d	$\sigma^2e + 2r\,\sigma^2d$
Error	(n–1)(r–1)	Ms_e	σ^2e

Where, r = replication; n = genotypes; MS_r, MS_d, Ms_e = mean squares of replication, genotype (differences) and error, respectively; σ^2e and σ^2d = expected MS of error and genotypes (differences).

The observed mean squares were substituted into the equations as:

$$\sigma^2d = (MS_d - MS_e)/2r$$

$$\sigma^2d = (1/8)H$$

$$H = 8(MS_d - MS_e)/2r$$

3.5.1.2.2.3. Degree of dominance

Degree of dominance was calculated as $(H/D)^{1/2}$, where H and D are the dominance and additive variance components, respectively.

3.5.1.2.2.4. Correlation coefficient ($r_{s,d}$)

The correlation coefficient ($r_{s,d}$) between the sum ($L_{1i} + L_{2i}$) and the genotypic differences ($L_{1i} - L_{2i}$) was calculated. Significant positive or negative correlations would indicate a predominant direction towards decreasing or increasing values of the trait, respectively (Jinks *et al*., 1969).

3.5.1.3. Estimation of components of variances and heritabilities

The component of variances and heritabilities were estimated for the set 3 data (pooled across all the three blocks) from the generation means trials evaluated during 2006 rainy season and 2007 summer season.

3.5.1.3.1. Variance estimates

On the assumption of absence of non-allelic interactions, phenotypic variances of the six generations were used to compute four parameters $E_{w,}$ D, H, and F as per the method suggested by Mather and Jinks (1971).

$$E_w = \frac{1}{4}(VP_1 + VP_2 + 2VF_1)$$
$$D = 2VF_2 - (VB_1 + VB_2)$$
$$H = VB_1 + VB_2 - VF_2 - E_w$$
$$F = VB_2 - VB_1$$

Where, $VP_{1,}$ $VP_{2,}$ VF_1 and $V B_2$ are variance of respective generations.

E_w = non-heritable variance due to environment.

D = fixable variance due to additive genes

H = non-fixable variance due to dominance

Dominance ratio = $(H/D)^{1/2}$

3.5.1.3.2. Heritability estimates

The heritabilities were estimated based on the method suggested by Mather and Jinks (1971).

Broad-sense heritability $= 100 \times (VF_2 - E_w)/VF_2$

Narrow-sense heritability $= 100 \times \{2VF_2 - (VB_1 + VB_2)\}/VF_2$

The range of heritabilities was characterized as low (<30%), moderate (31 - 60%) and high (> 61%) as described by Robinson *et al.* (1949).

3.5.1.4. Correlation analysis

The association between yield and its component traits as well as inter-association among the yield component traits were worked out following the method suggested by Goulden (1952) for the observed traits in F_2 population of all the crosses evaluated for generation means during summer season of 2007.

$$\text{Correlation coefficient } (r_{xy}) = \frac{\sum XY - \sum X \sum Y}{\sqrt{\sum X^2 - (\sum X)^2}\sqrt{\sum Y^2 - (\sum Y)^2}}$$

Where, r_{xy} is simple correlation coefficient between the traits 'X' and 'Y'

The significance of correlation coefficient was tested using 't' value with (n–2) degrees of freedom given by Snedecor and Cochran (1967).

3.5.2. QTL analysis

3.5.2.1. Phenotypic analysis

The analysis of variance for phenotypic data sets observed from $F_{2:3}$ progenies were performed using the Residual Maximum Likelihood algorithm (ReML), which provides the Best Linear Unbiased Predictors (BLUPs) of the performance of the genotypes (Patterson and Thompson, 1971) using GenStat Ver 8.0 (2005). ReML estimates the components of variance by maximizing the likelihood of all contrasts with zero expectations. The predicted means were calculated with each trait and each entry with entries considered as fixed effects, and block and entry × replication interaction as random effects, in the analysis. The methodology for estimating the heritability (broad-sense) and correlation coefficients for the observed traits are detailed in sections 3.5.1.3.2 and. 3.5.1.4.

3.5.2.2. Linkage map construction

Marker classes at each locus were summarized for all individuals into the three different genotypic classes expected for an F_2 population. The segregation of each marker was tested with a chi-square test for goodness of fit to the expected Mendelian segregation ratio (1:2:1) of the parental configuration.

The linkage map was constructed with MAPMAKER/EXP V.3.0 (Lander *et al.,* 1987). The Haldane (1919) mapping function was used to convert the recombination frequencies to genetic distances in centiMorgans (cM). The analysis was carried out by evaluating the mapping population as an F_2 population using two-point analysis to identify linked pairs at a LOD score of 3.5. The 'Sequence all' command was used for two-point (or pair-wise) linkage analysis. While the 'Group' command was used to group the marker in a sequence into different linkage groups. The 'Compare' command was used to calculate the maximum likelihood map for each specified order of markers and reported the orders sorted by likelihood of their maps. One sequence can specify more than one order of loci. For example, the sequence '{1 2 3}' specifies three different orders '1 2 3', '1 3 2' and '2 1 3'. MAPMAKER reports only the 20 most likely orders. The order having a log-likelihood of 0.0 was selected as the best order. The obtained order was then analyzed further using a three-point linkage analysis via the 'Ripple' command. Other markers were added using the 'Try' command and their positions were fine-tuned using the 'Ripple' command. The 'Ripple' command was used to assign exact orders of markers. Marker loci on linkage groups that were more than 50 cM apart were considered to be unlinked. The markers used in the present study have been already mapped in previous studies and they were used as reference for the map obtained in the present study to compare the linkage distance, marker position and marker order in linkage groups. Markers used in the

present study were therefore assigned to linkage groups based on their known chromosome locations and their order in the present $F_{2:3}$ mapping population was verified using MAPMAKER.

3.5.2.3. Quantitative trait loci (QTL) analysis

The data sets of 188 F_2 population and the predicted means of $F_{2:3}$ progenies and their genotyping data from 44 markers were used to identify genomic regions associated with the traits using composite interval mapping (CIM) analysis. Computations were performed using the software package PLABQTL ver 1.1 (Utz and Melchinger, 1995), which performs CIM using a regression approach (Haley and Knott, 1992) with selected markers as cofactors. Markers to serve as cofactors were identified using step-wise regression with an F-to-enter and F-to-delete threshold value of 2.5. The presence of putative QTL in an interval was tested using a critical LOD threshold as determined by PLABQTL using the Boneferroni chi-sqaure approximation (Zeng, 1994) corresponding to a genome-wise type I error of 0.25. Since the mapping population used in the present study constituted F_2 and $F_{2:3}$ progenies, along with the additive (A) model, additive-dominance (A+D) and epistatic (A+D+AA+AD+DD) genetic models were also included. The detection of QTL in the epistatic model is conducted without epistatic effects. Only in the final simultaneous fit all specified digenic epistatic effects are estimated for the detected set of QTLs using a stepwise regression procedure whereby the F-to-Enter value (and F-to-Drop) is obtained by using the Bonferroni bound at alpha = 0.05.

Genetic effect was positive if alleles from male parent contributed to the trait of interest and negative if alleles from female parent contributed towards the trait of interest. Estimate of genetic effects of each of detected QTL, the LOD score, and the total proportion of phenotypic variances explained jointly by all detected QTLs were

obtained by fitting a multiple linear regression model that simultaneously included all the detected QTL. After the QTL analysis, the identified QTLs for traits were assigned to the linkage groups based on position of makers using MapChart 2.1.

4. Results

The results of genetic and QTL analyses for panicle length, panicle diameter, grain size and other agronomic traits are presented in this chapter.

4.1. Genetic analysis

4.1.1. Generation means analysis

The observations recorded on the six basic generations of two trait-specific crosses each for panicle length, panicle diameter and grain size evaluated during 2006 rainy season and 2007 summer season were subjected to generation means analysis to elucidate the genetic architecture underlying these traits.

4.1.1.1. Analysis of variance

Analysis of variance for non-segregating generations (P_1, P_2 and F_1) of six basic generations of all the crosses evaluated during 2006 rainy season and 2007 summer season, revealed non-significant variation between blocks for the observed traits (Table 6). Since, there was no significant difference between the blocks, the observations from each block of a season were pooled as three sets of different population sizes.

(i) Set 1 consisted of three individual blocks (B_1, B_2 and B_3) with a population size of 20, 100 and 350 plants for parents and their F_1's, backcrosses and F_2 population, respectively.

(ii) Set 2 consisted of three two-way block combinations [*i.e.* block 1 and 2 (B_{12}), block 1 and 3 (B_{13}), and block 2 and 3 (B_{23})] with a population size of 40, 200 and 700 plants for parents and their F_1's, backcrosses and F_2 population, respectively.

(iii) Set 3 consisted of observations pooled across all the three blocks (B_{123}) with a population size of 60, 300 and 1050 plants for parents and their F_1's, backcrosses and F_2 population, respectively.

4.1.1.2. Mean performance of six generations

The mean performance of six basic generations (P_1, P_2, F_1, F_2, $BC_1$and BC_2) for panicle length, panicle diameter and grain size are presented in Table 7 to 9. The mean performance of six-generations for the set 3 population size (pooled over three blocks) are illustrated here (Figure 2).

4.1.1.2.1. Panicle length

Panicle length varied from 16.5 cm to 17.5 cm in P_1 and from 67.1 to 66.5 cm in P_2, across the two seasons (2006 rainy and 2007 summer seasons) in cross 1, while in F_1 it varied from 36.7 to 39.1 cm, which was 12% and 7% less than the mid-parental values, suggesting apparently the presence of partial dominance of genes for smaller panicle over those for the longer panicle. (Table 7). The panicle length in F_2 was between 35.3 cm and 35.8 cm, which was 4% and 8% less than the F_1, during 2006 rainy season and 2007 summer season, respectively. The BC_1 population had 25.4 cm and 26.1cm long panicles, which was 55% and 49% more than the P_1, whereas in BC_2 population it was between 42.7 cm and 41.9 cm, which was 36% and 37% less than P_2, during 2006 rainy season and 2007 summer season, respectively.

The mean performance for panicle length in cross 2 is presented in Table 7. The panicle length in P_1 varied from 16.3 cm to 17.4 cm across the two seasons, while in P_2 it varied from 46.8 cm to 44.9 cm. The panicle length in F_1 varied between 29.6 cm and 30.5 cm, which was 6% and 1% less than the mid-parental values, while in F_2 population it varied from 28.7 to 28.5 cm, which was 3% and 7% less than the F_1

during 2006 rainy season and 2007 summer season, respectively. The panicle length in BC_1 varied from 21.7 to 22.4 cm, which was 33% and 29% more than the P_1, whereas in BC_2 population it varied from 37.5 to 35.9 cm, which was 20% and 19% less than the P_2 during 2006 rainy season and 2007 summer season, respectively.

4.1.1.2.2. Panicle diameter

The observed mean performance for panicle diameter of six generations in cross 1 are presented in Table 8. Panicle diameter of the thin-panicle parent (P_1) was between 17.9 mm and 18.5 mm, while in the thick-panicle parent (P_2) it was 49.0 mm and 48.3 mm during 2006 rainy season and 2007 summer season, respectively. The panicle diameter in F_1 varied between 35.1 and 34.9 mm, which was 5% and 4% higher than the mid-parental values, while in F_2, it decreased by 15% (29.7 mm) and 18% (28.7 mm) as compared to F_1 during 2006 rainy season and 2007 summer season, respectively. In the BC_1 population, it was 22.7 mm in both the seasons, which was 26% and 22% more than the P_1 during 2006 rainy season and 2007 summer season, respectively, while in BC_2 it varied from 38.6 to 37.3 mm, which was 21% and 23% less than the P_2 during 2006 rainy season and 2007 summer season, respectively.

The mean performance for panicle diameter of six generations in cross 2 are presented in Table 8. The panicle diameter in P_1 varied from 14.4 to 15.6 mm, while in P_2 it varied from 41.4 to 40.1 mm during 2006 rainy season and 2007 summer season, respectively. The panicle diameter in F_1 varied from 26.7 to 27.3 mm, which was 4% and 2% less than the mid-parental values across two seasons, while in F_2 population it varied from 24.3 to 24.5 mm, which was 9% and 10% less than the F_1, during 2006 rainy season and 2007 summer season, respectively. The panicle diameter in BC_1 population varied from 18.9 to 19.1 mm, which was 31% and 22% more than P_1, while in BC_2 population it varied from 32.2 to 32.5 mm, which was

22% and 19% less than P_2, during 2006 rainy season and 2007 summer season, respectively.

4.1.1.2.3. Grain size

Small grain size parent (P_1) had 4.4 g and 5.0 g of 1000-grain weight, while in the large grain size parent (P_2) had 13.1 g and 13.50 g during 2006 rainy season and 2007 summer season, respectively (Table 9). The grain size (1000-grain weight) in F_1 varied from 8.3 to 8.7 g, which was 5% and 6% less than the mid-parental values, while in F_2 it varied from 7.9 to 8.6 g, which was 5% and 1% less than the F_1 during 2006 rainy season and 2007 summer season, respectively. In the BC_1 population, 1000-grain weight varied from 6.3 g to 6.8 g, which was 42% and 36% more than P_1, while in BC_2 population, it was between 9.0 g and 9.8 g, which was 32% and 27% less than P_2 during rainy season of 2006 and summer season of 2007, respectively.

The mean performance for grain size in cross 2 during 2006 rainy and 2007 summer is presented in Table 9. The grain size (1000-grain weight) in the small-seed parent (P_1) was between 5.2 g and 5.9 g, while in the large-seed parent (P_2) it was 13.8 g and 14.9 g during 2006 rainy season and 2007 summer season, respectively. The F_1 had 8.6 to 9.7 g of 1000-grain weight, which was 10% and 12% less than the mid-parental values, while the F_2 population had 8.5 to 9.5 g, which was 2% less than the F_1 during 2006 rainy season and 2007 summer season, respectively. In the BC_1 population, 1000-grain weight varied from 7.0 to 7.4 g, which was 34% and 26% more than P_1, while in the BC_2 population it varied from 9.6 to 11.3 g, which was a decrease of 30% and 24% compared to P_2 during 2006 rainy season and 2007 summer season, respectively.

4.1.1.3. Three -parameter model estimates

First, the observed mean of six generations for panicle length, panicle diameter and grain size were subjected to a scaling test to assess the adequacy of the additive-dominance model. Scales A, B and C were estimated and tested with their respective standard errors. The joint scaling test was used for the estimation of the three parameters (mid-parent [m], additive [d] and dominance [h] effects), and their goodness of fit was tested following a chi-square distribution with three degrees of freedom.

4.1.1.3.1. Panicle length

In cross 1, A, B and C scale estimates were highly significant across sets and seasons, except scale A which was non-significant in B_3 block of set 1 during 2006 rainy season (Table 10). The joint scaling test showed that m, d and h components were highly significant across sets and seasons. However, the magnitude of additive genetic component was 78% (–24.9) and 85% (–23.9) higher than the magnitude of dominance gene effect for set 3 during 2006 rainy season and 2007 summer season, respectively. This trend was also observed in other sets across seasons. The χ^2 values were highly significant for all the sets across seasons.

The scaling tests for panicle length in cross 2 showed significant values for all the three scales across different sets and seasons except during 2006 rainy season, where scale A showed a non-significant value in B_1 block of set 1 and scale B showed a non-significant value in B_2 and B_3 blocks of set 1 and B_{13} and B_{23} blocks of set 2 (Table 11). Highly significant values for m, d and h components were obtained through joint scaling test across sets and seasons. However, the additive component for set 3 was 84% (–15.2) and 92% (–13.5) higher than the dominance component

across seasons. This trend was also observed in other sets. The χ^2 values were found to be significant across all sets and seasons.

4.1.1.3.2. Panicle diameter

The scaling test for panicle diameter in cross 1 showed highly significant values for A, B and C across sets and seasons. Joint scaling test showed highly significant estimates for the additive effects across sets and seasons (Table 12). The dominance component, which was non-significant in all the three sets in rainy season of 2006, was highly significant in summer season of 2007 except in B_3 block of set 1. However, χ^2 values were highly significant for all the sets in both the seasons. It indicates the inadequacy of additive-dominance model for this trait.

In cross 2, the scaling test showed significant values for all the three scales across sets in both the seasons, except for scale B in B_3 block of set 1 during summer season of 2007 (Table 13). Joint scaling test showed highly significant d and h components across sets and seasons, except B_1 block of set 1 during 2006 rainy season, where dominance component showed a non-significant value. The estimated additive component (–13.2) was higher in magnitude as compared to the dominant component (–1.9) for set 3 in 2006 rainy season. This trend was also observed in all other sets across seasons. The χ^2 values to test the goodness of fit of three-parameter model were found to be significant in all the sets across seasons.

4.1.1.3.3. Grain size

For this trait in cross 1, scale A was non-significant in all the sets in both the seasons (Table 14). However, scales B and C were significantly differed from zero in all the sets except scale C in B_2 block of set 1, which showed a non-significant value during 2007 summer season. The three parameters (m, d and h) obtained using joint scaling

test were found to be highly significant for this trait across all the sets in both the seasons. The additive gene effect (–3.9) was higher in magnitude than the dominance gene effects (–1.0), and the χ^2 values were also found to be highly significant for set 3 during 2006 rainy season. This trend was observed in all sets across seasons.

For cross 2, scale A was non-significant in all the sets during 2006 rainy season. However, it was significant in all the sets except in B_1 bock of set 1 during 2007 summer season (Table 15). Scales B and C were significant in all the sets in both the seasons. The estimates of (d) and (h) were highly significant, and the additive effect (–3.9) was 85% higher than the dominance effect (–0.57) in set 3 during 2006 rainy season. Similar trend was also observed in other sets and seasons. The χ^2 values were also significant in all the sets across seasons.

4.1.1.4. Six-parameter model estimates

Simple additive-dominance model was inadequate, as revealed by significant values for A, B and C scales, suggesting the presence of non-allelic interactions in the genetic control of all the three sink size traits. These results were also supported by the three-parameter model of joint scaling test, showing large and significant χ^2 values. Therefore, six-parameter model was used to determine the type and magnitude of gene action involved in the inheritance of sink size traits.

4.1.1.4.1. Panicle length

For panicle length in cross 1, both additive and dominance effects were significant and negative in all the sets across seasons (Table 16). The additive effect (–17.3) was higher in magnitude than the dominance effect (–9.8) in set 3 during 2006 rainy season. This pattern was also observed in all other sets in both the seasons. The additive x additive (i) interaction was significant in most of the blocks and sets in both

the seasons, except blocks B_1 and B_3 in set 1, block B_{13} in set 2 during 2006 rainy season and B_2 block of set 1 during 2007 summer season. Additive x dominance (j) and dominance x dominance (l) interactions were positive and significant in all the three sets in both the seasons. The magnitude of dominance x dominance interaction (25.5) was 81% and 68% higher than additive x additive (–4.72) and additive x dominance (8.06) interactions, respectively in set 3 during 2006 rainy season, and this pattern was also observed across other sets and seasons. The negative sign of dominance effect and positive sign of dominance x dominance interaction indicated a duplicate type of epistasis in all sets in both the seasons.

In cross 2, the average effect was highly significant for this trait in all the three sets in both the seasons (Table 17). The additive component was negative and highly significant in all the sets in both the seasons. Conversely, the dominance component was found to be non-significant across all sets in both the seasons. Among the interacting components, the additive x additive interaction was non-significant in set 1 across seasons, however in set 2, it was significant in all the three blocks during rainy season of 2006 and in block B_{12} during summer season of 2007. Significance of this interaction was also detected in set 3 in both the seasons. The other interacting components, additive x additive and dominance x dominance effects were non-significant in all the sets across seasons. Both the dominance and dominance x dominance components were in the positive direction, indicating the presence of complementary type of epistasis, however, these components were non-significant in this cross.

4.1.1.4.2. Panicle diameter

The additive effect for this trait in cross 1 was highly significant in all the three sets in both the seasons (Table 18). The dominance effect was significant to highly

significant across the seasons in set 1, whereas in set 2 and set 3, this component was highly significant in both the seasons. The magnitude of additive effect (–15.9) was higher than the dominance effect (5.5) in set 3 during 2006 rainy season. Similar trend was observed across the other sets in both the seasons. The additive x additive interaction was significant only in B_1 block of set 1 during both the seasons. However, it was significant for all the block combinations in set 2 at varying levels in both the seasons. This interaction was also found to be highly significant in set 3 across seasons. The additive x dominance interaction was non-significant in all the sets in both the seasons except B_3 block of set 1 during 2007 summer season. However, the dominance x dominance interaction was highly significant and positive across all the sets in both the seasons, except B_3 block of set 1 during 2006 rainy season. The dominance x dominance interaction (10.8) was 65% higher in magnitude than the additive x additive interaction (3.8) in set 3 during 2006 rainy season. Similar trend was observed across all the sets in both the seasons. Complementary type of epistasis was inferred for this trait, as signs of both dominance (h) and dominance x dominance (l) component were in the positive direction.

In cross 2, the average effect was highly significant in all the sets in both the seasons (Table 19). The additive effect for this trait was highly significant and negative in all the sets in both the seasons. Significant and positive dominance effect was observed in block B_1 of set 1 during 2006 rainy season and in all the three blocks of set 1 during 2007 summer season with varying levels of significance. However, this component was highly significant in set 2 and set 3 across all the blocks in both the seasons except in block B_{23} of set 2 during 2006 rainy season. The magnitude of additive effect (–13.3) was higher than dominance effect (3.9) in set 3 during 2006 rainy season. Similar trend was observed across other sets and seasons. The additive x

additive interaction was positive and significant in all sets across seasons. The additive x dominance interaction was non-significant in all the sets during rainy season of 2006, whereas it was significant in 2007 summer season for B_2 and B_3 blocks in set 1 and B_{13} and B_{23} blocks in set 2 with varying levels of significance. However, it was detected with high level of significance in set 3. The dominance x dominance interaction was non-significant across sets and seasons. As dominance component was negative and dominance x dominance components was positive, duplicate type of epistasis was inferred for this trait in this cross.

4.1.1.4.3. Grain size

In cross 1, the additive effect for this trait was highly significant in all the sets in both seasons (Table 20). The dominance component was negative and significant in B_1 and B_2 blocks of set 1 during 2006 rainy season and B_2 and B_3 blocks during 2007 summer season with varying level of significance. However in set 2 and 3, this component was highly significant in both the seasons. The magnitude of additive effect (–2.7) was higher than dominance effect (–1.5) in set 3 during 2006 rainy season. Similar trend was also observed across other sets and seasons. Additive x additive interaction was significant only in B_1 block during 2006 rainy season and B_2 block during 2007 summer season in set 1 at varying levels. Similarly, in set 2 also this interaction was significant in two blocks in both 2006 rainy season (B_{12} and B_{13}) and 2007 summer season (B_{12} and B_{23}). However in set 3, this component was highly significant across the seasons. Both the additive x dominance and dominance x dominance interaction were significant across all the sets and seasons. The dominance x dominance interaction (4.8) was 77% and 65% higher in magnitude than the additive x additive (–1.1) and additive x dominance (1.7) interaction components, respectively in set 3 of 2006 rainy season. This interacting component also showed a higher magnitude as

compared to all other components across all the sets and seasons. The negative sign of dominance component and positive sign of dominance x dominance component indicated the presence of duplicate epistasis in this cross.

The additive effect for grain size in cross 2 was highly significant in all the sets in both the seasons (Table 21). The dominance component was significant in set 1 for block B_2 and B_3 during 2006 rainy season and block B_2 during 2007 summer season. This component in set 2 was significant at varying levels across seasons. However, it was highly significant in set 3 across seasons. The additive component (–2.6) was 43% higher than the dominance component (–1.5) in set 3 of 2006 rainy season. This trend was also evidenced across all other sets and seasons. The additive x additive interaction was non-significant in all the sets in both the seasons except in set 3 during 2007 summer season. The additive x dominance interaction was highly significant in all the three sets during 2006 rainy season. During summer season of 2007, this component was significant at varying levels in set 1 and set 2 population sizes. However in set 3, it was highly significant across seasons. The dominance x dominance interaction was significant across all the three sets. The dominance x dominance interaction (3.46) was 51% higher in magnitude than the additive x dominance interaction (1.7) component in set 3 during 2006 rainy season. This interacting component was also higher as compared to all other components across all the sets and seasons. The negative sign of dominance component and positive sign of dominance x dominance interaction indicate a duplicate type of epistasis for this trait.

4.1.2. Triple test cross analysis

The detection, estimation and interpretation of epistasis has progressed much faster at the level of first degree statistics (Mather and Jinks, 1982) which has certain limitations due to the cancellation of genetic effects. The TTC technique of Kearsey

and Jinks (1968) tests the presence of epistasis and estimates additive (D) and dominance (H) components with a higher degree of precision in the absence of epistasis. Even in the presence of epistasis, it provides estimates of additive and dominance components, which are useful for comparison with variance estimates from filial generation data. In the present study, the detection of epistasis and additive and dominance components (assuming no epistasis) for panicle length, panicle diameter and grain size in one cross (cross 1) from each trait-specific group was carried as per the method given by Kearsey and Jinks (1968), and the results are presented in Table 22 to 24.

4.1.2.1. Panicle length

Analysis of variance showed that the interaction of blocks with additive x additive (i) as well as additive x dominance and dominance x dominance (j + l) epistatic component was non-significant. Therefore, the individual epistatic components were tested against total epistasis x block interactions. Total epistasis was highly significant for this trait. Further, partitioning of the total epistasis showed the highly significant contribution of additive x additive (i) and additive x dominance and dominance x dominance (j + l) interactions (Table 22). However, the relative magnitude of mean squares due to additive x dominance and dominance x dominance (j + l) interaction was higher as compared to additive x additive interaction.

On the assumption of absence of epistasis, analysis of variances for sums (L_{1i} + L_{2i}) and differences (L_{1i} – L_{2i}) revealed the significance of their respective mean squares. Accordingly, the additive effect and dominance component were highly significant for this trait. The relative magnitude of additive effect (371.6) was lower than the dominance component (465.1). However, as this cross gave evidence of significant epistasis for this trait, the estimates of the additive effect and dominance

component were biased by unknown extent. The average degree of dominance was in the range of overdominance, as the estimate was greater than unity (1.12). The correlation coefficient (r_{sd}) of sum ($L_{1i} + L_{2i}$) and differences ($L_{1i} – L_{2i}$) was negative and significant (–0.59) for this trait, indicating that dominant allele have increasing effects on the trait.

4.1.2.2. Panicle diameter

Analysis of variance showed that the interaction of blocks with additive x additive (i) as well as additive x dominance and dominance x dominance (j + l) epistatic component was non-significant (Table 23). Therefore, the individual epistatic components were tested against total epistasis x block interactions. The mean squares due to total epistasis was highly significant. The partitioning of the epistatic variance showed significant additive x additive and additive x dominance and dominance x dominance interactions for this trait.

The significance of mean squares due to sums and differences revealed the importance of both additive and dominance components. The estimate of additive effect (157.4) component was relatively higher than the dominance component (135.4). The degree of dominance was partial as evident from the estimate being less than unity (0.93) for this trait. The estimated value for correlation coefficient (r_{sd}) of sum and differences was negative and significant (–0.67) for this trait, indicating that dominant alleles have increasing effects on the trait.

4.1.2.3. Grain size

Analysis of variance showed that interaction of blocks with additive x additive (i) as well as additive x dominance and dominance x dominance (j + l) epistatic component was non-significant. Therefore, the individual epistatic components were tested

against total epistasis x block interactions. Mean square for epistasis provided evidence for significant total epistatic effect (Table 24). When the overall epistasis was partitioned, the results showed non-significant additive x additive (i) epistasis for this trait. However, the additive x dominance and dominance x dominance (j + l) epistasis was highly significant.

Analysis of variance for sums and differences, on the assumption of no epistasis indicated significant mean squares for this trait. These results provide evidence for the presence of both additive and dominance genetic components for this trait. The estimated additive (9.87) component was lesser than the dominance component (12.34). The degree of dominance being more than unity (1.12) revealed overdominance for this trait. The estimated value for correlation coefficient (r_{sd}) of sums and differences was non-significant, indicating symmetrical distribution of dominant alleles among parents.

4.1.3. Estimation of components of variances and heritabilities

The variance components attributed to total genetic, additive and dominance variation along with dominance ratio and heritabilities (broad and narrow sense) were estimated for panicle length, panicle diameter and grain size. These estimates were obtained from the variance of six generations of trait-specific crosses evaluated during 2006 rainy and 2007 summer seasons. The results are presented in Table 25.

4.1.3.1. Panicle length

Panicle length in cross 1 had an additive variance (D) of 83.2 and 90.3 and dominance variance (H) of 14.2 and 9.0, while in cross 2 the additive variance was 43.0 and 55.8 and dominance variance was 4.9 and 3.2 during 2006 rainy season and 2007 summer season, respectively. The magnitude of additive variance was much greater than the

dominance variance, and the dominance ratio was less than unity in both the crosses and the seasons. Broad-sense heritability was high in both the crosses, ranging from 94.9 to 98.1% across seasons. Narrow-sense heritability ranged from 83.8 to 90.5% across the crosses and the seasons.

4.1.3.2. Panicle diameter

Panicle diameter in cross 1 had an additive variance (D) of 31.0 and 29.9 and dominance variance (H) of 13.82 and 14.8, while in cross 2 the additive variance was 27.7 and 29.5 and dominance variance was –0.1 and –1.7 during 2006 rainy season and 2007 summer season, respectively. The magnitude of additive variance was higher than the dominance variance component, and the dominance ratio was less than unity in both the crosses across seasons. The estimated values for heritabilities in broad and narrow sense ranged between 90.5% and 95.9% and 64.0% and 91%, respectively across the crosses and the seasons.

4.1.3.3. Grain size

The additive variance for grain size was 9.1 and 5.2, while the dominance variance was –3.1 and –0.5 in cross 1 during 2006 rainy season and 2007 summer season, respectively. In cross 2, the additive variance was 5.2 and 7.1 and the dominance variance was –0.03 and –0.8 during 2006 rainy season and 2007 summer season, respectively.

The dominance ratio was found to be positive and less than unity in both the crosses across seasons. The estimated values for broad-sense heritability varied from 88.0 to 95.8% and narrow-sense heritability ranged between 89.0% and 96.1% across crosses and seasons for this trait.

4.1.4. Character association in trait-specific crosses

Simple correlation coefficients were estimated for the observed traits in F_2 population of trait-specific crosses evaluated during 2007 summer season and the results are presented in Table 26.

4.1.4.1. Panicle length crosses

The association of panicle length in cross 1 with panicle diameter (0.11), days to 50% flowering (0.02) and productive tiller number (0.01) was non-significant. However, it showed significant and positive associations with grain size (0.17), plant height (0.25) and single plant yield (0.22).

In cross 2, panicle length showed positive and significant association with panicle diameter (0.23), whereas it had positive and non-significant association with grain size (0.12). However, days to 50% flowering (0.36), plant height (0.38) and single plant yield (0.32) exhibited highly significant positive correlation with this trait, whereas, productive tiller number (0.09) showed positive non-significant associations.

4.1.4.2. Panicle diameter crosses

Panicle diameter in cross 1 exhibited positive significant correlation with panicle length (0.19) and positive and highly significant correlation with grain size (0.28) and single plant yield (0.43). However, the association of panicle diameter with days to 50% flowering (–0.28) was negative and highly significant. Whereas, plant height (0.04) and productive tiller number (0.14) showed positive but non-significant associations with this trait. In cross 2, association of panicle diameter with panicle length (0.48), grain size (2.70) and single plant yield (0.35) was positive and highly significant. Positive and significant association of panicle diameter with days to 50%

flowering (0.20) and plant height (0.22) was also observed. However, the productive tiller number (0.08) showed non-significant association with this trait.

4.1.4.3. Grain size crosses

Grain size showed positive and highly significant association with panicle diameter (0.49) and single plant yield (0.32) in cross1. In cross 2 also grain size showed a highly significant association with panicle diameter (0.67) and single plant yield (0.31). This trait showed non-significant association with all other traits. However, the association of grain size was positive with panicle length (0.05) and days to 50% flowering (0.09) and negative with plant height (–0.01) and productive tiller number (–0.03) in cross 1. Similar association was also observed in cross 2 except with days to 50% flowering (–0.16), where the association was negative.

4.2. QTL analysis

QTL analysis was performed for the observed traits in the F_2 and $F_{2:3}$ mapping populations and their results are presented below.

4.2.1. Phenotypic analysis

Data were recorded on sink size and agronomic traits from the F_2 and $F_{2:3}$ population trials conducted during 2006 rainy season and 2007 summer season, respectively.

4.2.1.1. Analysis of variances

The analysis of variance for the replicated phenotypic data sets from the $F_{2:3}$ trial was performed using the Residual Maximum Likelihood algorithm (ReML), which provides the Best Linear Unbiased Predictors (BLUPs) of the performance of the genotype (Patterson and Thompson, 1971). The predicted means were calculated considering entries as fixed effects and the block and entry × replication interaction as

random effects. The variances due to $F_{2:3}$ progenies were highly significant ($P \geq 0.01$) for all the observed traits except productive tiller number which showed significance only at 5% level and panicle harvest index which was non-significant.

4.2.1.2. Mean performances

The mean performance of parents across 2006 rainy season (F_2 trial) and 2007 summer season (F_2:$_3$ trial) and means of F_2 and $F_{2:3}$ mapping populations for the observed traits are presented in Table 27.

The panicle length in P_1 was 28.6 cm and for P_2 it was 24.6 cm, while in F_2 and $F_{2:3}$ it was 27.8 cm and 27.4 cm, respectively. Panicle diameter in P_1 was 18.4 mm and in P_2 it was 35.1 mm, while it was 26.4 mm in F_2 population and 26.0 mm in $F_{2:3}$ progenies. Grain size (1000-gran weight) in P_1 was 5.4 g, while in P_2 it was 12.0 g, whereas in F_2 population, it was 8.1 g and in $F_{2:3}$ progenies it was 8.7 g.

Plant height in P_1 was 101.2 cm. while in P_2 it was 108.6 cm, whereas in F_2 population it was 114.2 cm and in $F_{2:3}$ progenies it was 108.9 cm. Productive tiller number in P_1 was 2.7 and in P_2 it was 1.3, while it was 2.4 in the F_2 population and 1.7 in $F_{2:3}$ progenies.

Panicle grain weight in P_1 was 15.1 g and in P_2 it was 22.8 g, while the $F_{2:3}$ population had a mean of 21.7 g. Panicle weight in P_1 was 21.2 g and in P_2 it was 37.2 g, while it was 33.6 g for $F_{2:3}$ progenies. Panicle harvest index was 63.3% and 65.8 % in P_1 and P_2, respectively, while in $F_{2:3}$ it was 64.8%.

4.2.1.3. Heritability estimates

Heritability in broad-sense was estimated for the observed traits in both F_2 and $F_{2:3}$ mapping populations (Table 27). High heritability estimates were obtained for panicle length (0.71), panicle diameter (0.72) and plant height (0.87) in the F_2 population data

set, however for grain size (0.59) the heritability estimate was moderate and for productive tiller number (0.35) it was low. In $F_{2:3}$ progenies, the heritability estimates were high for all the traits ranging between 0.62 and 0.92, except for panicle harvest index (0.56) which showed a moderate heritability. As expected, the heritability estimates from the replicated data sets of $F_{2:3}$ for all the observed traits were comparatively higher than that of F_2 population data set. Except for productive tiller number, the heritability estimates were significantly greater than 0.50 for all the observed traits in both F_2 and $F_{2:3}$ populations, which is a prerequisite for effective QTL mapping.

4.2.1.4. Frequency distributions

The variation observed for the sink size and agronomic traits in F_2 and $F_{2:3}$ mapping progenies are presented graphically through frequency distributions (Figure 3 to 6). Panicle length in F_2 population showed a bimodal distribution whereas in $F_{2:3}$ it showed a continuous symmetrical distribution. For panicle diameter an irregular distribution was observed in F_2 population, however in $F_{2:3}$ progenies it showed a symmetrical distribution. Grain size presented a continuous symmetrical distribution in both the F_2 and $F_{2:3}$ populations.

For plant height, the distribution was slightly skewed to the left in F_2 population, while in $F_{2:3}$ progenies, it presented a normal distribution. Similarly, productive tiller number presented a skewed distributions toward left in both F_2 and $F_{2:3}$ mapping populations.

Panicle grain weight, panicle weight and panicle harvest index in $F_{2:3}$ progenies presented a moderately symmetrical distribution. The continuous symmetrical distribution for traits panicle length, panicle diameter and grain size indicated that they are likely to be polygenic in nature and quantitatively inherited

Transgressive segregants were observed for panicle length and plant height in both F_2 and $F_{2:3}$ mapping populations. Panicle grain weight, panicle weight and panicle harvest index also showed transgressive segregants in $F_{2:3}$ progenies. The occurrence of transgressive segregants might be the result of complementation of favourable and unfavourable alleles from either of the parents.

4.2.1.5. Phenotypic correlations

Phenotypic correlations were calculated on $F_{2:3}$ mapping population for all the traits under study (Table 28). Correlation coefficient revealed the degree of association among traits and also indicated the chances of identifying co-mapped QTLs for the correlated traits.

A positive significant correlation was observed for panicle length with panicle grain weight (0.221), plant height (0.427) and productive tiller number (0.210); panicle diameter with grain size (0.553), panicle grain weight (0.276) and panicle weight (0.438); grain size with panicle weight (0.271) and panicle grain weight (0.200); panicle grain weight with panicle weight (0.867), panicle harvest index (0.406) and productive tiller number (0.244); panicle harvest index with productive tiller number (0.267). Similarly, significant negative correlation was observed for panicle length with panicle diameter (−0.300); panicle diameter with panicle harvest index (−0.225), plant height (−0.226) and productive tiller number (−0.302); and grain size with productive tiller number (−0.239).

4.2.2. Molecular analysis

4.2.2.1. Parental polymorphism

A total of 331 primer pairs, which included 96 primer pairs each of SNP, SSR and EST-SSR markers, and 43 STS markers were initially screened on parental lines to

detect polymorphism, which resulted in identification of 109 primer pairs. A total of 44 markers with known map positions, evenly distributed across the seven pearl millet linkage groups and showing clear allelic differentiation between the parents were finally selected for genotyping the $F_{2:3}$ mapping population. The selected markers included 24 SNPs, 10 SSRs, 6 EST-SSRs and 4 STSs (Table 29).

4.2.2.2. Marker segregation in mapping population

The segregation pattern of 44 marker loci in $F_{2:3}$ mapping population were tested for the goodness of fit to the expected ratio 1:2:1 [1= Homozygote for female parent (P_1), 2 = Heterozygote, 1= Homozygote for male parent (P_2)] using χ^2 test. The calculated χ^2 values were compared with table value at 2 degree of freedom for each marker locus and the results are present in Table 30.

A total of 4 markers out of 44 marker loci showed significant χ^2 value for segregation distortion when compared with table value at 1% probability level. These markers were located on LG 2 (*Xicmp*3063), LG 3 (*Xpsms*31), LG 5 (*Xpsms*18) and LG 7 (*Xpsmp*2027).

4.2.3 Linkage map construction

A linkage map was constructed using the genotypic data of 44 markers genotyped on 188 $F_{2:3}$ individuals by applying the F_2 model in MAPMAKER/EXP V.3.0 software programme. Linkage distances in terms of centimorgan (cM) values were calculated using the Haldane function. The linkage map was constructed using a LOD default value of 3.5 and maximum inter-marker distance of 50.0 cM. The details of linkage groups (LG) to which 44 markers were assigned and linkage distances between the marker loci in each group are given in Table 30 and Figure 7.

The number of markers mapped per linkage group and the length of each

linkage group are presented in Table 31. The highest number of markers were found on LG 3 (9 markers), followed by LG 1 (8 markers), LG 2 (8 markers), LG 5 (7 markers) and LG 6 (6 markers). The linkage groups 7 (4 markers) and 4 (2 markers) had lowest number of mapped markers. The linkage map thus constructed had a total length of 1018.7 cM. The average distance between the marker pairs was 23 cM with a range of 14 cM on LG 1 to 38 cM on LG 6. However, there were some gaps wider than 50 cM on LG 2, 3, 5 and 6.

4.2.3.1. Linkage group 1

The number of polymorphic loci for LG 1 was 18% of the total markers mapped. There was no clustering of markers on this linkage group. It comprised of 5 SNPs, 1 SSR, 1 EST-SSR and 1 STS markers, which together covered a total distance of 110 cM with an average distance of 13.8 cM between marker loci. The distribution of markers in this linkage group is ideal for QTL mapping, except for the large gap (44.1 cM) between markers *Xpsms*58 and *Xpsms*29.

4.2.3.2. Linkage group 2

The linkage map of chromosome 2 comprised of 8 markers covering a total distance of 195 cM with an average distance of 24.4 cM between marker loci. LG 2 is relatively well covered with 5 SNPs, 1 SSR, 1 EST-SSR and 1 STS marker. The marker distribution in this linkage group was also ideal for QTL mapping, except for the large gap (95.3 cM) between *Xpsms*75 and *Xpsms*73.

4.2.3.3. Linkage group 3

Nine markers were mapped on LG 3 covering a distance of 180 cM with 5 SNPs, 2 SSRs, 1 EST-SSR and 1 STS marker. The average inter-marker distance was 20 cM, however, gaps of more than 30 cM exists between marker loci *Xicmp3073 -Xpsms68*

and between *Xpsms31* - *Xpsmp2068*.

4.2.3.4. Linkage group 4

This linkage group had only two mapped markers (1 SNP and 1 SSR) with an interval of 37.7 cM, with poorest coverage among the seven linkage groups. Clearly more polymorphic markers need to be identified that can map to this linkage group, so that QTL detection on this linkage group will be practical.

4.2.3.5. Linkage group 5

In this linkage group seven markers were mapped, including 3 SNPs, 2 SSRs, 1 EST-SSR and 1 STS markers. The total length of this linkage group is 170.0 cM with an average inter-marker distance of 24.6 cM. Three large gaps exist in this linkage group, which were found between the marker loci *Xicmp3027* - *Xpsmp2064*; *Xpsms2* - *Xpsmp345* and between *Xpsmp345* - *Xpsms18*.

4.2.3.6. Linkage group 6

Six markers constitute linkage group 6, which includes 3 SNP, 1 SSR and 2 EST-SSR markers covering a total length of 228 cM. This linkage group was the longest among the seven linkage groups with an average inter-marker distance of 38 cM. While the centromeric region of this linkage group is well covered, the proximal and distal arms needs much higher density of markers.

4.2.3.7. Linkage group 7

This linkage group contains only four markers and covers a distance of 96 cM with 2 SNP and 2 SSR markers. The average distance between adjacent markers was 24.0 cM, but additional markers need to be mapped onto this linkage group before QTL detection will be effective across its entire length.

4.2.4. Mapping QTLs for sink size and agronomic traits

Mapping of QTLs was carried out using phenotypic and genotypic data for 188 $F_{2:3}$ mapping population and also for F_2 population. QTL analysis was carried out in $F_{2:3}$ progenies for panicle length, panicle diameter, grain size, panicle weight, panicle grain weight, panicle harvest index, plant height and productive tiller number, and in F_2 population for panicle length, panicle diameter, grain size, plant height, and productive tiller number. PLABQTL was used to analyze the data by composite interval mapping (CIM) procedure using different genetic models (additive, additive-dominance and epistasis). The CIM method was implemented using a LOD of 2.5 as the threshold value for identifying significant QTLs.

Individual putative QTLs were detected for both sink size components and agronomic characters. According to Tanksley *et al.* (1996), regions of the genome are identified to contain a putative QTL if the results meet one or more of the following criteria: (i) a significant effect observed for a single marker/trait combination at a single environment with $P<0.001$ (ii) significant effects observed in the same direction (i.e. either all positive effects or all negative effects) for a single marker/trait combination at one or two environments with $P<0.01$ and (iii) significant effects observed in the same direction for a single marker/trait combination at one or two environments with $P<0.1$. Based on these criteria in the present study, a total of 27 putative QTLs were identified for a total of seven targeted characters.

4.2.4. 1. Panicle length

Additive genetic model identified seven QTLs, one each on LG 1, 2, 3, 4 and 7 and two each on LG 6 using $F_{2:3}$ progeny data for this trait (Table 32). The LOD scores for these ranged from 2.9 to 8.0. The variation explained by these individual QTLs due to their additive effect ranged from 6.8 to 25.9%. The favourable alleles for the QTLs on

LG 1, 2, 6 and 7 were from P_1 parent while for the QTLs on LG 3 and LG 4, the positive effect was from male P_2 parent. The total variation explained by additive model was 40.7%. This model failed to detect any QTLs for the F_2 data set.

The additive-dominance model identified eight QTLs using the $F_{2:3}$ progenies, one each on LG 1, 2, 4 and 7 and two each on LG 3 and LG 6. The LOD scores for these QTLs ranged from 2.6 to 8.2 (Figure 8). The additive effects individually explained 4.5 to 26.9% of the variation while the dominance effects explained 0.0 to 1.4% of the variation. Panicle length QTLs on LG 3 and 4 had favourable alleles from P_2 parent, while for the other loci the favourable alleles came from P_1 parent. In the F_2 population, two QTLs on LG 2 and 6 were detected with LOD values of 3.3 and 3.7, respectively explaining 0.5 and 3.4% additive effect of variation and dominance effects explaining 0.7% and 3.4% of variation, respectively. The total variation explained by the model was 42.7 % in the $F_{2:3}$ data set and 13.1% in F_2 data set.

Epistatic model revealed additive × additive and dominance × dominance QTL pair interactions for this trait in the $F_{2:3}$ progeny data set. This pair-wise epistatic interactions explained between 6.5 to 12.3% of observed variation. No significant epistatic interactions were detected in the F_2 population. A total of 40.1% variation in the $F_{2:3}$ progenies and 12.5% variation in F_2 population was explained through this model.

On the whole, QTL analysis identified eight genomic regions distributed one each on LG 1, 2, 4 and 7 and two each on LG 3 and LG 6 that contributed significantly to the genetic control of panicle length. The variation explained by individual QTLs ranged from 6.1 to 18.2%. Though significant interaction among the QTLs on LG 1, 3, 6 and 7 were detected using the epistatic model, the total variation explained through the additive-dominance model for this trait was high in both F_2

population (13.1%) and $F_{2:3}$ progenies (42.7%).

4.2.4.2. Panicle diameter

Four QTLs were detected for this trait on LG 2, 3, 6 and 7 using $F_{2:3}$ progenies data following additive model (Table 33). The LOD scores ranged from 3.6 to 14.7 and the variation explained by these individual QTLs ranged between 8.9 and 28.6%. The favourable alleles for all QTLs were contributed from P_2 parent. The portion of observed variation explained through this model was 44.3%.

Additive-dominance model identified five QTLs for this trait, distributed across LG 2, 3, 5, 6 and 7 using $F_{2:3}$ progeny data set. The LOD score ranged between 2.6 and 14.7 (Figure 8). The portion of phenotypic variation explained by additive effects ranged from 3.6 to 29.1% and those explained by dominance effects ranged between 0.1 to 2.8%. The favourable alleles were all contributed by the P_2 parent. The portion of observed phenotypic variation explained by this model was 45.8%.

Using the epistatic model, a QTL on LG 2 showed significant additive x dominance interaction effects with a QTL on LG 3 and this interaction explained 2.8% of the observed phenotypic variation for panicle diameter. The epistatic model explained 41.0% of observed phenotypic variation.

In total, five QTLs significantly associated with panicle diameter were found on LG 2, 3, 5, 6 and 7 using the $F_{2:3}$ progenies data set. The variation explained by these individual QTLs ranged from 6.3 to 30.2% with LOD values of 2.6 to 14.7. Additive x dominance interaction was observed between QTLs located on LG 2 and LG 3. However, the additive-dominance model explained the highest portion of observed variation (45.8 %) for this trait. All three models failed to detect any significant QTLs for this trait using the F_2 data set.

4.2.4.3. Grain size

The additive model detected two QTLs for this trait using the $F_{2:3}$ progeny data set (Table 34). These were mapped on LG 1 and 3 with LOD values of 2.8 and 3.1, and explained 6.4 and 10.4% of variation, respectively. This model also detected two QTLs using the F_2 population data set, one each on LG 3 and 6 with LOD values of 8.8 and 4.8, respectively, and explained 22.9 and 9.9% of observed variation. The favourable alleles for all these QTLs were from P_2 parent. This model explained a total of 13.3% of observed variation in the $F_{2:3}$ progenies and 32.2% in the F_2 population.

The additive-dominance model detected five QTLs using the $F_{2:3}$ progenies data set (LG 1, 3, 5, 6 and 9) and the LOD scores for these QTLs ranged from 2.5 to 3.7 (Figure 8). The variation explained by these QTLs ranged from 0.3 to 9.7% due to additive effects and ranged from 0.1 to 4.2% due to dominance effects. However, only two QTLs were detected for this trait using the F_2 data set. These QTLs on LG 3 and 6 had LOD scores of 9.4 and 6.6, and additive effects explaining 24.0% and 13.2% of observed variation and dominance effects explaining 2.3% and 3.9%, respectively. The favourable alleles for all QTLs for this trait were contributed by P_2 parent. The portion of observed variation explained by this model was 23.6% in the $F_{2:3}$ progenies and 35.6% in the F_2 population.

The epistatic model detected significant interaction among the identified QTLs. Dominance x dominance interaction and additive x dominance interaction was noticed among the QTLs detected in the $F_{2:3}$ progenies. The variation explained by significant pair-wise epistatic interactions ranged between 3.1% and 4.3%. In the F_2 population, dominance x dominance interaction was observed between the two detected QTLs and this explained 7.9% of the observed variation for grain size. This

model explained observed variation of 29.6% and 41.1% for the $F_{2:3}$ progenies and F_2 population data sets, respectively.

In general, across F_2 and $F_{2:3}$ progeny populations using the three genetic models, a total of 5 QTLs were identified using all the three genetic models for grain size. These QTLs were distributed across LG 1, 3, 5, 6 and 7. The QTLs on LG 3 and LG 6 were detected in both F_2 and $F_{2:3}$ data sets. Individual QTLs explained 6.1 to 21.2% of the observed phenotypic variation. The epistatic model detected significant interactions among all the detected QTLs. The observed variation for this trait was best explained through the epistatic model in both $F_{2:3}$ progenies (29.6%) and F_2 population (41.4%).

4.2.4.4. Panicle weight

The additive model failed to detect any QTL for this trait. However, the additive-dominance model detected a single QTL on LG 3 using $F_{2:3}$ progenies data set (Table 35). This QTL had a LOD value of 2.6 and explained only 2.8% of total variation observed (Figure 9). The additive and dominance effect for this QTL explained 3.8% and 0.3% of observed variation, respectively. The favourable allele for this QTL was from P_2 parent.

4.2.4.5. Panicle harvest index

A single QTL for plant harvest index was identified using $F_{2:3}$ progeny data set for both additive and additive-dominance models (Table 35). This significant QTL was mapped on LG 3 between marker loci *Xicmp*3073 and *Xpsms*68. This QTL had a LOD value of 3.0 and explained 3.7% of variation through the additive model. Using the additive-dominance model, it explained 4.7% of observed variation *via* additive effect and 1.6% *via* dominance effect (Figure 9). The favourable allele was from P_2.

4.2.4.6. Plant height

The additive model for plant height detected six QTLs, two on LG 1 and one each on 2, 3, 4 and 5 using $F_{2:3}$ progeny data set, with LOD scores ranging from 3.1 to 8.3 (Table 36). The portion of observed variation explained by these individual QTLs ranged from 4.1% to 26.7%. Using F_2 population data set, two QTLs were detected, one each on LG 1 and 5 with LOD values of 6.5 and 4.9 that explained 13.6% and 9.1% of variation, respectively. The favourable alleles for the QTLs on LG 1, 2 and 3 were contributed by P_2 parent, while those on LG 4 and 5 were from P_1. The total portion of variation explained by this model was 42.1% in $F_{2:3}$ progenies and 18.2% in F_2 population.

The additive-dominance model revealed same QTLs as obtained by the additive model except for an additional QTL on LG 1 detected in $F_{2:3}$ progeny data set. The favourable allele for this additional QTL was from P_2 parent. In the $F_{2:3}$ data set, QTLs were detected at LOD scores ranging between 3.3 and 11.1 with additive effects explaining between 5.1% and 22.3% and dominance effects explaining between 0.0% and 4.4% of the total variation (Figure 10). Using the F_2 population data set, two QTLs on LG 1 and 5 were detected with LOD values of 8.0 and 5.3, with additive effects explaining 14.1% and 9.9% and dominance effects explaining 3.9% and 0.9% of the total variation, respectively. The QTLs on LG 1 and 5 had favourable alleles from P_1 and P_2 parent, respectively. The total variation explained by this model was 49.3% and 20.1% for $F_{2:3}$ progeny and F_2 population, respectively. The epistatic model did not detect any significant interaction among the identified QTLs in either of the populations.

In general a total of six QTLs for plant height were detected, two on LG 1 and one each on LG 2, 3, 4 and 5 using all the three models in $F_{2:3}$ data set, however, only two QTLs were identified on LG 1 and LG 5 using F_2 data set. The proportion of

observed phenotypic variation explained by these individual QTLs ranged from 7.3 to 23.6%. The LOD scores ranged from 3.1 to 11.0. The additive-dominance model explained a total of 49.3% observed variation for this trait.

4.2.4.7. Productive tiller number

The additive model failed to detect any QTLs for this trait. However, the additive-dominance model detected a single QTL, which was flanked by marker loci *Xpsm*6 and *Xpsmp*2203 on LG 7 (Table 37). This QTL had a LOD score of 3.5 and its additive effect explained 5.5% and the dominance effect explained 3.3% of the total observed variation (Figure 10). The favourable allele for this trait was contributed by P_1 parent.

5. Discussion

The increase in grain yield potential through improvement of sink size component traits is suggested to be a logical breeding objective in crop plants including pearl millet. However, these traits are complex, quantitative in nature, affected by many genes and environment along with interaction between genes and environments. In the present study, the genetic architecture of panicle length, panicle diameter and grain size were characterized through genetic and QTL analyses. The inferences drawn from the results are discussed here.

5.1. Genetic analysis

Plant genetics needs a methodology, which can provide reliable information on the nature of gene action from the quantum of genetic materials handled. Although many mating designs are available to generate wide genetic variability in experiments, the basic requisite is the information on the genetic mechanism involved in the inheritance of a trait in the base population (Comstock and Robinson, 1952). In the present study, the nature of gene action for sink size traits was investigated using generation means and variances and triple test cross analyses. These methodologies provide information on the relative importance of additive, dominance and epistatic gene effects, in determining genotypic values of individuals. The variance components also allow the estimation of broad-sense and narrow-sense heritabilities. Narrow-sense heritability estimates are based on additive genetic variance (fixable component) and are better predictors of the effectiveness of selection in genetically heterogeneous population than broad-sense heritability.

The inferences drawn from the results of generation means and variance analyses of two crosses performed for each of panicle length, panicle diameter and

grain size traits along with those from TTC analysis performed for one cross for each of these traits are discussed below.

5.1.1. Genetic parameters for sink size traits

5.1.1.1. Panicle length

The mean values of the parents for panicle length of the two crosses studied across seasons (2006 rainy season and 2007 summer season) differed significantly. However, the difference was greater in parental lines of cross 1. The means of F_1, F_2 and backcross generations also substantially differed from one another. The F_1s mean in both the crosses were lower than the mid-parental values, suggesting apparently the presence of partial dominance of genes with small panicle (P_1) over those with longer panicle (P_2).

Both scaling and joint scaling tests revealed that a simplistic additive-dominance model did not adequately explain the observed variation for panicle length in both the crosses across seasons, providing an evidence for the presence of digenic or higher order interactions.

The six-parameter model in the estimation of various genetic components for panicle length revealed that the additive effect was highly significant in both the crosses and the seasons. The dominance effect was significant in cross 1 in both the seasons, but its magnitude was lower than the additive effect. The significant effects of both additive and dominance components for panicle length in cross 1 was similar to the reports of Singh and Sagar (2001), Ramamoorthy and Das (1994) and Desale (1993). It was also substantiated from the TTC results where both additive and dominance gene effects contributed significantly to panicle length in cross 1. These results were in agreement with those of Singh *et al.* (1990a) who found significant additive and dominance effects for panicle length through triple test cross analysis.

The magnitude of additive component was higher than dominance component as evidenced from generation means and variances analyses, indicating the relative importance of additive gene effects in governing panicle length. The preponderance of additive gene effects for panicle length was also reported by Singh *et al.* (2000), Joshi and Desale (1996), Mangath *et al.* (1994), Shinde and Patil (1987), Singh and Singh (1972) and Singh *et al.* (1972).

The degree of dominance for panicle length was found to be partial through generation means and variances in both the crosses across both seasons, whereas TTC analysis revealed overdominance for this trait. However, the estimates of dominance components through TTC may not be unbiased because of the presence of significant additive x dominance and dominance x dominance epistasis for this trait, which was evident from both TTC and generation means analyses. Influence of partial dominance for this trait was also reported by Gill *et al.* (1968). The significant dominance component and significant correlation between sums and differences for this trait through TTC analysis indicates unidirectional distribution of dominant alleles among the parents. The negative sign of correlation between sums and differences indicate that the dominant alleles carry positive effect on the trait.

As a result of low magnitude of dominance and environmental variances, the broad and narrow-sense heritability estimates were high in both the crosses across seasons. High heritability for panicle length in pearl millet was also reported by Varu *et al.* (2005), Hepziba *et al.* (1993), Vyas and Srikant (1986), Singh *et al.* (1979) and Madhava *et al.* (1971).

All the three types of interaction effects were significant in cross 1 for at least in one block of set 1 and two blocks of set 2, and were highly significant for set 3 in both the seasons. However, the magnitude of dominance x dominance interaction was

greater followed by additive x dominance and additive x additive interactions. These findings were in agreement with the results of Ramamoorthy and Das (1994) who reported higher magnitude of dominance x dominance interaction and Singh and Singh (1972) who reported a higher magnitude of additive x dominance than the additive x additive interaction for this trait. The test of epistasis through TTC analysis for this cross also revealed the presence of total epistasis. Partitioning of the total epistasis revealed that both additive x additive (i) and additive x dominance and dominance x dominance (j + l) epistasis were significant. However in cross 2, only additive x additive interaction was found to be significant in both the seasons. Significance of additive x additive interaction for this trait was also reported by Singh *et al.* (2000), Gandhi *et al.* (1999) and Shinde and Patil (1987).

The negative sign of (h) and positive sign of (l) components revealed the presence of duplicate epistasis for this trait in cross 1. Shinde and Patil (1987) and Sheoran *et al.* (2000) also reported duplicate epistasis for this trait. However in cross 2, complementary epistasis was detected as both (h) and (l) components were in same direction (positive). Presence of complementary epistasis for this trait was also reported by Ramamoorthy and Das (1994).

In general, for panicle length, all the six genetic components were significant in cross 1. Previous studies have not reported the significance of all the genetic components for this trait in any single cross. This may be because the parental lines did not represent extreme contrast for this trait in earlier studies, which in turn represents the dispersal of like genes between the parental lines. The dispersal of alleles among the parental lines may cause the canceling of some genetic effects, resulting in the underestimation of additive (d), additive x additive (i) and additive x dominance (j) interactions. Further, in cross 2, additive and additive x additive

interaction were significant. The attrition of other genetic components might be because the parental lines utilized in this cross may have been subjected to optimizing selection, and hence are expected to have a predominantly additive architecture with less pronounced dominance components (Gilchrist and Partridge, 2001).

The estimated genetic components for panicle length under different sets of population size through six-generation means showed that the additive x additive interaction was frequently non-significant in the blocks of set 1 population size. However, this component was detected to be significant in the blocks of set 2 and detected with high levels of significance in set 3 population size in both the crosses across both seasons. The consistency in detection of significant genetic component under different population sizes are in part due to the change in allelic frequency with respect to population size, which would be precise in large population. Hence for panicle length, the optimum population size to detect all the genetic component reliably would be at least as that of set 2 (700 plants of F_2 population and 200 plants each of BC_1 and BC_2 populations).

The results of generation means and TTC analysis had a substantial agreement with each other in relation to the relative importance of additive, dominance and epistasis for panicle length. Thomas and Tapsell (1983) also reported similarly in the results of generation means and TTC analysis while studying the genetics of quantitative traits in barley.

5.1.1.2. Panicle diameter

The mean difference for panicle diameter between the parental lines was comparatively higher in cross 1 than cross 2. As expected, the means of six generations differed significantly in both the crosses across the sets and the seasons. The presence of partial dominance of genes with thick panicle over those of thin

panicle was inferred, as F_1 means in both the crosses were higher than the mid-parental values.

The significance of individual scales in scaling test, and χ^2 value of joint scaling test indicated that simple additive-dominance model was not sufficient to explain the total genetic variation for panicle diameter in both the crosses. The lack of fit of additive-dominance model might reveal the presence of non-allelic interactions for this trait.

Both the additive and dominance gene effects for panicle diameter were found to be highly significant in the six-parameter model. The importance of both additive and dominance gene effects for panicle diameter was also reported by Singh *et al.* (2000), Sagar and Singh (1996) and Desale (1993). TTC analysis of variances for sums and differences indicated significance of their mean squares for panicle diameter of cross 1. These results also provide evidence for the presence of both additive and dominance gene effects in the genetic control of panicle diameter. However, higher estimates of additive component than the dominance component were observed through all different methods (generation means and variances and TTC). This suggests the presence of partial degree of dominance and additive x additive (i) epistasis for this trait. Presence of partial dominance for this trait was confirmed, as the estimates were less than unity in both the crosses across different methods. The significant dominant component and negative significant correlation between sums and differences from TTC suggests that dominant alleles are predominantly unidirectional among parents, and the dominant alleles more frequently carry the increasing effect for panicle diameter. As the additive variance estimates were higher than the dominance variance, the estimates of heritabilities were high for this trait in both the crosses across seasons suggesting that this trait is likely to show modest

response to the selection. High heritability for panicle diameter was also reported by Varu *et al.* (2005) and Gupta *et al.* (1988).

Among the interaction components, additive x additive and dominance x dominance interactions were found to be the most important component for cross 1, being significant in both the seasons across different sets of population sizes. However, the magnitude of dominance x dominance interaction was higher than additive x additive interaction. The results of TTC analysis for cross 1 also revealed significant additive x additive and additive x dominance and dominance x dominance (j + l) epistasis. In cross 2, additive x additive interaction was highly significant in both the seasons. The additive x dominance interaction was also found to be significant during 2007 summer season. Singh and Singh (1972) also reported the significant additive x dominance interaction for this trait. The dominance x dominance interaction for panicle diameter in this cross was non-significant across seasons. The lack of dominance x dominance component in cross 2 may be accounted to the selection pressure which results in fixation of additive x additive component in the parental lines, as evidenced from comparatively lesser contrast between the parental lines of cross 2 than cross 1.

The complementary type of epistasis was evidenced from the same sign (positive) of dominance and dominance x dominance components. This type of epistatsis was also reported by Sheoran *et al.* (2000). Conversely, Gandhi *et al*. (1999) and Ramamoorthy (1996) reported duplicate epistatsis for this trait.

The estimated genetic components for panicle diameter under different sets of population size through six-generation means showed that the additive x additive interaction and additive x dominance components varied for their significant levels across different sets of population sizes. These interacting components were

frequently non-significant in the blocks of set 1 population size. However, these components were significant in the blocks of set 2 and set 3 population size. The consistency in detection of significant genetic component in both set 2 and set 3 indicated that the set 2 population size is optimum for the detection all the components precisely.

Both generation means and TTC analysis emphasized the importance of both additive and dominance components for panicle diameter. However their magnitude was larger in TTC analysis than generation means. For the interaction components also both approaches showed substantial agreement with each other. Similar conclusions were also drawn by Bakheit *et al.* (2002) in faba bean crosses and Kearsey *et al*. (1987) in *Lolium perenne* comparing the estimates of TTC and generation means analysis.

5.1.1.3. Grain size

In both the crosses, mean of parents showed large contrast for this trait. The mean of F_1, F_2 and backcross generations considerably varied from parents across seasons. The F_1 mean was higher than the mid parental values suggesting partial dominance of genes with large grain size parent (P_2) over those with small grain size parent (P_1).

The scaling test for adequacy of additive-dominance model for genetic control of grain size indicated the presence of epistasis. The result of joint scaling test also confirmed that three-parameter model did not adequately explain the genetic control of grain size as the goodness of fit for this model through χ^2 test showed highly significant values, and thus warranted the use of the six-parameter model.

Six-parameter model revealed that both additive and dominance effects were significant for this trait in both the crosses across seasons. The role of both additive and dominance gene effects for the inheritance of grain size confirmed the earlier

reports of Gotmare and Govila (1999), Chand *et al.* (1973) and Phul and Athwal (1969). TTC analysis for cross 1 also revealed the significance of both additive and dominance components. The generation means and variance analysis revealed higher magnitude of additive component than dominance component in both the crosses across season suggesting the presence of partial dominance. However, TTC analysis revealed overdominance for this trait, as the additive effect was lower in magnitude than the dominance. Estimates of additive and dominance variances for grain size may not be free from bias, since both the generation means and TTC analyses showed the presence of epistatic interactions. If the genes of like effect are not completely associated in the parents, it is possible that additive gene effects are underestimated as a result of the cancellation of additive (d), additive x additive (i) and additive x dominance (j) effects. However, dominance (h) effects are not influenced by the distribution of the alleles in the parents (Mather and Jinks, 1982). The significant dominance component and negative non-significant correlation coefficient between sums and differences in TTC, indicates an ambidirectional distribution of dominant and recessive allele among the parents and the dominance allele have increasing effects on the grain size.

As generation means and variances revealed the higher magnitude of additive component than dominance component for this trait, the estimated broad and narrow-sense heritability values were high, which suggested a large proportion of genetic effects on the phenotypic expression of the trait, and that selection for this trait would be expected to be highly efficient. Similar high heritability estimates for this trait was also reported by Borkhataria *et al.* (2005) and Kunjir and Patil (1986). Conversely, Sachan and Singh (2001) reported moderate heritability for this trait.

The additive x additive interaction was significant only in cross 1, however its

magnitude was lower compared to other types of interactions. This type of interaction was also revealed by Sheoran *et al.* (2000) for this trait. In the interaction components, dominance x dominance followed by additive x dominance interaction contributed significantly towards the inheritance of grain size in both the crosses across both the seasons. The partitioning of significant total epistatic variation in TTC analysis also revealed that the additive x dominance and dominance x dominance (j + l) epistasis was significant while the fixable component additive x additive epistasis was non-significant. Chand *et al.* (1973) also reported the significance of both additive x dominance and dominance x dominance interactions for grain size. Whereas, Gill *et al.* (1974) reported only additive x dominance (j) interaction for this trait. However, Phul and Athwal (1969) carried out the most intensive study on the inheritance of grain size in pearl millet, which revealed that additive and additive x dominance interaction effects are of primary importance for grain size.

Large significant dominance component and their interactions for grain size is a recognizable pattern underlying genetic parameters for fitness traits that might be the result of directional selection (Mather, 1966; 1983). Willis and Orr (1993) opined that when a number of loci are controlled by dominant or overdominant loci for a trait, intense directional selection and to some extent stabilizing selection will not erode as much additive variance as it would if the trait were controlled purely by additive effects, and an additional expectation is that duplicate epistasis should also arise in directionally selected traits. Opposite sign of dominance (negative) and dominance x dominance (positive) components for grain size confirms the expectation of presence of duplicate interaction in both the crosses across both seasons. Singh *et al.* (1972) also reported duplicate epistasis for this trait. However, Phul and Athwal (1969) indicated the presence of complementary epistasis for grain size.

Estimates of gene effects for grain size under different sets of population sizes through generation means showed significant additive x additive component in cross 1 and dominance x dominance component in cross 2 which varied within set 1 blocks in both seasons. However, these components were significant under the population size of set 2, and were further detected with high level of significance in set 3. Hence, the population size of set 2 could be optimum to detect all the genetic components determining the inheritance of grain size.

TTC analysis revealed the importance of epistasis, particularly additive x dominance and dominance x dominance (j + l) epistasis along with additive and dominance components in the genetic control of grain size. The results of generation means analysis also confirmed the above interpretation to a large extent, except for additive x additive interaction, which was found to be significant in generation means analysis. However its magnitude was lower than additive x dominance and dominance x dominance interactions. Nanda *et al*. (1990) also reported a general agreement between the results of TTC and generation means analyses while studying the inheritance of quantitative traits in bread wheat.

5.1.2. Character associations in trait-specific crosses

The most rapid improvement of economic traits is expected from selection applied simultaneously to all the component traits together. The association between two traits can be directly observed through the correlation coefficient of phenotypic values. The genetic cause of correlation is chiefly pleiotropy, though linkage is a cause of transient correlation particularly in populations derived from crosses between diverse lines (Falconer, 1960).

In the present study, the results of correlation estimates revealed that panicle length, panicle diameter and grain size were positively and significantly associated

with grain yield in both the crosses in their respective trait-specific groups. Similar positive significant association for panicle length, panicle diameter and grain size with grain yield were reported by Salunke *et al.* (2006), Unnikrishnan *et al.* (2004), Anarase and Ugale (2001) and Poongodi and Palaniswamy (1995). Inter-correlation among these traits in their respective groups showed that panicle length in panicle length group had significant positive association with grain size in cross 1 and with panicle diameter in cross 2. Panicle diameter of panicle diameter had highly significant association with panicle length and grain size in both the crosses. Grain size of grain size group exhibited positive and significant association with panicle diameter in both the crosses studied. Positive and significant inter-correlation of grain size with panicle diameter was also reported by Poongodi and Palaniswamy (1995) and Borole and Patil (1991). The inter-correlation of these traits with other yield component traits in their respective groups revealed that panicle length had positive correlation with plant height in both the crosses; panicle diameter showed negative significant association with days to 50% flowering in cross 1, whereas in cross 2 it exhibited significant positive associations with days to 50% flowering and plant height.

The observation of positive significant association among sink size traits and also with grain yield signified the possibilities for the improvement of grain yield through simultaneous selection of these traits in breeding programmes. Though panicle diameter and grain size exhibited strong positive correlation across groups and crosses, the associations of panicle length with panicle diameter and grain size were inconsistent across groups. Weak or negative association of panicle length with panicle diameter and grain size could be attributed to linkage or due to yield component compensation. Improvement of panicle diameter and grain size is quite

possible through simultaneous selection. However inclusion of panicle length with panicle diameter and grain size in simultaneous selection could have few obstacles due to their compensatory associations, which can be overcome through selective mating in early segregating population to break linkages and recombine genes, if the association is the result of somewhat tight linkage blocks.

5.2. QTL analysis

QTL analysis is an important approach for studying the genetic architecture of complex traits, facilitating estimation of number of genomic regions that significantly affect a trait, the distribution of gene effects and the relative importance of additive, dominant and epistatic effects (Mackay, 2001 and Laurie *et al.,* 2004). In pearl millet, the major determinants of sink size traits such as panicle length, panicle diameter and grain size have been sparingly subjected to QTL analysis.

In the present study, three important sink size traits, panicle length, panicle diameter and grain size, and their related traits like panicle weight, panicle grain weight and panicle harvest index, as well as agronomic traits like plant height and productive tiller number, were genetically dissected through QTL analysis in an F_2 and $F_{2:3}$ mapping populations, primarily developed for grain size. Several interesting aspects regarding the number, genomic loci and genetic effects affecting sink traits and their component traits were revealed and their merits are further discussed here.

5.2.1. Phenotypic analysis

Phenotypic characterization of quantitative traits is a pre-requisite to the application of molecular genetic knowledge for broadening our understanding of their genetic control. As a first step in the analysis, the mean performance of sink size traits and other agronomic traits of parents and mapping populations were recorded. The mean

performance of the parents displayed substantial differences for all the observed traits. The analysis of variance for different traits under study revealed highly significant variation except for panicle harvest index among the $F_{2:3}$ progenies, indicating that sufficient variation for these traits existed in the mapping population.

The sink size component traits like panicle length, panicle diameter, grain size, panicle grain weight, panicle weight and plant height showed continuous distribution among the $F_{2:3}$ progenies. Continuous distribution or absence of discrete segregating classes for a trait suggests that its inheritance is either determined by a large number of genes with small effects or a few major genes with substantial environmental effects. For productive tiller number and panicle harvest index, the distribution was skewed indicating the lack of enough variability for these two traits among the parental lines. Transgressive segregants were observed for panicle length, panicle grain weight, panicle weight and plant height. The presence of transgressive segregants might suggest that the parental lines had desirable and undesirable alleles in various proportions for loci governing these traits. However, it should be noted that the segregant plants in the F_2 and $F_{2:3}$ are still heterozygous at a large number of loci, so what might appear like a transgressive segregant could very much be the result of hybrid vigour

Heritability estimates measures the relative importance of heredity in determining the expression of a trait (Allard, 1960). The reliability of QTL mapping also largely depends upon the heritability of individual traits (Kearsey and Farquhar, 1998). Heritability estimates (broad-sense) in F_2 population (0.59 - 0.87) and from the replicated evaluations of the $F_{2:3}$ progeny population (0.56 - 0.92) revealed that all the observed traits except productive tiller number had heritability value greater than 50%, which is a prerequisite for effective QTL mapping. As expected, the heritability

estimates for all the observed traits from the replicated data sets of $F_{2:3}$ progenies were comparatively higher than the F_2 population data set.

The knowledge of correlations among the observed traits gives an idea about the assessment of changes brought about by selection that simultaneously influence correlated traits (Falconer, 1989). The genetic cause of correlation among the traits is either due to pleiotropy and /or linkage. In pleiotropy, the same genes determine the expression of more than one character and linkage is an association feature by which the traits are inherited together. The correlation arising from linkages are transient and can be manipulated. In the present study, correlation coefficients were estimated among the observed traits in the $F_{2:3}$ progenies. Significant positive correlation was observed for panicle length with productive tiller number and panicle grain weight. However its association with panicle diameter was negative but significant. Similarly, panicle diameter had positive significant associations with grain size, panicle weight and panicle grain weight and negative significant associations with panicle harvest index, plant height and productive tiller number. There was also positive significant association of grain size with panicle grain weight and panicle weight and negative significant association with productive tiller number. In addition, panicle grain weight had positive significant correlations with panicle weight and panicle harvest index, and panicle harvest index had positive significant correlation with productive tiller number. These results indicate that by carefully selecting parental alleles associated with increasing or decreasing expression of traits, it should be possible to improve both the traits simultaneously. The correlations among these traits also give an indication of the common loci that might be mapped through QTL analysis.

5.2.2 Molecular analysis

5.2.2.1. Parental polymorphism

Mapping populations in many crop plant that involved crosses between unrelated parental lines with large contrast have shown high level of polymorphism than those which were closely related (Anderson *et al.,* 1993 and Devos *et al.,* 1995). In the present study also, the large contrast between the parental lines of mapping population provided a high degree of polymorphism for markers across most of the linkage groups. Among the 331 markers (96 each of SSCP-SNP, SSR, EST-SSR and 43 STS) assayed on parental lines for polymorphism, SSCP-SNP markers showed the highest level of polymorphism (41.7%), followed by SSRs (37.5%), EST-SSRs (29.2%) and STS markers (11.6%). This finding is contradictory to the report of Bertin *et al.* (2005) who observed comparatively low polymorphism for SNPs than the SSRs, as evident from the reported mean PIC values of 0.49 for SNP and 0.72 for SSR markers tested on the same genotypic panel of pearl millet inbreds. However, Rafalski (2002) reported 86% SNP polymorphism in maize inbreds, and found that the frequency of nucleotide change among inbreds was high, at around one in every 48 bp in non-coding regions and one in every 130 bp in coding regions. SNPs are reported as an essentially inexhaustible source of polymorphic markers for use in high-resolution genetic mapping. SNP markers also have great advantages in unraveling detailed syntenic relationships in specific parts of the genome in comparative mapping applications (Rafalski, 2002).

Although both genomic SSRs and EST-SSRs showed less polymorphism than SNPs in this study, they were very informative, since they are co-dominant, locus specific and evenly distributed (Roder *et al.,* 1995). In pearl millet, Qi *et al.* (2004) reported an average PIC value of 0.71 for SSR markers, which suggests that

microsatellite markers could be used successfully for many types of investigations.

The STS marker system showed very less polymorphism (11.6%) in the present study. The low level of polymorphism of the STS marker system was observed 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 (Money *et al.,* 1993). However, these markers have proven to be useful to cover the telomeric regions of the chromosomes, where other marker systems showed relatively poor coverage. Once identified, polymorphic markers from different systems can be successfully utilized for various types of investigations including genome mapping, gene tagging, evolutionary studies and germplasm characterization.

5.2.2.2. Marker segregation in the mapping population

Identification of sufficient number of markers revealing polymorphism among parental lines is a prerequisite for the construction of a genetic linkage map. In the present study, the mapping population was based on a pair of genetically diverse inbreds, for which a higher number of polymorphic markers (109) with wide and better genome coverage were identified. From this, a set of 44 polymorphic markers well distributed across the seven pearl millet linkage groups were finally selected based on their positions in earlier linkage maps for genotyping a $F_{2:3}$ mapping population with a population size of 188 progenies to construct the linkage map. The larger the mapping population, higher is the confidence in the estimates of recombination frequencies and map distances. The use of a large mapping population also gives a higher chance in detecting the QTLs with small effects and estimates the genetic effects of QTLs accurately (Kicherer *et al.,* 2000). However, the optimum size of mapping population may depend on the genome size of the organism, the generation of mapping population and the nature of the trait under study (Beavis,

1998). A population size of 188 $F_{2:3}$ progenies used in the present study appears to be reasonably large compared to those used in several earlier studies, but not too large compared to the plant numbers in other studies (Poncet *et al*., 2000).

Chromosomal regions that cause distorted segregation ratios may be detected as segregation distortion of mapped loci (Vogl and Xu, 2000). If segregation-distorted locus (SDL) segregates in a population, markers linked to this SDL will also show distorted segregation. In the present study, segregation pattern of 44 marker loci in the 188 $F_{2:3}$ mapping progenies were tested for the goodness of fit to the expected ratio of 1:2:1 using χ^2 test. A total of 4 markers out of 44 markers loci showed distorted segregation as revealed by the significant χ^2 values. These markers were *Xicmp*3063, *Xpsms*31, *Xpsms*18 and *Xpsmp*2027. Markers that show obvious distortion are often excluded from the linkage analysis. However, this usually leads to reduction in genome coverage and failure in detection of few QTLs. An eclectic way would be to add segregation-distorted markers in the linkage analysis along with normally segregating markers and construct the map to determine map position of the distorted markers and then determine whether they need to be included or removed from the linkage map based on their relative positions in the map. In the present study, markers showing distorted segregation were mapped on LG 2 (*Xicmp*3063), LG 3 (*Xpsms*31), LG 5 (*Xpsms*18) and LG 7 (*Xpsmp*2027). Liu *et al.* (1994) and Yadav *et al*. (2004) also reported distorted marker segregation in pearl millet. No attempt was made to investigate the cause of these distortions, as most distortions appear to be cross-specific. A possible mechanism suggests that there may be a gene present in the distorted segregation region that affects gametophtyic or zygotic competitiveness (Qi *et al.,* 2004). For a correctly inferred marker order and map distance, influence of segregation distortion on QTL analysis could be negligible. However, if the

recombination fraction or the orders of marker loci are inferred incorrectly, basic assumptions of QTL analysis do not hold and the results may be imprecise (Sandbrink et al., 1995). The detection of QTLs through composite interval mapping which involves step-wise regression, however, would not be affected by segregation distortion of marker loci (Dao-Hua *et al.,* 2005).

5.2.3. Construction of linkage map

The first molecular marker-based genetic map published for pearl millet had a genetic length of only 303 cM (Liu *et al.,* 1994) and recently Yadav *et al.* (2004) constructed a linkage map consisted of 91 markers covering 476 cM. The present map spans 1018.7 cM, covering all the seven linkage groups with an average marker interval of 23.4 cM. Thus, the present map covered a substantially larger proportion of the pearl millet nuclear genome compared to the earlier maps reported by Devos *et al*. (2000), Poncet *et al*. (2002), Yadav *et al*. (2002), Qi *et al.* (2004), Yadav *et al*. (2004) and Bidinger *et al.* (2007). LG 1, which had a length of about 110 cM, was comparable to the map length reported by Devos *et al.* (2000). The other linkage groups that were expected to provide nearly complete chromosome coverage are LG 2, 3, 5 and 6, which carried markers in the centromeric and distal regions. The two linkage groups that are short are LG 4 and 7 with genetic lengths of 37.7 cM and 96.0 cM, respectively. The unexpectedly shorter length of these two linkage groups were probably due to lack of enough polymorphic markers between the parents used in this study and/or an indication that recombination is unusually low in these linkage groups.

A wide genome coverage was achieved mainly because the relative positions of most of these markers were already known and the markers selected were evenly distributed in the centromeric regions and distal ends across the linkage groups. The

percentage of markers assigned to the respective linkage groups (at least those having good coverage in this study) is in good agreement with estimates obtained by other researchers (Devos *et al*., 2000; Yadav *et al*., 2004). The number of markers assigned to each linkage group and their map distances is in part a reflection of the relative amount of genetic variation present among the linkage groups. The low marker coverage of LG 4 and LG 7 in present study might be due to parents used in this study being more similar for genomic regions in these linkage groups.

The present map had large gaps in the distal regions for which the most probable reason could be that in the pearl millet recombination is extremely localized in the distal regions of chromosomes. According to Qi *et al.* (2004) the large gaps in the distal regions indeed represent regions of high recombination, rather than a general lack of markers in those regions. It is, however, possible on the other hand, that the pearl millet linkage maps are still incomplete and genomic sequences of rice and sorghum can be used to develop new markers that are located on distal regions of the linkage groups, provided colinearity is maintained in the distal chromosomal regions between rice, sorghum and pearl millet (Devos *et al.,* 2000; Qi *et al.,* 2004).

5.2.4. Mapping quantitative trait loci (QTL)

5.2.4.1. Mapping QTL for sink size traits

The identification of markers associated with sink size trait QTLs would help breeders to construct beneficial allelic combinations and accelerate breeding programmes for the development of improved cultivars. Characterization of QTLs for sink size traits will also provide insight into the mode of their inheritance. The most practical application of the QTLs for sink size traits would be to perform marker-assisted selection aimed at efficient pyramiding of favourable QTL alleles to improve sink size structures in pearl millet. The QTLs mapped for sink size traits in the present study

are presented in Figure 11.

5.2.4.1.1. Panicle length

Earlier QTL mapping studies examining the panicle length in pearl millet have demonstrated that this trait is affected by genomic regions across LG 1, 2, 4 and 7 (Poncet *et al.*, 2000; 2002 and Nepolean, 2002). In the present study, using $F_{2:3}$ data set, eight genomic regions, each on LG 1, 2, 4 and 7 and two each on LG 4 and 6 showed associations with panicle length. Using the F_2 data set two QTLs on LG 2 and 6 were identified for panicle length. Alleles derived from the female parent (P_1) were favourable for QTLs on LG 1, 2, 3, 6 and 7, while for the QTLs on LG 3 and 4, favourable alleles were contributed by the male parent (P_2). Among the detected QTLs in the present study, the largest portion of variation (26.9%) was explained by a QTL on LG 2 (*Xpsmp*2237 – *Xpsms*89) followed by a QTL on LG 6 (*Xpsms*88 – *Xpsmp2270*), which explained 12.8% of observed variation.

Overall, the number of significant QTLs detected for panicle length varied between the two populations (2 QTLs in F_2 and 8 QTLs in $F_{2:3}$). The detection of the additional QTLs (all with small effect) in $F_{2:3}$ progenies may be the result of higher heritability estimates obtained from the replicated progeny trials.

The total phenotypic variation explained by the additive-dominance model was marginally greater than that explained by the additive or epistatic models. However, significant additive x additive and dominance x dominance epistatic interactions were observed among the detected QTLs through epistatic model. In the presence of epistasis the expected magnitude of the estimates of marginal effects (additive and dominance) of a QTL may substantially differ between the different genetic models. For example in the present study, the sign reversal for the additive effects of panicle length QTLs on LG 6 was observed between the additive -

dominance and epistatic model. In the additive - dominance model, the estimate of additive effects of the QTL may be confounded by additive x additive and additive x dominance interactions. Similarly, the estimate of dominance effects may be confounded by the additive x dominance and dominance x dominance interactions.

5.2.4.1.2. Panicle diameter

Five QTLs were detected and mapped on LG 2, 3, 5, 6 and 7 for panicle diameter using $F_{2:3}$ progeny data set across all the genetic models. For all these QTLs, favourable allele was contributed from P_2 parent. The QTLs on LG 5, 6 and 7 corresponds with the previous reports for QTL positions of this trait (Poncet *et al.*, 2000; 2002). However, the additional QTLs on LG 2 between "*Xpsms*78 – Xpsms592 and on LG 3 between "*Xpsmp*2272 - *Xpsms*17" had a LOD score greater than 14.0 and cumulatively explained large proportion of observed phenotypic variation for this trait.

All the three genetic models failed to detect any significant QTL(s) for panicle diameter using F_2 data set. This may be due to the uncontrolled environmental influence on expression of the trait in single plants. However, the QTLs identified using the replicated $F_{2:3}$ phenotypic data set could be highly reliable as progeny means from replicated field plots were used as the unit of phenotypic measurement for QTL analysis (Paterson, 1997).

5.2.4.1.3. Grain size

There were five putative QTLs detected for grain size on LG 1, 3, 5, 6 and 7 using $F_{2:3}$ data set while using the F_2 data set detected two QTLs on LG 3 and 6. The QTLs mapped on LG 1 and LG 3 appear to be comparable to those reported by Bidinger *et al.* (2007) for this trait, and were highly under additive control. The QTLs found on

LG 6 and LG 7 appear to be similar to those reported by Yadav *et al.* (2002). The present study also mapped an additional QTL for grain size on LG 5, which has not been identified in earlier studies. However, this QTL also contributed significantly (6.4%) to the total phenotypic variation observed for grain size in this study.

Significant, additive x dominance and dominance x dominance interactions were observed among the detected QTLs, suggesting that the marginal effects of these QTLs could may biased. According to Carlborg and Haley (2004), epistatic model is necessary for validating the importance of the detected QTLs. Further, the knowledge of the type of interactions can guide a researcher to choose the appropriate genetic background of recipient lines in MAS to obtain maximal gain.

The lower level of observed phenotypic variation explained by the individual QTLs in the present study confirms the quantitative nature of grain size and its inheritance. It is also in agreement with the hypothesis that polygenes controlling important metric traits such as grain size are usually distributed among several genomic regions that may not be linked to one another (Fatokun *et al.,* 1992). These results imply that transfer of large grain size cannot be approached easily through conventional breeding programmes. Therefore, the consistent QTLs identified in both F_2 population and $F_{2:3}$ progenies would be useful targets for marker-assisted selection for large grain size in pearl millet breeding programmes.

5.2.4.2. Mapping QTLs for traits related to sink size.

Although the primary purpose of this study was to identify QTLs for sink size traits (panicle and grain size), few other traits that are related to sink size such as panicle weight, panicle grain mass and panicle harvest index were also evaluated for QTL mapping. Poncet *et al.* (2000; 2002) have reported that LG 2, 5 and 6 were associated with panicle weight variation. Nepolean (2002) observed that panicle grain weight

variation is associated with QTLs on LG 4 and 7. Similarly, QTLs for panicle harvest index were identified across all seven linkage groups (Yadav *et al.,* 2004 and Bidinger *et al.,* 2007). Panicle harvest index used specifically as a measure of tolerance to terminal drought, indicates the ability to set and fill grains under limited moisture.

In the present study, only a single QTL for each of panicle weight and panicle harvest index was detected on LG 3 (Figure 11). For panicle grain weight no QTLs were identified. The probable reason for the detection of only a single QTL or no QTLs for these traits may be the lack of enough variation among the parental lines and mapping population in this study.

5.2.4.3. Mapping QTLs for agronomic traits

QTL analysis was also made to map QTLs for agronomic traits such as plant height and productive tiller number. The identified QTLs for these traits are represented in Figure 11.

5.2.4.3.1. Plant heightPoncet *et al.* (2000; 2002) mapped QTLs for plant height on LG 1, 2, 5, 6 and 7. Similarly, Nepolean (2002) located QTLs for plant height on LG 4, 6 and 7. In addition, Azhaguvel *et al.* (2003) mapped two major semi-dwarfing genes, d_1 and d_2 on LG 1 and 4, respectively.

In the present study based d_2 x d_2 crosses, six QTLs were detected for plant height across five linkage groups (two on LG 1 and one each on LG 2, 3, 4 and 5) using $F_{2:3}$ data set. Only two QTLs each on LG 1 and 5 were detected using F_2 data set. The position of these QTLs is in agreement with other studies (Poncet *et al.,* 2000; 2002, Nepolean, 2002, Azhaguvel *et al.* 2003), except for the QTL on LG 3, which was not identified in earlier studies. However, the QTL on LG 3 also explained a larger portion of the phenotypic variance (21% in additive-dominance model) and

hence may be considered as another dwarfing gene locus. The recessive alleles for QTL on LG 1 and LG 2 were associated with reduced plant height, and were contributed by P_2 and P_1 parents, respectively. Two QTLs on LG 6 and 7 reported in earlier studies (Poncet *et al.,* 2000; 2002, Nepolean, 2002) were not detected in the present study. These results indicate that QTLs for plant height are wide spread over the pearl millet genome. However, QTLs on LG 1, 3, 4 and 5 are important in controlling this trait in the population used in this study.

Although five different sources of dwarfing genes were reported (Burton and Fortson, 1966), there is wide use of only d_2 gene in breeding programmes. However, d_2 dwarfing gene in pearl millet carries a yield penalty due to pleotropic association with reduced individual grain mass (Bidinger *et al.,* 2001; Rai and Hana, 1999), although this can be overcome by manipulating the genetic background.

5.2.4.3.2. Productive tiller number

QTLs for this trait were previously mapped on LG 1, 2, 4, 6 and 7 (Poncet *et al.,* 2000, Yadav *et al.,* 2002, Poncet *et al.,* 2002, Nepolean, 2002 and Yadav *et al.,* 2003), which suggests that productive tiller number is affected by several loci. In the present study, use of $F_{2:3}$ progeny data set identified a single QTL for productive tiller number on LG 7, which might correspond to the QTL identified in earlier studies. The lack of detection of other QTLs in the present study may be due to less variation in the mapping population and low heritability (< 50%) observed for this trait.

5.2.4.4. Co-mapped QTLs

The quantitative traits affected by pleiotropism and linkage tend to reveal significant correlation among them. This, in turn, generally leads to identification of co-mapped QTLs. However, it is not possible to distinguish between pleiotropy and linkage as a

cause of a correlated effect on two traits until one has mapped the QTN (Quantitative Trait Nucleotide) responsible for phenotypic variation of each trait (Mackay, 2001).

In the present study, QTLs for panicle length and plant height were co-mapped on LG 2 (*Xpsmp2237* - *Xpsms89*) and LG 4 (*Xpsms77* - *Xpsmp2084*), and the favourable allele for of these QTLs were contributed by parent P_1. Panicle diameter and panicle weight QTLs were co-mapped on LG 3 between *Xpsmp2222* and *Xpsms17* markers. The additive effects for these traits were contributed by parent P_2. Similarly, genomic regions associated with panicle length, panicle diameter and grain size were co-mapped on LG 6 between *Xpsms88* and *Xpsms2270* markers. The panicle length QTL showed negative association with both panicle diameter and grain size. However, the panicle diameter QTL showed positive association with grain size QTL and their favourable alleles were contributed from P_2 parent. Panicle length and productive tiller number had a common QTL on LG 7 between *Xpsm6* and *Xpsmp2203* markers and the allele for increased panicle length and reduced productive tiller number were contributed from P_2 parent.

The results of present study suggested that many of the QTL regions appear to affect multiple traits. Further research needs to be done to learn whether there is a single gene with pleiotropic effect underlying such common QTLs or there is a cluster of tightly linked genes affecting several traits. As expected, co-mapped QTLs for the traits in present study also had significant correlations among them.

There were additional QTLs for several pairs of associated traits that did not co-map. One of the reasons for not detecting common QTLs for significantly associated traits could be that the QTLs identified could explain only a part of the total variation. Further, it is possible that there are a number of QTLs with small effects that may be responsible for a large portion of the trait variation that are

common among those traits, but could not be detected with the size of mapping population used and heritabilities achieved in the present study.

The co-mapped QTLs demonstrated the existence of genes or gene clusters with major effects, which are involved in the control of significant proportions of the phenotypic variation in several quantitatively inherited traits related to sink size components. Where favourable effects across several traits can be obtained with the allele of one parent of the population, such as the QTL for panicle diameter and grain size co-mapped on LG 6, then these QTL become obvious target for marker-assisted selection.

5.2.5. Prospects for marker-assisted selection

Early generation selection for quantitative traits in the field is difficult, its efficiency is unpredictable, and genotype screening for such traits requires replicated trials, which is resource intensive. Consequently, marker-assisted selection could be used to facilitate the transfer of genes for quantitative traits into well-adapted genotypes. However, there are certain considerations that need to be taken when deciding which QTL should be targeted in a MAS strategy. First of all, it is necessary to decide the genomic region (s) for which there is enough evidence for the presence of a major QTL. This can be achieved by setting appropriate thresholds (LOD more than 3) for the identification of QTLs, by detecting QTLs in the same genomic region under different conditions, and/or across several mapping studies. In the present study, the QTLs identified for panicle length on LG 2 and 6 (LOD greater than 3 in both F_2 and $F_{2:3}$ data sets); for panicle diameter on LG 2 and 3 with (LOD greater than 14 in $F_{2:3}$ data set); for grain size on LG 3 and 6 (LOD greater than 3 in both F_2 and $F_{2:3}$ data sets) along with two QTLs identified for plant height on LG 1 and 5 (LOD greater than 5 in both F_2 and $F_{2:3}$ data sets) fulfilled these criterion. However, the QTLs on

LG 6 for panicle length and grain size do not appear to be suitable for MAS because the favorable alleles at these tightly linked (and perhaps identical) QTLs are from the opposite parents, and both QTLs exhibited epistatic effects. Hence, it can be concluded that the QTLs on LG 2 for panicle length, LG 2 and 3 for panicle diameter, LG 3 for grain size and on LG 1 and 5 for plant height are promising candidates for MAS. Jefferies *et al*. (1997) demonstrated that molecular markers closely linked to genes of agronomic importance are useful tools for indirect selection in barley breeding. Marker-assisted selection is time-efficient, and depending on linkage relationships, has low selection error. However, MAS should be done on a case by case basis. Further investigation will be required to validate the importance of the genomic regions identified in the present study in other genetic backgrounds. This can be done simultaneously with exploratory MAS to introgress the favourable alleles identified for sink size traits in the current study, into a range of genetically diverse agronomically elite backgrounds.

5.3. Genetic versus QTL analysis

Genetic (generation means and TTC analysis) and QTL analysis were compared for their efficiency in elucidating the gene actions for grain size in cross 1. Based on the results of classical genetic and QTL analysis, the most noticeable finding was the prevalence and importance of epistasis, particularly dominance interactions for grain size as it explained a greater proportion of the total variation. The models of gene actions including epistasis between different loci developed for conventional quantitative genetics and recent QTL mapping studies do not have the same level of applicability because the genetic effects in these model are defined with reference to different types of population (Yang *et al*., 2004). In both generation means and TTC analysis, the estimated parameters capture the net contribution of gene effects.

However, the consequences of summation over loci are quite different for various parameters. With the TTC design, it is possible to separate epistatic variance components from those of additive and dominance components, but one cannot clearly discriminate between additive x dominance and dominance x dominance variances. This is because coefficients for these two variances are almost identical in the genetic expectations. TTC analysis revealed the importance of epistasis, particularly additive x dominance and dominance x dominance (j + l) epistasis, along with additive and dominance components in the genetic control of grain size. The results of generation means analysis confirmed this interpretation to a large extent, except that additive x additive interaction was also found to be significant in generation means. However, its magnitude was less than the additive x dominance and dominance x dominance interactions. Nanda *et al*. (1990) also reported a general agreement between the results of TTC and generation means analyses while studying the inheritance of quantitative traits in bread wheat. The results of biometrical genetic analysis on grain size reveals the presence of epistasis, dispersion and ambidirectional dominance, which must imply multiple QTL for this trait. The results of QTL analysis agrees with this hypothesis as five QTLs were detected for grain size in this study. QTL analysis results were also consistent with classical genetic approaches for the type and direction of epistatic interactions. Thus, dominance x dominance and additive x dominance epistasis were predominantly observed among the detected QTLs, in great agreement with both generation means and TTC analysis. However, there are also inconsistencies. The generation means indicated significant additive x additive epistasis, although TTC and QTL analyses didn't revealed this epistasis for grain size. This may reflect the low power of TTC analysis and QTL detection with

given restricted sets of genotypes, as compared to generation means analysis, which used a much larger population size.

5.4. Breeding strategies

Quantitative genetic information of the traits provides much of the framework for the design and analysis of selection methods used in breeding programmes (Allard, 1960; Falconer and Mackay, 1996; and Cooper *et al.,* 1999). In the present study, the quantitative genetic parameters for three sink size traits (panicle length, panicle diameter and grain size) were determined using genetic and QTL analyses in crosses involving parents with large contrasts for these traits. Based on the relative importance of genetic parameters obtained for each trait, suitable breeding approaches for their improvement are discussed below.

Pearl millet breeding comprises three broad activities (i) population improvement (ii) inbred development and (iii) hybrid or open-pollinated variety development. The objective of population improvement is to increase the frequency of favourable alleles while maintaining genetic variation. These populations then can serve as a potential source of superior inbreds without any genetic ceiling for future hybrid improvement.

In the present study, additive genetic effects and additive x additive interactions were found to be of prime importance for panicle length in cross 2 and panicle diameter in both the crosses. Dominance effects were also observed, but were lower in magnitude than the additive effects. Under such conditions, mass selection can be effective, but S_1 or S_2 selection is likely to be more effective.

Mass selection for panicle size (panicle length and diameter) and grain size has been practiced in pearl millet since the first deliberate attempts to improve this crop (Krishnaswamy, 1962). However, mass selection has not always produced the

gains desired. For instance, three cycles of mass selection for increased grain size and grain yield of pearl millet gave inconsistent responses (Khadr and Qyinloye, 1978). The extent to which mass selection can change a particular trait, however, is expected to correspond with its heritability. The heritability estimates (broad and narrow-sense) in the present study for panicle length and panicle diameter were high suggesting that mass selection could be successful for manipulating these traits. However, heritability estimates in the present study may be biased upwards as portions of epistatic variation were also exhibited by these traits. The presence of non-additive gene action for these traits suggests that mass selection would be less effective. The effectiveness of mass selection, however, could be maximized by any technique that increases trait heritabilities on a single plant basis. One such technique is stratified mass selection, whereby environmentally induced plant-to-plant variations are limited to those occurring within relatively small strata of the overall experimental plot (Gardner, 1961).

Selections based on progeny performance using an inbred tester are of particular interest to breeders to hasten additive genetic variances in the population. Selection among S_1 or S_2 progenies is attractive on theoretical grounds because, in the absence of overdominance, it is expected to be appreciably more effective than testcross method for changing population gene frequencies (Comstock, 1964; Wright, 1980). In pearl millet, Sastry *et al.* (1987) and Dutt and Bainiwal (2005) reported that S_1 progeny selection method appears to be superior to either of half sib or full sib methods of progeny selection in exposing the hidden variability.

In the present study, epistatic interactions were found to be the important genetic components for panicle length in cross 1 and grain size in both crosses. Along with epistatic interactions, additive and dominance gene effects were also found to be

significant for these traits. Hence, the successful breeding method for these traits would be the one that can capitalizes on epistatic as well as additive and dominance genetic variation. Reciprocal recurrent selection theoretically improves both additive and non-additive gene actions (Comstock *et al*., 1949). In pearl millet, Zaveri *et al.* (1988) reported that reciprocal full sib scheme might prove more rewarding, as in addition to improving the base population by selection, it is the most effective method for breeding hybrids from the inbred lines derived from each cycle of selection. However, because the testcross population is used for selection, the population performance *per se* will be improved only indirectly. In the absence of overdominance, progeny selection using either S_1 or S_2 lines is considered to be superior to other methods of recurrent selection for improvement of *per se* performance (Lamkey, 1992). In pearl millet, Bidinger and Raju (2000b) also reported that it is possible to make a reasonable progress in increasing grain mass by S_1 progeny selection, wherein alleles are fixed rapidly and deleterious homozygous alleles are exposed and eliminated early in selection (Weyhrich *et al.,* 1998). Combining the progeny selection and reciprocal recurrent selection methods simultaneously should permit the benefits of non-additive and additive genetic effects (Goulas and Lonnquist, 1976; Dhilon, 1991). Progress from simultaneous selection is expected to be the summation of expected progress from each individual methods (Hallauer and Miranda, 1988).

Quantitative theory in plant breeding has generally been supported by retrospectively analyzing data derived from populations that lack controls. New tools have recently been added to the arsenal of applied quantitative genetics, namely molecular markers. Molecular markers are now increasingly being employed to trace the presence of target alleles from non-recurrent parent (foreground selection) as well

as accelerating the recovery of the recurrent parent genome (background selection) in backcross programmes. In the present study, QTL analysis for sink size traits revealed 9 QTLs for panicle length, 5 QTLs for panicle diameter and 5 QTLs for grain size, of which QTLs on LG 2 for panicle length, LG 2 and 3 for panicle diameter, LG 3 for grain size and LG 1 and 5 for plant height are identified as promising candidates for MAS. Transfer of these QTLs from donor to recipient line could be approached through marker-assisted backcrossing (MAB), which would lead to the significant improvement of these traits in the recurrent parent.

The linked markers to known QTL of a trait can also be effectively utilized in population improvements. Recurrent selection was developed to allow gradual increase in the frequency of favourable alleles (Allard, 1960) and has proven effective for increasing mean performance while maintaining genetic variability (Hallauer and Miranda, 1981). However, high-intensity recurrent selection leads to the loss of genetic variability in population. Through the use of molecular markers linked to the target trait in high intensity recurrent selection, changes in trait variability at every cycle of selection could be monitored effectively and can avoid the loss of genetic variability between cycles of selection (Labate *et al*., 1999; Pinto *et al*., 2003). In maize, marker-QTL associations was successfully exploited in population improvement through marker-assisted recurrent selections (Edwards and Johnson, 1994; Johnson, 2004).

The breeding methods suggested based on conventional and molecular approaches would vary in effectiveness across populations for selection. Integrating molecular marker technologies such as MAS into breeding could become increasingly important in the coming years, to realize genetic gains with greater speed and precision. However, the method of choice for evaluation of individual plants from a

population would primarily depend on the cost of conducting each method. Hoeck *et al.* (2003) reported that phenotypic selection was estimated to be US$ 0.35 per plant for seed size in soybean. The cost for MAS was estimated to be a minimum of US$ 0.75 per plant, assuming six multiplexed markers run on one lane for each plant. On the basis of these estimates, phenotypic selection would be preferred, however it also depends on traits under selection, and for few traits MAS could be warranted where phenotypic selection is least efficient. Although MAS is currently used widely for simply inherited traits than for polygenic traits, with the development and access to reliable PCR-based marker such as SNPs and SSRs, which are simple to use, the cost of such techniques is progressively decreasing and may become affordable in the frameworks of breeding programmes for complex traits. Surely, better methods of gene mapping and estimation of breeding values through molecular markers are still needed, and integrating QTL mapping in applied breeding deserves further work.

6. Summary

The present study was carried out to characterize the genetic architecture of three sink size component traits such as panicle length, panicle diameter and grain size in pearl millet through genetic and QTL analyses. The experimental material for genetic studies comprised of two trait-specific crosses each for panicle length, panicle diameter and grain size traits. The six basic generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) were produced for all the crosses. Also, triple test cross (TTC) families [L_{1i} (P_1 x F_{2i}), L_{2i} (P_2 x F_{2i}) and L_{3i} (F_1 x F_{2i})] were generated for each trait-specific cross. The six basic generations were evaluated in a randomized complete block design with three blocks in 2006 rainy season and 2007 summer season. The TTC families were evaluated in a randomized complete block design with three replications during the 2007 summer season. For QTL analysis, the mapping population comprised of 188 F_2 and their $F_{2:3}$ progenies, developed from a cross between two inbred lines selected primarily with large contrast for grain size, but these also differed for panicle length and panicle diameter. The F_2 and $F_{2:3}$ mapping populations were evaluated during the 2006 rainy season and 2007 summer season, respectively. The 188 $F_{2:3}$ progenies were genotyped with 44 markers (24 SNP, 10 SSR, 6 EST-SSR and 4 STS) and linkage map was constructed using MAPMAKER/EXP V.3.0. software. Composite interval mapping (CIM) procedure was used for the QTL analysis using PLABQTL software.

The salient findings of the study are as follows: -

- Scaling and joint scaling tests revealed that a simplistic additive-dominance model did not adequately explain the observed variation for the three sink size traits in both the crosses across both seasons, providing an evidence for the presence of digenic or higher order interactions, and thus warranted the use of the six-parameter model.

- For panicle length in cross 1, six-parameter model revealed significant additive (d) and dominance (h) components. All three types of epistatic interactions were significant in both seasons in this cross. These results were in agreement with the results of TTC analysis. However in cross 2, only additive effects and additive x additive interaction were significant for this trait. The presence of partial dominance was inferred for this trait, as the magnitude of additive component was greater than dominance component in both crosses.

- For panicle diameter, generation means analysis revealed that the additive (d) and dominance (h) gene effects were highly significant in both crosses across the two seasons. TTC analysis for this trait also showed significant estimates for both additive and dominance components. As the magnitude of additive component was higher than the dominance component in both the crosses across both seasons, presence of partial dominance was inferred for this trait. Among the digenic interactions, additive x additive (i) interaction was found to be the most important component for this trait, being significant in both the crosses, followed by dominance x dominance (l) interaction which was significant in cross 1 across both seasons. However, test of epistasis in TTC analysis showed that all the three interactions components were significant.

- For grain size, six-parameter model and TTC analysis revealed the importance of both additive (d) and dominance (h) effects. The higher magnitude of additive component than dominance component in both the crosses suggested the presence of partial dominance. All three types of interactions were significant in cross 1. However, TTC analysis showed that only additive x dominance and dominance x dominance interaction components were significant. In cross 2, additive x dominance and dominance x dominance interaction components were significant.

- The estimates of narrow-sense heritability for panicle length, panicle diameter and grain size were found to be high in both crosses across seasons suggesting the predominance of additive genetic variances.
- Correlation coefficient estimates in the trait-specific crosses revealed that panicle length, panicle diameter and grain size were positively and significantly associated with grain yield in both the crosses of their respective trait-specific group. Inter-correlation among these traits in their respective trait-specific groups showed that panicle length in panicle length group had significant positive association with grain size in cross 1 and with panicle diameter in cross 2. Panicle diameter of panicle diameter group had highly significant association with panicle length and grain size in both the crosses. Grain size of grain size group exhibited positive and significant association with panicle diameter in both the crosses.
- The linkage map revealed that the highest number of markers were on LG 3 (9 markers), followed by LG 1 (8 markers), LG 2 (8 markers), LG 5 (7 markers) and LG 6 (6 markers). The linkage group 7 (4 markers) and 4 (2 markers) had lower number of mapped markers. The basic map had a total length of 1018.7 cM. The average distance between the marker pairs was 23 cM.
- QTL analysis identified eight genomic regions for panicle length using $F_{2:3}$ data set, one each on LG 1, 2, 4, and 7 and two each on LG 3 and LG 4. The variation explained by individual QTLs ranged from 6.1 to 18.2%. Though significant interaction among the QTLs on LG 1, 3, 6 and 7 were detected using the epistatic model, the total variation explained through the additive-dominance model was high in both the F_2 population (13.1%) and $F_{2:3}$ progenies (42.7%).
- For panicle diameter, five QTLs were found on LG 2, 3, 5, 6 and 7 using the $F_{2:3}$

progeny data set. The variation explained by these individual QTLs ranged from 6.3 to 30.2%. Additive x dominance interaction was observed between QTLs located on LG 2 and LG 3. The additive-dominance model explained the highest proportion of observed variation (45.8%) for this trait.

- For grain size, a total of 5 QTLs were identified on LG 1, 3, 5, 6 and 7 across F_2 and $F_{2:3}$ progenies. QTLs on LG 3 and 6 were detected in both F_2 and $F_{2:3}$ data sets. Individual QTLs explained 6.1 to 21.2% of the observed phenotypic variation. Variation for this trait was best explained through the epistatic model for both F_2 population (41.4%) and $F_{2:3}$ progenies (29.6%).

The findings from genetic study suggest that improvement of panicle length and panicle diameter through mass selection or family selection (S_1 or S_2) is expected to have a high degree of efficiency, as these traits revealed predominance of additive genetic effects than dominance and epistatic effects along with high heritability. Higher magnitude of dominance x dominance interaction along with additive effects for grain size suggests that the successful breeding method for this trait will be the one like reciprocal recurrent selection, which can capitalize on additive as well as non-additive genetic effects.

From the QTLs mapped in this study, one QTL for panicle length on LG 2 (LOD greater than 3 in both F_2 and $F_{2:3}$ data sets); two QTLs for panicle diameter each on LG 2 and 3 (LOD greater than 14 in $F_{2:3}$ data set) and one QTL for grain size on LG 3 (LOD greater than 3 in both the F_2 and $F_{2:3}$ data sets) were identified as the candidate QTLs for validation and possible use in marker-assisted selection experiments.

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Table 1. QTLs reported for sink size and agronomic traits in pearl millet.

Trait	Author	LG	Flanking markers	LOD	% Variation (R^2)	Additive effect	Population details
Panicle length (cm)							
	Poncet *et al.* (2000)	1	*Xpsm858* - *Xpsm515*	2.45	13.20	1.27	250 F_2 individuals derived from wild x cultivated lines (Molli x Sonu)
		2	*Xpsm592* - *Xpsm738*	7.37	35.90	-2.13	
		7	*Xpsm655*			0.42	
	Poncet *et al.* (2002)	7	*Xpsm526* - *rz404*	2.35	8.60	-1.71	168 F_2 individuals derived from domesticated x wild hybrid (Thiotande x Wild)
	Nepolean (2002)	4	*Xpsm568* - *Xpsm512*	6.22	22.10	-0.48	136 $F_{2:4}$ testcross progenies derived from a cross of PT 732B x P 1449-2
Panicle diameter (mm)							
	Poncet *et al.* (2000)	5	*Xpsm735*	2.62	15.40	-0.34	250 F_2 individuals derived from wild x cultivated cross (Molli x Sonu)
		7	*Xpsm655*	15.58	62.00	-0.54	
	Poncet *et al.* (2002)	6	*Est-E* - *Xpsm579*	4.17	14.40	-2.03	168 F_2 individuals derived from domesticated x wild cross (Thiotande x Wild)
		7	*Xpsm526* - *rz404*	2.16	6.40	1.33	
	Nepolean (2002)	4	*Xpsm568* - *Xpsm512*	2.46	10.50	0.51	136 $F_{2:4}$ testcross progenies derived from a cross of PT 732B x P 1449-2
		4	*Xpsm512* - *Xpsm344*	5.97	21.00	0.71	

Contd..

Trait	Author	LG	Flanking markers			LOD	% Variation (R^2)	Additive effect	Population details
Grain size (1000-grain weight)									
	Yadav *et al.* (2002)	2	*Xpsm394*	-	*Xpsm214*	4.46	21.80	0.05	150 $F_{2:3}$ testcross progenies derived from H 77/833-2 x PRLT 2/89-33
		6	*Xpsm459*	-	*Xpsm588*	2.76	13.80	-0.04	
		6	*Xugt1*	-	*Xpsm87.1*	1.90	11.30	-0.04	
		7	*Xpsm718*	-	*Xpsm266*	2.96	17.20	-0.05	
	Bidinger *et al.* (2007)	1	*Xpsm761*	-	*Xpsm756*	6.90	57.20	0.40	79 $F_{2:4}$ testcross progenies derived from ICMB 841 x 863B
		2	*Xpsm322*	-	*Xpsmp2059*	6.60	34.60	0.30	
		3	*Xpsm108*	-	*Xpsmp2214*	4.30	17.60	-0.20	
		6	*Xpsm588*	-	*Xpsm713*	3.00	6.10	0.10	
	Nepolean (2002)	4	*Xpsm568*	-	*Xpsm512*	3.00	11.60	0.54	136 $F_{2:4}$ testcross progenies derived from a cross of PT 732B x P 1449-2
Panicle weight (g)									
	Poncet *et al.* (2000)	2	*Xpsm176*	-	*Xpsm592*	16.04	60.90	-1.92	250 F_2 individuals derived from wild x cultivated lines (Molli x Sonu)
		5	*Xpsm651*	-	*Xpsm735*	2.99	22.50	-1.11	
		6	*Xpsm696*	-	*Est-E*	-	-	-0.60	
	Poncet *et al.* (2002)	6	*Est-E*	-	*Xpsm579*	4.06	16.20	-1.17	168 F_2 individuals derived from domesticated x wild cross (Thiotande x Wild)

Contd..

Trait	Author	LG	Flanking markers			LOD	% Variation (R^2)	Additive effect	Population details
Panicle grain weight (g)									
	Nepolean (2002)	7	*Xrm9-2b*	-	*Xpsms618*	2.40	9.00	0.41	136 $F_{2:4}$ progenies derived from a cross of PT 732B x P 1449-2
		4	*Xpsm84*	-	*Xpsm612*	3.58	12.40	0.54	
		6	*Xpsm579*	-	*Xpsm613b*	2.73	12.30	0.51	
Panicle harvest index (%)									
	Yadav *et al.* (2004)	1	*Xpsm573*	-	*Xpsm761*	2.13	13.80	-	79 $F_{2:3}$ testcross progenies derived from ICMB 841 x 863B
		2	*Dhn4*	-	*Xpsm443*	3.79	38.40	-	
		3	*Xpsm325*	-	*Xpsmp2070*	4.17	22.30	-	
		5	*Xpsmp2064*	-	*Xpsm345*	4.93	25.60	-	
		6	*Xpsm514*	-	*Xpsm870*	3.03	35.60	-	
		7	*Xpsmp2074*	-	*Xpsm526*	13.50	72.00	-	
	Bidinger *et al.* (2007)	1	*Xpsm 761*	-	*Xpsm756*	5.30	49.50	1.30	79 $F_{2:4}$ testcross progenies derived from ICMB 841 x 863B
		2	*Xpsmp2059*	-	*Xpsmp250*	6.00	50.80	1.60	
		3	*Xpsm108*	-	*Xpsmp2214*	6.00	25.20	-0.50	
		4	*Xpsm588*	-	*Xpsm713*	5.10	18.40	0.50	

Contd..

Trait	Author	LG	Flanking markers			LOD	% Variation (R^2)	Additive effect	Population details
Plant height (cm)									
	Poncet *et al.* (2000)	1	*Xpsm516*	-	*Xpsm756*	2.68	15.70	4.61	250 F_2 individuals derived from wild x cultivated cross (Molli x Sonu)
		2	*Xpsm176*	-	*Xpsm592*	-	-	-10.44	
		5	*Xpsm345*	-	*Xpsm731*	5.58	29.90	-21.41	
		5	*Xpsm651*	-	*Xpsm735*	6.65	34.70	-24.75	
		6	*Est-E*	-	*al6*	-	-	19.30	
		7	*Xpsm655*			2.73	16.10	-16.42	
		7	*Xpsm812*			2.47	14.20	-16.16	
	Poncet *et al.* (2002)	6	*Xpsm579*			4.16	12.50	14.87	168 F_2 individuals derived from domesticated x wild cross (Thiotande x Wild)
		7	*Xpsm562*	-	*rz404*	2.41	8.20	-19.85	
	Nepolean (2002)	4	*Xpsm512*	-	*Xpsm344*	5.97	21.00	0.71	136 $F_{2:4}$ testcross progenies derived from a cross of PT 732B x P 1449-2
	Azhaguvel *et al.* (2003)	1	*Xpsm515*	-	*Xpsm280*	-	-	-	142 $F_{2:3}$ testcross progenies derived from IP 18293 x Tift $238D_1$
		4	*Xpsm84*	-	*Xpsm413.2*	-	-	-	

Contd..

Trait	Author	LG	Flanking markers			LOD	% Variation (R^2)	Additive effect	Population details
Productive tiller number									
	Poncet *et al.* (2000)	1	*Xpsm461*	-	*Xpsm858*	-	-	-1.27	250 F_2 individuals derived from wild x cultivated cross (Molli x Sonu)
		2	*Xpsm856*	-	*Xpsm176*	2.96	15.20	8.85	
		6	*Est-E*	-	*al6*	4.33	37.00	13.79	
		7	*Xpsm655*			2.33	12.90	5.79	
	Poncet *et al.* (2002)	2	*Xpsm592*	-	*Xpsm662*	2.36	6.30	2.98	168 F_2 individuals derived from domesticated x wild cross (Thiotande x Wild)
		4	*Xpsm409.1*	-	*Xpsms464*	2.42	28.60	-6.76	
	Yadav *et al.* (2002)	2	*Xpsm443*	-	*Xpsm356*	4.26	19.30	-2.15	150 F_2 testcross progenies derived from H 77/833-2 x PRLT 2/89-33
		2	*Xpsm214*	-	*Xpsm25*	2.40	11.50	-1.11	
		2	*Xpsm443*	-	*Xpsm356*	2.58	10.70	-0.93	
		4	*Xpsm716*	-	*Xpsm265*	2.18	16.40	-1.23	
		4	*Xpsm686*	-	*Xpsm525*	2.08	10.00	-0.79	
		6	*Xpsm95*	-	*Xpsm575*	7.57	31.60	-2.92	
		7	*Xpsm618*	-	*Xpsm717*	2.56	13.00	-1.15	
	Nepolean (2002)	7	*Xrm9-2b*	-	*Xpsm618*	2.45	9.20	-0.42	136 $F_{2:4}$ testcross progenies derived from a cross of PT 732B x P 1449-2
	Yadav *et al.* (2003)	1	*Xpsm858*	-	*Xpsm565*	2.65	-	0.68	104 $F_{2:3}$ testcross progenies derived from H 77/833-2 x PRLT 2/89-33
		2	*Xpsm592*	-	*Xpsm443*	8.29	-	-1.87	
		6	*Xpsm95*	-	*Xpsm575*	2.34	-	-1.07	
		7	*Xpsm618*	-	*Xpsm717*	4.04	-	-1.31	

Table 2. Pedigree of parental lines involved in the development of trait-specific genetic populations.

Group	Trait / Cross	Female parent		Male Parent
1	**Panicle length**			
	Cross 1	(ICMB 89111 x IPC 1466)-21-1-3-6-B-5	x	{((SRC II C3 S1-19-3-2xHHVBC)-5-3-1) x (IP19626-4-1-3)}-B-7-1-1
	Cross 2	{(81B x SRL 53-1) x 843B}-30-2-B	x	{ICMV-IS 94206-7 x (SRC II C3 S1-1-1-2 x HHVBC)-1-3-3}-B-10-1-1
2	**Panicle diameter**			
	Cross 1	(96111Bx4017-3-3-B)-4-5-4-1-1-1-1-B-3	x	HHVBC HS-10-1-2-1-1-1-1
	Cross 2	NCD2 BC7F14- 12-1--3-5-5	x	HHVDBC dwarf HS-249-1-2-1-B-3
3	**Grain size**			
	Cross 1	(81Bx4025-3-2-B)-11-5-2-2-B-2	x	HHVBC II D2 HS-302-3-1-6-8-2-6-2-B
	Cross 2	{(ICMB 96555 x IP 10437)-8 x ICMB 97444}-6-4-1-1	x	MC 94 C2 -S1-3-1-3-3-1-2-4-B

Table 3. Crosses subjected for genetic and QTL studies.

Genetic / QTL studies	Group 1		Group 2		Group 3	
	Panicle length		Panicle diameter		Grain size	
Generation means analysis	Cross 1	Cross 2	Cross 1	Cross 2	Cross 1	Cross 2
Triple test cross analysis	Cross 1	-	Cross 1	-	Cross 1	-
QTL analysis	-	-	-	-	Cross 1	-

Table 4. Sequence information of selected polymorphic markers.

S.No.	Marker	Type	Forward primer sequence	Reverse primer sequence	Allele size		Reference
					P_1	P_2	
1	Xpsms2	SSCP-SNP	TGATGATCAATTGATTCATCCG	TATTCAGCTGGACAATGTGCG	1450	1460	Bertin *et al.* (2005)
2	Xpsms6	SSCP-SNP	TGTCCCACTCTCTACAGATTC	TATACACCACTCAACTTACTCA	420	750	Bertin *et al.* (2005)
3	Xpsms17	SSCP-SNP	CCCTTCATGGTGAGGATGAG	GACAGAGAAGCTTATCCTGC	340	338	Bertin *et al.* (2005)
4	Xpsms18	SSCP-SNP	TGTGCCATCATCATTCTTGG	CGAGATAGCATCTATGGTGC	220	218	Bertin *et al.* (2005)
5	Xpsms29	SSCP-SNP	CCCTGCGTCAGCATCTCCTG	GGTGGAGGACATCCTCAAAG	690	680	Bertin *et al.* (2005)
6	Xpsms31	SSCP-SNP	ACGAGACCTTCATCTTCACTG	CTTGACGACTGGGTGAGCTG	390	370	Bertin *et al.* (2005)
7	Xpsms32	SSCP-SNP	TGGTAAGGCCAAGAAGATGG	AAATCCGTCCATGTTCACGC	260	258	Bertin *et al.* (2005)
8	Xpsms39	SSCP-SNP	CCTGAACGATGTCTCAATACC	ATCAATGAGCCAGAGCTTGC	290	310	Bertin *et al.* (2005)
9	Xpsms41	SSCP-SNP	TGAGGAGCATTTGTACAGGC	CCATCGATGAGCTTCAGTTC	210	290	Bertin *et al.* (2005)
10	Xpsms58	SSCP-SNP	GTTTCATGTCTGATCTCGACG	AGACTCTTTCTGCCGTTGCG	360	350	Bertin *et al.* (2005)
11	Xpsms59	SSCP-SNP	CTTTCACGTGTCTGCCAAGC	TCAATCCTCTTGCTCGCAAC	290	300	Bertin *et al.* (2005)
12	Xpsms61	SSCP-SNP	CTGGCTTCACACCTAGAGATG	GGATAGCATTGCGAATGGTG	590	580	Bertin *et al.* (2005)
13	Xpsms68	SSCP-SNP	AGGAGGTGGAGTCGATAAGG	CTTTGCTCCTCTCGTTGTACG	290	288	Bertin *et al.* (2005)
14	Xpsms73	SSCP-SNP	TTCACTTGCAAGCAAGATGG	CTTGTATCCAGAGCTAAGACC	350	340	Bertin *et al.* (2005)
15	Xpsms74	SSCP-SNP	TTCTGACACTGTGCCTTTAGC	AGACCCAGCATGCACTCAAC	1100	1110	Bertin *et al.* (2005)
16	Xpsms75	SSCP-SNP	AAGAGGGCCTTGAACTGTTG	CAGATCTTTCAGGCTGTCTCC	385	380	Bertin *et al.* (2005)
17	Xpsms76	SSCP-SNP	CAACCATGCTACTCTATCTGG	GCAATGTCTGTCATGAACTG	400	398	Bertin *et al.* (2005)
18	Xpsms77	SSCP-SNP	GGATGCTACCTTCTCCTTCAC	AACCTTCTACAGCTTCGCTG	290	310	Bertin *et al.* (2005)
19	Xpsms78	SSCP-SNP	GCGCGATCTTGAACCACTCG	GCCATCTTCCTTGACCGCATC	300	298	Bertin *et al.* (2005)
20	Xpsms80	SSCP-SNP	GTACAAGGAGATCGAGAACG	GACGGAAGGTGTCAACAATG	850	800	Bertin *et al.* (2005)
21	Xpsms86	SSCP-SNP	CGTACAAGGAGATCGAGAAC	AATGTCGACATCAACAGCTC	750	740	Bertin *et al.* (2005)
22	Xpsms88	SSCP-SNP	AATGCACTAGTCCACCGTCC	CCTACACCACACGCTTCCTC	280	282	Bertin *et al.* (2005)

contd..

S.No.	Marker	Type	Forward primer sequence	Reverse primer sequence	Allele size		Reference
					P_1	P_2	
23	Xpsms89	SSCP-SNP	AGGGACACGCGAATACAAGC	CTTGAGAAGGAGAGTTGTCTTC	580	578	Bertin *et al.* (2005)
24	Xpsms92	SSCP-SNP	TGGTGATGCTGCTGCTTTAG	CGACCGAGTACATCTTCTGG	380	385	Bertin *et al.* (2005)
25	Xpsmp2027	Genomic SSR	AGCAATCCGATAACAAGGAC	AGCTTTGGAAAAGGTGATCC	290	250	Qi *et al.* (2004)
26	Xpsmp2064	Genomic SSR	ACCGAATTAAAGTCATGGATCG	TTGATTCTTCTGACACAAATGAG	110	120	Qi *et al.* (2004)
27	Xpsmp2068	Genomic SSR	CAATAACCAAACAAGCAGGCAG	CTTCACTCCCACCCTTTCTAATTC	90	110	Qi *et al.* (2004)
28	Xpsmp2069	Genomic SSR	CCCATCTGAAATCTGGCTGAGAA	CCGTGTTCGTACATGGTTTTGC	210	212	Qi *et al.* (2004)
29	Xpsmp2078	Genomic SSR	CATGCCCATGACAGTATCTTAAT	ACTGTTCGGTTCCAAAATACTT	130	160	Qi *et al.* (2004)
30	Xpsmp2084	Genomic SSR	AATCTAGTGATCTAGTGTGCTTCC	GGTTAGTTTGTTTGAGGCAAATGC	240	250	Qi *et al.* (2004)
31	Xpsmp2203	Genomic SSR	GAACTTGATGAGTGCCACTAGC	TTGTGTAGGGAGCAACCTTGAT	185	190	Qi *et al.* (2004)
32	Xpsmp2222	Genomic SSR	TGGCTTCCAGACTAATCATCAC	TTATTTTAGCGGCGAGATTGAC	150	148	Qi *et al.* (2004)
33	Xpsmp2237	Genomic SSR	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTAGTCCACACCCCA	250	240	Allouis *et al.* (2001)
34	Xpsmp2270	Genomic SSR	AACCAGAGAAGTACATGGCCCG	CGACGAACAAATTAAGGCTCTC	140	142	Allouis *et al.* (2001)
35	Xicmp3017	EST-SSR	CACCAAACAGCATCAAGCAG	AGGTAGCCGAGGAAGGTGAG	190	192	Senthilvel *et al.* (2004)
36	Xicmp3027	EST-SSR	ACACCATCACCGACAACAAA	AGTGACCTGGGGTACAGACG	210	212	Senthilvel *et al.* (2004)
37	Xicmp3063	EST-SSR	TCCGGTAGAGACCGTAATGG	GGCACTCCCTAGCAAAATGA	178	180	Senthilvel (Unpublished)
38	Xicmp3073	EST-SSR	GCACGAGGGCCAGATTCTA	TACACGGTGATGACACGACA	150	160	Senthilvel (Unpublished)
39	Xicmp3081	EST-SSR	ACGCCGTTTTCGTGTAGTCT	TCCACAAGGTGACCTCACTG	200	180	Senthilvel (Unpublished)
40	Xicmp3086	EST-SSR	ACCAAACGTCCAAACCAGAG	ATATCTCTTCGCTGCGGTGT	140	142	Senthilvel (Unpublished)
41	Xpsm37	RFLP-STS	AAAGGTGTCGTTGTTGTGCC	GACTGCTGGTCGGTCACG	498	500	JIC (Unpublished)
42	Xpsm345	RFLP-STS	CTGGGGGAGAGAGAAGGG	AAAAGGCTGGGAGAGTAGGC	195	200	JIC (Unpublished)
43	Xpsm592	RFLP-STS	GCCACAGAAACACTGAAGATG	GGAAGGCATCCAAGAGCC	820	815	JIC (Unpublished)
44	Xpsm669	RFLP-STS	TAATGGGTAGGAAAACCTCGC	GAAAAAGAGGCAGGCAAATG	885	900	JIC (Unpublished)

Table 6. ANOVA for parents and F_1's for sink size traits

Traits / Crosses	Parent / F_1	Season	Mean Squares		F ratio
			Replication	Error	
Panicle length					
Cross 1	P_1	R 06	1.36	0.79	1.71
		S 07	1.55	0.80	1.94
	P_2	R 06	0.69	3.03	0.23
		S 07	6.03	2.63	2.29
	F_1	R 06	2.14	1.85	1.16
		S 07	0.80	2.16	0.37
Cross 2	P_1	R 06	1.12	1.69	0.67
		S 07	1.95	1.97	0.99
	P_2	R 06	1.95	3.26	0.60
		S 07	5.59	3.06	1.83
	F_1	R 06	3.87	2.68	1.44
		S 07	1.12	2.90	0.38
Panicle diameter					
Cross 1	P_1	R 06	2.76	3.31	0.83
		S 07	3.51	2.80	1.25
	P_2	R 06	1.08	3.93	0.27
		S 07	1.30	1.98	0.66
	F_1	R 06	6.21	3.69	1.68
		S 07	1.43	1.44	0.99
Cross 2	P_1	R 06	3.64	1.32	2.75
		S 07	1.29	1.20	1.07
	P_2	R 06	7.67	4.47	1.71
		S 07	2.73	4.41	0.62
	F_1	R 06	4.31	1.89	2.28
		S 07	0.29	3.14	0.09
Grain size					
Cross 1	P_1	R 06	0.07	0.31	0.21
		S 07	0.49	0.42	1.19
	P_2	R 06	0.16	0.51	0.31
		S 07	0.26	0.53	0.49
	F_1	R 06	0.33	0.65	0.52
		S 07	1.03	0.81	1.27
Cross 2	P_1	R 06	0.19	0.38	0.50
		S 07	0.29	0.32	0.91
	P_2	R 06	0.62	0.78	0.79
		S 07	0.95	0.39	2.43
	F_1	R 06	0.00	0.08	0.06
		S 07	0.08	0.11	0.72

R 06 - Rainy 2006 S 07 -Summer 2007

Table 7. Mean performance of parents, F_1, F_2 and backcross generations for panicle length.

Cross / Season		Set 1				Set 2				Set 3	
		N	B_1	B_2	B_3	N	B_{12}	B_{13}	B_{23}	N	B_{123}
Cross 1											
R 06	P_1	20	16.15 ± 0.20	16.29 ± 0.21	16.91 ± 0.22	40	16.22 ± 0.14	16.53 ± 0.16	16.60 ± 0.16	60	16.45 ± 0.13
	P_2	20	67.04 ± 0.38	67.14 ± 0.37	67.40 ± 0.41	40	67.09 ± 0.26	67.22 ± 0.28	67.27 ± 0.27	60	67.19 ± 0.22
	F_1	20	37.04 ± 0.28	36.39 ± 0.32	36.78 ± 0.31	40	36.72 ± 0.22	36.91 ± 0.21	36.59 ± 0.22	60	36.74 ± 0.18
	F_2	350	35.04 ± 0.53	34.98 ± 0.53	35.75 ± 0.54	700	35.01 ± 0.38	35.39 ± 0.38	35.36 ± 0.38	1050	35.26 ± 0.31
	BC_1	100	25.52 ± 0.47	24.35 ± 0.47	26.38 ± 0.49	200	24.93 ± 0.33	25.95 ± 0.34	25.36 ± 0.34	300	25.42 ± 0.28
	BC_2	100	43.59 ± 0.94	41.91 ± 0.97	42.70 ± 0.96	200	42.75 ± 0.68	43.14 ± 0.67	42.30 ± 0.68	300	42.73 ± 0.55
S 07	P_1	20	17.54 ± 0.19	17.77 ± 0.20	17.22 ± 0.21	40	17.65 ± 0.14	17.38 ± 0.14	17.49 ± 0.15	60	17.51 ± 0.12
	P_2	20	67.64 ± 0.39	66.20 ± 0.37	65.51 ± 0.34	40	66.92 ± 0.29	66.57 ± 0.31	65.85 ± 0.25	60	66.45 ± 0.24
	F_1	20	38.88 ± 0.32	39.28 ± 0.30	39.10 ± 0.36	40	39.08 ± 0.22	38.99 ± 0.24	39.19 ± 0.23	60	39.09 ± 0.19
	F_2	350	35.41 ± 0.53	35.18 ± 0.55	36.80 ± 0.53	700	35.30 ± 0.38	36.10 ± 0.38	35.99 ± 0.38	1050	35.80 ± 0.31
	BC_1	100	25.95 ± 0.48	25.93 ± 0.50	26.35 ± 0.48	200	25.94 ± 0.34	26.15 ± 0.34	26.14 ± 0.34	300	26.08 ± 0.28
	BC_2	100	40.54 ± 0.92	43.52 ± 0.95	41.62 ± 0.93	200	42.03 ± 0.67	41.08 ± 0.65	42.57 ± 0.67	300	41.89 ± 0.55
Cross 2											
R 06	P_1	20	16.23 ± 0.26	16.56 ± 0.32	16.10 ± 0.29	40	16.39 ± 0.20	16.16 ± 0.19	16.33 ± 0.22	60	16.29 ± 0.17
	P_2	20	46.82 ± 0.42	46.50 ± 0.43	47.12 ± 0.35	40	46.66 ± 0.30	46.97 ± 0.27	46.81 ± 0.28	60	46.81 ± 0.23
	F_1	20	29.98 ± 0.34	29.71 ± 0.39	29.12 ± 0.37	40	29.84 ± 0.25	29.55 ± 0.26	29.41 ± 0.27	60	29.60 ± 0.21
	F_2	350	28.57 ± 0.38	28.73 ± 0.39	28.71 ± 0.38	700	28.65 ± 0.27	28.64 ± 0.27	28.72 ± 0.27	1050	28.67 ± 0.22
	BC_1	100	22.18 ± 0.50	21.32 ± 0.51	21.50 ± 0.51	200	21.75 ± 0.36	21.84 ± 0.36	21.41 ± 0.36	300	21.67 ± 0.29
	BC_2	100	36.97 ± 0.58	37.52 ± 0.57	37.89 ± 0.58	200	37.24 ± 0.41	37.43 ± 0.41	37.70 ± 0.41	300	37.46 ± 0.33
S 07	P_1	20	17.37 ± 0.31	17.68 ± 0.31	17.05 ± 0.33	40	17.52 ± 0.22	17.21 ± 0.22	17.37 ± 0.23	60	17.37 ± 0.18
	P_2	20	45.09 ± 0.40	44.22 ± 0.38	44.14 ± 0.39	40	44.66 ± 0.28	44.61 ± 0.29	44.18 ± 0.27	60	44.48 ± 0.23
	F_1	20	30.67 ± 0.38	30.58 ± 0.38	30.22 ± 0.38	40	30.62 ± 0.27	30.44 ± 0.27	30.40 ± 0.27	60	30.49 ± 0.22
	F_2	350	28.40 ± 0.40	28.52 ± 0.39	28.53 ± 0.41	700	28.46 ± 0.28	28.47 ± 0.28	28.53 ± 0.28	1050	28.48 ± 0.23
	BC_1	100	22.73 ± 0.51	22.31 ± 0.52	22.26 ± 0.49	200	22.52 ± 0.36	22.50 ± 0.36	22.28 ± 0.36	300	22.43 ± 0.29
	BC_2	100	35.87 ± 0.53	35.98 ± 0.54	35.98 ± 0.54	200	35.92 ± 0.38	35.92 ± 0.38	35.98 ± 0.38	300	35.94 ± 0.31

N – Population size R 06 - Rainy 2006 S 07 - Summer 2007

Table 8. Mean performance of parents, F_1, F_2 and backcross generations for panicle diameter.

Cross / Season		Set 1				Set 2				Set 3	
		N	B_1	B_2	B_3	N	B_{12}	B_{13}	B_{23}	N	B_{123}
Cross 1											
R 06	P_1	20	17.79 ± 0.42	18.36 ± 0.44	17.66 ± 0.35	40	18.07 ± 0.30	17.72 ± 0.27	18.01 ± 0.28	60	17.94 ± 0.23
	P_2	20	49.31 ± 0.46	48.94 ± 0.44	48.88 ± 0.44	40	49.12 ± 0.31	49.09 ± 0.31	48.91 ± 0.31	60	49.04 ± 0.25
	F_1	20	35.10 ± 0.44	35.72 ± 0.44	34.61 ± 0.41	40	35.41 ± 0.31	34.86 ± 0.30	35.17 ± 0.31	60	35.14 ± 0.25
	F_2	350	29.38 ± 0.37	29.66 ± 0.37	30.09 ± 0.38	700	29.52 ± 0.26	29.74 ± 0.26	29.87 ± 0.27	1050	29.71 ± 0.21
	BC_1	100	22.23 ± 0.46	22.61 ± 0.50	23.21 ± 0.53	200	22.42 ± 0.34	22.72 ± 0.35	22.91 ± 0.36	300	22.68 ± 0.29
	BC_2	100	38.79 ± 0.65	38.02 ± 0.65	39.09 ± 0.62	200	38.41 ± 0.46	38.94 ± 0.45	38.55 ± 0.45	300	38.63 ± 0.37
S 07	P_1	20	18.82 ± 0.39	18.05 ± 0.36	18.70 ± 0.36	40	18.43 ± 0.27	18.76 ± 0.26	18.37 ± 0.26	60	18.52 ± 0.22
	P_2	20	48.20 ± 0.36	48.15 ± 0.31	48.62 ± 0.27	40	48.18 ± 0.23	48.41 ± 0.22	48.38 ± 0.21	60	48.32 ± 0.18
	F_1	20	34.74 ± 0.27	35.19 ± 0.24	34.72 ± 0.29	40	34.96 ± 0.18	34.73 ± 0.20	34.95 ± 0.19	60	34.88 ± 0.16
	F_2	350	29.05 ± 0.35	29.42 ± 0.37	27.46 ± 0.37	700	29.24 ± 0.25	28.26 ± 0.26	28.44 ± 0.26	1050	28.65 ± 0.21
	BC_1	100	22.21 ± 0.45	22.72 ± 0.47	23.05 ± 0.49	200	22.46 ± 0.33	22.63 ± 0.33	22.88 ± 0.34	300	22.66 ± 0.27
	BC_2	100	38.39 ± 0.65	37.50 ± 0.64	35.98 ± 0.63	200	37.95 ± 0.46	37.19 ± 0.46	36.73 ± 0.45	300	37.29 ± 0.37
Cross 2											
R 06	P_1	20	13.99 ± 0.28	14.04 ± 0.27	15.17 ± 0.26	40	14.01 ± 0.19	14.58 ± 0.21	14.60 ± 0.21	60	14.40 ± 0.17
	P_2	20	40.70 ± 0.49	41.47 ± 0.45	41.93 ± 0.47	40	41.09 ± 0.34	41.31 ± 0.35	41.70 ± 0.32	60	41.37 ± 0.28
	F_1	20	27.47 ± 0.36	26.04 ± 0.29	26.52 ± 0.35	40	26.75 ± 0.25	26.99 ± 0.26	26.28 ± 0.23	60	26.67 ± 0.21
	F_2	350	23.61 ± 0.29	24.02 ± 0.30	25.27 ± 0.28	700	23.81 ± 0.21	24.44 ± 0.20	24.65 ± 0.21	1050	24.30 ± 0.17
	BC_1	100	18.81 ± 0.33	18.58 ± 0.30	19.42 ± 0.30	200	18.69 ± 0.22	19.11 ± 0.22	19.00 ± 0.21	300	18.93 ± 0.18
	BC_2	100	32.55 ± 0.48	31.28 ± 0.50	32.87 ± 0.47	200	31.91 ± 0.35	32.71 ± 0.33	32.08 ± 0.34	300	32.23 ± 0.28
S 07	P_1	20	15.58 ± 0.25	15.41 ± 0.28	15.91 ± 0.19	40	15.49 ± 0.19	15.74 ± 0.16	15.66 ± 0.17	60	15.63 ± 0.14
	P_2	20	40.47 ± 0.49	39.96 ± 0.49	39.75 ± 0.43	40	40.21 ± 0.34	40.11 ± 0.33	39.85 ± 0.32	60	40.06 ± 0.27
	F_1	20	27.23 ± 0.39	27.28 ± 0.40	27.46 ± 0.40	40	27.25 ± 0.28	27.34 ± 0.28	27.37 ± 0.28	60	27.32 ± 0.23
	F_2	350	24.93 ± 0.29	23.97 ± 0.30	24.64 ± 0.30	700	24.45 ± 0.21	24.78 ± 0.21	24.31 ± 0.21	1050	24.51 ± 0.17
	BC_1	100	20.00 ± 0.26	18.24 ± 0.30	19.01 ± 0.28	200	19.12 ± 0.21	19.51 ± 0.19	18.63 ± 0.21	300	19.08 ± 0.17
	BC_2	100	32.09 ± 0.51	32.34 ± 0.49	33.20 ± 0.46	200	32.22 ± 0.35	32.64 ± 0.34	32.77 ± 0.34	300	32.54 ± 0.28

N – Population size R 06 - Rainy 2006 S 07 - Summer 2007

Table 9. Mean performance of parents, F_1, F_2 and backcross generations for grain size.

Cross / Season		Set 1				Set 2				Set 3	
		N	B_1	B_2	B_3	N	B_{12}	B_{13}	B_{23}	N	B_{123}
Cross 1											
R 06	P_1	20	4.45 ± 0.12	4.34 ± 0.12	4.42 ± 0.14	40	4.40 ± 0.08	4.44 ± 0.09	4.38 ± 0.09	60	4.40 ± 0.07
	P_2	20	13.06 ± 0.12	13.23 ± 0.17	13.13 ± 0.18	40	13.15 ± 0.11	13.09 ± 0.11	13.18 ± 0.12	60	13.14 ± 0.09
	F_1	20	8.42 ± 0.15	8.17 ± 0.21	8.37 ± 0.18	40	8.30 ± 0.13	8.40 ± 0.12	8.27 ± 0.14	60	8.32 ± 0.10
	F_2	350	7.97 ± 0.14	7.87 ± 0.14	7.84 ± 0.14	700	7.92 ± 0.10	7.90 ± 0.10	7.85 ± 0.10	1050	7.89 ± 0.08
	BC_1	100	6.33 ± 0.15	6.24 ± 0.16	6.23 ± 0.16	200	6.28 ± 0.11	6.28 ± 0.11	6.24 ± 0.11	300	6.27 ± 0.09
	BC_2	100	8.95 ± 0.12	8.98 ± 0.12	9.02 ± 0.12	200	8.97 ± 0.08	8.98 ± 0.08	9.00 ± 0.08	300	8.98 ± 0.07
S 07	P_1	20	5.03 ± 0.14	4.88 ± 0.17	5.19 ± 0.13	40	4.95 ± 0.11	5.11 ± 0.09	5.04 ± 0.11	60	5.03 ± 0.08
	P_2	20	13.52 ± 0.13	13.40 ± 0.14	13.63 ± 0.21	40	13.46 ± 0.10	13.58 ± 0.12	13.52 ± 0.13	60	13.52 ± 0.09
	F_1	20	8.49 ± 0.11	8.74 ± 0.23	8.94 ± 0.24	40	8.62 ± 0.13	8.72 ± 0.14	8.84 ± 0.16	60	8.73 ± 0.12
	F_2	350	8.53 ± 0.13	8.63 ± 0.12	8.69 ± 0.12	700	8.58 ± 0.09	8.61 ± 0.09	8.66 ± 0.09	1050	8.62 ± 0.07
	BC_1	100	6.96 ± 0.16	6.53 ± 0.18	6.98 ± 0.15	200	6.75 ± 0.12	6.97 ± 0.11	6.76 ± 0.12	300	6.82 ± 0.09
	BC_2	100	9.91 ± 0.17	9.70 ± 0.18	9.89 ± 0.16	200	9.81 ± 0.12	9.90 ± 0.12	9.79 ± 0.12	300	9.83 ± 0.10
Cross 2											
R 06	P_1	20	5.31 ± 0.12	5.24 ± 0.15	5.12 ± 0.14	40	5.28 ± 0.10	5.22 ± 0.09	5.18 ± 0.10	60	5.22 ± 0.08
	P_2	20	13.95 ± 0.19	13.79 ± 0.20	13.60 ± 0.20	40	13.87 ± 0.14	13.78 ± 0.14	13.70 ± 0.14	60	13.78 ± 0.11
	F_1	20	8.59 ± 0.06	8.60 ± 0.07	8.57 ± 0.06	40	8.59 ± 0.05	8.58 ± 0.04	8.59 ± 0.04	60	8.59 ± 0.04
	F_2	350	8.39 ± 0.13	8.51 ± 0.13	8.47 ± 0.12	700	8.45 ± 0.09	8.43 ± 0.09	8.49 ± 0.09	1050	8.46 ± 0.07
	BC_1	100	6.99 ± 0.16	7.09 ± 0.15	6.96 ± 0.16	200	7.04 ± 0.11	6.98 ± 0.11	7.02 ± 0.11	300	7.01 ± 0.09
	BC_2	100	9.78 ± 0.18	9.47 ± 0.17	9.61 ± 0.19	200	9.63 ± 0.13	9.70 ± 0.13	9.54 ± 0.13	300	9.62 ± 0.10
S 07	P_1	20	5.74 ± 0.11	5.93 ± 0.12	5.96 ± 0.14	40	5.83 ± 0.08	5.85 ± 0.09	5.94 ± 0.09	60	5.88 ± 0.07
	P_2	20	14.85 ± 0.16	15.14 ± 0.20	14.56 ± 0.14	40	15.00 ± 0.13	14.71 ± 0.11	14.85 ± 0.13	60	14.85 ± 0.10
	F_1	20	9.51 ± 0.08	9.78 ± 0.05	9.73 ± 0.06	40	9.65 ± 0.05	9.62 ± 0.05	9.76 ± 0.04	60	9.68 ± 0.04
	F_2	350	9.33 ± 0.15	9.52 ± 0.12	9.51 ± 0.14	700	9.42 ± 0.10	9.42 ± 0.10	9.52 ± 0.09	1050	9.45 ± 0.08
	BC_1	100	7.44 ± 0.14	7.25 ± 0.14	7.44 ± 0.15	200	7.34 ± 0.10	7.44 ± 0.10	7.35 ± 0.10	300	7.38 ± 0.08
	BC_2	100	11.14 ± 0.19	11.18 ± 0.22	11.42 ± 0.19	200	11.16 ± 0.14	11.28 ± 0.13	11.30 ± 0.15	300	11.25 ± 0.12

N – Population size R 06 - Rainy 2006 S 07 - Summer 2007

Table 10. Scaling and joint scaling test for panicle length in cross 1.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Scaling test							
Rainy 2006							
A	-2.14 * ± 1.01	-3.98 ** ± 1.01	-0.92 ± 1.04	-3.06 ** ± 0.72	-1.53 * ± 0.73	-2.45 ** ± 0.74	-2.35 ** ± 0.60
B	-16.91 ** ± 1.95	-19.71 ** ± 2.00	-18.78 ** ± 2.00	-18.31 ** ± 1.40	-17.85 ** ± 1.39	-19.25 ** ± 1.41	-18.47 ** ± 1.14
C	-17.11 ** ± 2.25	-16.30 ** ± 2.25	-14.86 ** ± 2.28	-16.70 ** ± 1.59	-15.99 ** ± 1.60	-15.58 ** ± 1.60	-16.09 ** ± 1.30
Summer 2007							
A	-4.52 ** ± 1.02	-5.19 ** ± 1.05	-3.60 ** ± 1.04	-4.85 ** ± 0.73	-4.06 ** ± 0.73	-4.40 ** ± 0.74	-4.44 ** ± 0.60
B	-25.44 ** ± 1.91	-18.44 ** ± 1.96	-21.36 ** ± 1.93	-21.94 ** ± 1.39	-23.40 ** ± 1.37	-19.90 ** ± 1.38	-21.74 ** ± 1.13
C	-21.30 ** ± 2.27	-21.80 ** ± 2.32	-13.72 ** ± 2.27	-21.55 ** ± 1.62	-17.51 ** ± 1.62	-17.76 ** ± 1.63	-18.94 ** ± 1.33
Joint scaling test							
Rainy 2006							
m	40.98 ± 0.21	41.08 ± 0.21	41.50 ± 0.22	41.04 ± 0.15	41.23 ± 0.15	41.29 ± 0.15	41.19 ± 0.12
(d)	-25.00 ** ± 0.21	-25.03 ** ± 0.21	-24.71 ** ± 0.23	-25.03 ** ± 0.15	-24.87 ** ± 0.16	-24.88 ** ± 0.15	-24.93 ** ± 0.12
(h)	-4.85 ** ± 0.35	-6.01 ** ± 0.37	-5.63 ** ± 0.38	-5.47 ** ± 0.26	-5.23 ** ± 0.26	-5.80 ** ± 0.27	-5.50 ** ± 0.21
χ^2 value	125.92 **	147.47 **	122.05 **	270.73 **	247.10 **	265.54 **	389.38 **
Summer 2007							
m	41.69 ± 0.21	41.30 ± 0.20	40.84 ± 0.19	41.42 ± 0.15	41.05 ± 0.16	41.05 ± 0.14	41.15 ± 0.13
(d)	-24.40 ** ± 0.21	-23.82 ** ± 0.20	-23.80 ** ± 0.19	-24.03 ** ± 0.16	-23.89 ** ± 0.16	-23.81 ** ± 0.14	-23.89 ** ± 0.13
(h)	-4.50 ** ± 0.38	-3.39 ** ± 0.36	-3.30 ** ± 0.39	-3.84 ** ± 0.26	-3.66 ** ± 0.28	-3.33 ** ± 0.27	-3.57 ** ± 0.22
χ^2 value	254.30 **	179.36 **	15.49 **	417.81 **	391.93 **	322.55 **	557.11 **

*, ** significance at 5% and 1% level, respectively χ^2 - Chi-square value

Table 11. Scaling and joint scaling test for panicle length in cross 2.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Scaling test							
Rainy 2006							
A	-1.84 ± 1.08	-3.61 ** ± 1.13	-2.21 * ± 1.12	-2.73 ** ± 0.78	-2.02 ** ± 0.78	-2.91 ** ± 0.80	-2.55 ** ± 0.64
B	-2.86 * ± 1.28	-1.16 ± 1.28	-0.46 ± 1.26	-2.01 ** ± 0.90	-1.66 ± 0.90	-0.81 ± 0.90	-1.49 * ± 0.73
C	-8.70 ** ± 1.72	-7.55 ** ± 1.81	-6.59 ** ± 1.74	-8.13 ** ± 1.25	-7.65 ** ± 1.23	-7.07 ** ± 1.26	-7.62 ** ± 1.02
Summer 2007							
A	-2.56 * ± 1.14	-3.64 ** ± 1.15	-2.76 * ± 1.11	-3.10 ** ± 0.81	-2.66 ** ± 0.79	-3.20 ** ± 0.80	-2.99 ** ± 0.65
B	-4.02 ** ± 1.20	-2.84 * ± 1.21	-2.40 ± 1.22	-3.43 ** ± 0.85	-3.21 ** ± 0.85	-2.62 ** ± 0.86	-3.09 ** ± 0.70
C	-10.17 ** ± 1.83	-8.97 ** ± 1.80	-7.52 ** ± 1.87	-9.57 ** ± 1.28	-8.84 ** ± 1.31	-8.24 ** ± 1.30	-8.89 ** ± 1.06
Joint scaling test							
Rainy 2006							
m	31.07 ± 0.23	31.12 ± 0.25	31.36 ± 0.21	31.10 ± 0.17	31.24 ± 0.16	31.25 ± 0.17	31.20 ± 0.13
(d)	-15.08 ** ± 0.23	-15.00 ** ± 0.25	-15.54 ** ± 0.22	-15.04 ** ± 0.17	-15.33 ** ± 0.16	-15.27 ** ± 0.17	-15.21 ** ± 0.14
(h)	-1.92 ** ± 0.41	-2.34 ** ± 0.46	-2.95 ** ± 0.42	-2.13 ** ± 0.30	-2.50 ** ± 0.30	-2.65 ** ± 0.31	-2.44 ** ± 0.25
χ^2 value	28.05 **	23.21 **	16.12 **	48.84 **	41.42 **	38.62 **	63.47 **
Summer 2007							
m	30.68 ± 0.24	30.50 ± 0.23	30.20 ± 0.24	30.58 ± 0.17	30.43 ± 0.17	30.35 ± 0.16	30.45 ± 0.14
(d)	-13.66 ** ± 0.24	-13.23 ** ± 0.23	-13.51 ** ± 0.24	-13.44 ** ± 0.17	-13.57 ** ± 0.17	-13.38 ** ± 0.17	-13.47 ** ± 0.14
(h)	-1.19 ** ± 0.44	-1.06 * ± 0.44	-0.88 * ± 0.44	-1.10 ** ± 0.31	-1.02 ** ± 0.31	-0.96 ** ± 0.31	-1.02 ** ± 0.25
χ^2 value	37.21 **	31.40 **	20.44 **	67.90 **	55.54 **	51.43 **	86.94 **

*, ** significance at 5% and 1% level, respectively χ^2 - Chi-square value

Table 12. Scaling and joint scaling test for panicle diameter in cross 1.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Scaling test							
Rainy 2006							
A	-8.42 ** ± 1.10	-8.86 ** ± 1.17	-5.85 ** ± 1.18	-8.64 ** ± 0.80	-7.14 ** ± 0.81	-7.36 ** ± 0.84	-7.71 ** ± 0.67
B	-6.82 ** ± 1.45	-8.61 ** ± 1.44	-5.32 ** ± 1.37	-7.72 ** ± 1.02	-6.07 ** ± 1.00	-6.97 ** ± 1.00	-6.92 ** ± 0.82
C	-19.77 ** ± 1.82	-20.12 ** ± 1.83	-15.39 ** ± 1.82	-19.95 ** ± 1.29	-17.58 ** ± 1.28	-17.75 ** ± 1.30	-18.43 ** ± 1.05
Summer 2007							
A	-9.14 ** ± 1.02	-7.80 ** ± 1.04	-7.31 ** ± 1.08	-8.47 ** ± 0.73	-8.23 ** ± 0.74	-7.56 ** ± 0.75	-8.09 ** ± 0.61
B	-6.15 ** ± 1.37	-8.35 ** ± 1.34	-11.37 ** ± 1.32	-7.25 ** ± 0.96	-8.76 ** ± 0.96	-9.87 ** ± 0.94	-8.63 ** ± 0.78
C	-20.28 ** ± 1.60	-18.88 ** ± 1.61	-26.92 ** ± 1.65	-19.58 ** ± 1.14	-23.59 ** ± 1.15	-22.89 ** ± 1.17	-22.02 ** ± 0.94
Joint scaling test							
Rainy 2006							
m	32.05 ± 0.28	32.07 ± 0.28	32.33 ± 0.26	32.07 ± 0.20	32.23 ± 0.19	32.25 ± 0.19	32.19 ± 0.16
(d)	-15.93 ** ± 0.29	-15.46 ** ± 0.29	-15.50 ** ± 0.26	-15.70 ** ± 0.20	-15.69 ** ± 0.19	-15.48 ** ± 0.20	-15.62 ** ± 0.16
(h)	-0.02 ± 0.52	0.53 ± 0.52	0.24 ± 0.48	0.24 ± 0.37	0.11 ± 0.35	0.24 ± 0.36	0.18 ± 0.29
χ^2 value	144.93 **	155.69 **	85.77 **	300.18 **	227.54 **	232.36 **	377.44 **
Summer 2007							
m	31.89 ± 0.24	31.82 ± 0.22	32.31 ± 0.21	31.85 ± 0.16	32.10 ± 0.16	32.05 ± 0.15	32.00 ± 0.13
(d)	-15.18 ** ± 0.25	-15.36 ** ± 0.23	-15.26 ** ± 0.22	-15.30 ** ± 0.17	-15.17 ** ± 0.16	-15.31 ** ± 0.16	-15.25 ** ± 0.13
(h)	1.22 ** ± 0.38	2.10 ** ± 0.34	0.10 ± 0.37	1.65 ** ± 0.25	0.73 ** ± 0.26	1.14 ** ± 0.25	1.18 ** ± 0.21
χ^2 value	213.69 **	196.37 **	325.24 **	405.00 **	519.99 **	502.62 **	706.48 **

*, ** significance at 5% and 1% level, respectively χ^2 - Chi-square value

Table 13. Scaling and joint scaling test for panicle diameter in cross 2.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Scaling test							
Rainy 2006							
A	-3.84 ** ± 0.81	-2.92 ** ± 0.72	-2.85 ** ± 0.74	-3.38 ** ± 0.55	-3.35 ** ± 0.56	-2.88 ** ± 0.53	-3.20 ** ± 0.45
B	-3.07 ** ± 1.13	-4.96 ** ± 1.13	-2.69 ** ± 1.10	-4.02 ** ± 0.81	-2.88 ** ± 0.80	-3.83 ** ± 0.79	-3.58 ** ± 0.66
C	-15.19 ** ± 1.48	-11.51 ** ± 1.45	-9.03 ** ± 1.44	-13.35 ** ± 1.06	-12.11 ** ± 1.05	-10.27 ** ± 1.03	-11.91 ** ± 0.86
Summer 2007							
A	-2.80 ** ± 0.70	-6.20 ** ± 0.78	-5.34 ** ± 0.71	-4.50 ** ± 0.53	-4.07 ** ± 0.50	-5.77 ** ± 0.53	-4.78 ** ± 0.43
B	-3.52 ** ± 1.19	-2.55 * ± 1.17	-0.82 ± 1.09	-3.04 ** ± 0.83	-2.17 ** ± 0.81	-1.69 ** ± 0.80	-2.30 ** ± 0.66
C	-10.80 ** ± 1.51	-14.03 ** ± 1.54	-12.00 ** ± 1.50	-12.42 ** ± 1.08	-11.40 ** ± 1.06	-13.02 ** ± 1.07	-12.28 ** ± 0.87
Joint scaling test							
Rainy 2006							
m	26.22 ± 0.25	26.74 ± 0.23	27.85 ± 0.24	26.54 ± 0.17	26.99 ± 0.18	27.26 ± 0.17	26.93 ± 0.14
(d)	-12.97 ** ± 0.25	-13.28 ** ± 0.24	-13.14 ** ± 0.24	-13.12 ** ± 0.17	-13.09 ** ± 0.18	-13.26 ** ± 0.17	-13.16 ** ± 0.14
(h)	-0.74 ± 0.44	-1.93 ** ± 0.39	-2.70 ** ± 0.42	-1.61 ** ± 0.31	-1.69 ** ± 0.32	-2.29 ** ± 0.29	-1.88 ** ± 0.25
χ^2 value	109.59 **	74.74 **	43.89 **	167.92 **	139.47 **	112.72 **	204.38 **
Summer 2007							
m	27.24 ± 0.25	26.73 ± 0.25	27.39 ± 0.21	26.99 ± 0.17	27.29 ± 0.16	27.06 ± 0.16	27.11 ± 0.14
(d)	-12.08 ** ± 0.24	-12.27 ** ± 0.25	-11.96 ** ± 0.21	-12.16 ** ± 0.17	-12.04 ** ± 0.16	-12.13 ** ± 0.16	-12.11 ** ± 0.14
(h)	-1.73 ** ± 0.44	-2.03 ** ± 0.46	-2.32 ** ± 0.41	-1.85 ** ± 0.32	-1.98 ** ± 0.30	-2.13 ** ± 0.31	-1.97 ** ± 0.25
χ^2 value	54.08 **	111.85 **	95.66 **	157.59 **	138.94 **	206.81 **	248.35 **

*, ** significance at 5% and 1% level, respectively χ^2 - Chi-square value

Table 14. Scaling and joint scaling test for grain size in cross 1.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Scaling test							
Rainy 2006							
A	-0.21 ± 0.36	-0.04 ± 0.40	-0.33 ± 0.40	-0.12 ± 0.27	-0.27 ± 0.27	-0.18 ± 0.28	-0.19 ± 0.22
B	-3.58 ** ± 0.31	-3.44 ** ± 0.36	-3.46 ** ± 0.35	-3.51 ** ± 0.23	-3.52 ** ± 0.23	-3.45 ** ± 0.25	-3.49 ** ± 0.19
C	-2.48 ** ± 0.64	-2.46 ** ± 0.71	-2.93 ** ± 0.69	-2.47 ** ± 0.48	-2.70 ** ± 0.47	-2.69 ** ± 0.50	-2.62 ** ± 0.39
Summer 2007							
A	0.40 ± 0.36	-0.55 ± 0.46	-0.18 ± 0.40	-0.08 ± 0.29	0.11 ± 0.27	-0.37 ± 0.31	-0.11 ± 0.24
B	-2.19 ** ± 0.39	-2.75 ** ± 0.44	-2.79 ** ± 0.45	-2.47 ** ± 0.29	-2.49 ** ± 0.30	-2.77 ** ± 0.31	-2.58 ** ± 0.25
C	-1.40 * ± 0.59	-1.26 ± 0.70	-1.94 ** ± 0.72	-1.33 ** ± 0.46	-1.67 ** ± 0.47	-1.60 ** ± 0.50	-1.53 ** ± 0.39
Joint scaling test							
Rainy 2006							
m	8.48 ± 0.08	8.40 ± 0.09	8.34 ± 0.10	8.43 ± 0.06	8.40 ± 0.06	8.38 ± 0.07	8.41 ± 0.05
(d)	-3.97 ** ± 0.08	-3.95 ** ± 0.09	-3.85 ** ± 0.10	-3.95 ** ± 0.06	-3.90 ** ± 0.06	-3.90 ** ± 0.07	-3.92 ** ± 0.05
(h)	-0.84 ** ± 0.16	-1.14 ** ± 0.20	-0.85 ** ± 0.20	-1.01 ** ± 0.13	-0.84 ** ± 0.13	-1.01 ** ± 0.14	-0.96 ** ± 0.11
χ^2 value	140.04 **	102.55 **	102.71 **	237.17 **	241.37 **	206.07 **	340.66 **
Summer 2007							
m	9.15 ± 0.09	9.02 ± 0.10	9.11 ± 0.11	9.09 ± 0.07	9.14 ± 0.07	9.08 ± 0.08	9.11 ± 0.06
(d)	-4.08 ** ± 0.09	-4.11 ** ± 0.10	-3.85 ** ± 0.10	-4.10 ** ± 0.07	-3.96 ** ± 0.07	-3.98 ** ± 0.07	-4.02 ** ± 0.06
(h)	-0.84 ** ± 0.14	-0.92 ** ± 0.22	-0.82 ** ± 0.23	-0.90 ** ± 0.13	-0.87 ** ± 0.14	-0.89 ** ± 0.16	-0.90 ** ± 0.12
χ^2 value	37.63 **	38.91 **	42.11 **	72.96 **	78.44 **	80.09 **	114.16 **

*, ** significance at 5% and 1% level, respectively χ^2 - Chi-square value

Table 15. Scaling and joint scaling test for grain size in cross 2.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Scaling test							
Rainy 2006							
A	0.09 ± 0.35	0.33 ± 0.35	0.23 ± 0.35	0.21 ± 0.25	0.16 ± 0.25	0.28 ± 0.25	0.22 ± 0.20
B	-2.98 ** ± 0.42	-3.45 ** ± 0.41	-2.95 ** ± 0.43	-3.21 ** ± 0.29	-2.96 ** ± 0.30	-3.20 ** ± 0.29	-3.12 ** ± 0.24
C	-2.87 ** ± 0.59	-2.21 ** ± 0.58	-1.99 ** ± 0.54	-2.54 ** ± 0.41	-2.43 ** ± 0.40	-2.10 ** ± 0.40	-2.36 ** ± 0.33
Summer 2007							
A	-0.38 ± 0.32	-1.21 ** ± 0.32	-0.81 * ± 0.33	-0.79 ** ± 0.23	-0.60 ** ± 0.23	-1.01 ** ± 0.23	-0.80 ** ± 0.19
B	-2.08 ** ± 0.41	-2.55 ** ± 0.48	-1.45 * ± 0.42	-2.32 ** ± 0.32	-1.77 ** ± 0.29	-2.00 ** ± 0.32	-2.03 ** ± 0.25
C	-2.31 ** ± 0.64	-2.55 ** ± 0.56	-1.94 ** ± 0.60	-2.43 ** ± 0.43	-2.13 * ± 0.44	-2.25 ** ± 0.41	-2.27 ** ± 0.35
Joint scaling test							
Rainy 2006							
m	9.20 ± 0.10	9.06 ± 0.10	8.94 ± 0.10	9.13 ± 0.07	9.06 ± 0.07	9.00 ± 0.07	9.07 ± 0.06
(d)	-3.95 ** ± 0.10	-3.81 ** ± 0.11	-3.84 ** ± 0.11	-3.89 ** ± 0.07	-3.89 ** ± 0.07	-3.83 ** ± 0.08	-3.87 ** ± 0.06
(h)	-0.72 ** ± 0.12	-0.58 ** ± 0.13	-0.44 ** ± 0.12	-0.65 ** ± 0.09	-0.57 ** ± 0.09	-0.51 ** ± 0.09	-0.57 ** ± 0.07
χ^2 value	64.31 **	79.03 **	54.20 **	141.38 **	117.64 **	132.11 **	194.60 **
Summer 2007							
m	10.05 ± 0.09	10.11 ± 0.10	10.04 ± 0.09	10.09 ± 0.07	10.05 ± 0.06	10.06 ± 0.07	10.07 ± 0.05
(d)	-4.39 ** ± 0.09	-4.44 ** ± 0.10	-4.28 ** ± 0.09	-4.41 ** ± 0.07	-4.34 ** ± 0.07	-4.34 ** ± 0.07	-4.36 ** ± 0.06
(h)	-0.66 ** ± 0.12	-0.41 ** ± 0.12	-0.40 ** ± 0.11	-0.57 ** ± 0.09	-0.54 ** ± 0.09	-0.39 ** ± 0.08	-0.51 ** ± 0.07
χ^2 value	34.56 **	52.98 **	24.07 **	81.968 **	55.99 **	73.618 **	103.91 **

*, ** significance at 5% and 1% level, respectively χ^2 - Chi-square value

Table 16. Estimates of the genetic components using six-parameter model for panicle length in cross 1.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Rainy 2006							
m	35.04 ± 0.53	34.98 ± 0.53	35.75 ± 0.54	35.01 ± 0.38	35.39 ± 0.38	35.36 ± 0.38	35.26 ± 0.31
(d)	-18.06 ** ± 1.06	-17.57 ** ± 1.07	-16.32 ** ± 1.08	-17.82 ** ± 0.75	-17.19 ** ± 0.75	-16.94 ** ± 0.76	-17.32 ** ± 0.62
(h)	-6.49 * ± 3.03	-12.72 ** ± 3.04	-10.22 ** ± 3.07	-9.60 ** ± 2.15	-8.35 ** ± 2.15	-11.47 ** ± 2.16	-9.81 ** ± 1.76
(i)	-1.93 ± 3.01	-7.40 * ± 3.01	-4.84 ± 3.04	-4.67 * ± 2.13	-3.39 ± 2.14	-6.12 ** ± 2.14	-4.72 ** ± 1.75
(j)	7.38 ** ± 1.08	7.86 ** ± 1.10	8.93 ** ± 1.11	7.62 ** ± 0.77	8.16 ** ± 0.77	8.40 ** ± 0.78	8.06 ** ± 0.63
(l)	20.98 ** ± 4.79	31.09 ** ± 4.85	24.55 ** ± 4.89	26.04 ** ± 3.41	22.76 ** ± 3.42	27.82 ** ± 3.45	25.54 ** ± 2.80
$(h/d)^{1/2}$	0.60	0.85	0.79	0.73	0.70	0.82	0.75
Summer 2007							
m	35.41 ± 0.53	35.18 ± 0.55	36.80 ± 0.53	35.30 ± 0.38	36.10 ± 0.38	35.99 ± 0.38	35.80 ± 0.31
(d)	-14.59 ** ± 1.04	-17.59 ** ± 1.07	-15.27 ** ± 1.05	-16.09 ** ± 0.75	-14.93 ** ± 0.74	-16.43 ** ± 0.75	-15.82 ** ± 0.61
(h)	-12.37 ** ± 3.00	-4.53 ** ± 3.10	-13.50 ** ± 3.00	-8.45 ** ± 2.16	-12.94 ** ± 2.13	-9.02 ** ± 2.16	-10.13 ** ± 1.76
(i)	-8.66 ** ± 2.98	-1.83 ± 3.08	-11.24 ** ± 2.97	-5.25 * ± 2.15	-9.95 ** ± 2.11	-6.53 ** ± 2.14	-7.24 ** ± 1.75
(j)	10.46 ** ± 1.06	6.63 ** ± 1.09	8.88 ** ± 1.06	8.54 ** ± 0.77	9.67 ** ± 0.76	7.75 ** ± 0.77	8.65 ** ± 0.63
(l)	38.62 ** ± 4.73	25.45 ** ± 4.88	36.20 ** ± 4.76	32.04 ** ± 3.42	37.41 ** ± 3.36	30.83 ** ± 3.42	33.42 ** ± 2.79
$(h/d)^{1/2}$	0.92	0.51	0.94	0.72	0.93	0.74	0.80

*, ** significance at 5% and 1% level, respectively

Table 17. Estimates of the genetic components using six-parameter model for panicle length in cross 2.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Rainy 2006							
m	28.57 ± 0.38	28.73 ± 0.39	28.71 ± 0.38	28.65 ± 0.27	28.64 ± 0.27	28.72 ± 0.27	28.67 ± 0.22
(d)	-14.79 ** ± 0.76	-16.20 ** ± 0.77	-16.39 ** ± 0.77	-15.49 ** ± 0.54	-15.59 ** ± 0.54	-16.29 ** ± 0.54	-15.79 ** ± 0.44
(h)	2.46 ± 2.19	0.95 ± 2.23	1.44 ± 2.20	1.70 ± 1.56	1.95 ± 1.55	1.19 ± 1.56	1.62 ± 1.27
(i)	4.01 ± 2.14	2.77 ± 2.18	3.93 ± 2.15	3.39 * ± 1.53	3.97 ** ± 1.52	3.35 * ± 1.53	3.57 ** ± 1.24
(j)	0.51 ± 0.80	-1.23 ± 0.81	-0.88 ± 0.80	-0.36 ± 0.57	-0.18 ± 0.57	-1.05 ± 0.57	-0.53 ± 0.46
(l)	0.69 ± 3.51	2.01 ± 3.56	-1.27 ± 3.54	1.35 ± 2.50	-0.29 ± 2.49	0.37 ± 2.50	0.48 ± 2.03
$(h/d)^{1/2}$	0.41	0.24	0.30	0.33	0.35	0.27	0.32
Summer 2007							
m	28.40 ± 0.40	28.52 ± 0.39	28.53 ± 0.41	28.46 ± 0.28	28.47 ± 0.28	28.53 ± 0.28	28.48 ± 0.23
(d)	-13.13 ** ± 0.74	-13.67 ** ± 0.75	-13.72 ** ± 0.73	-13.40 ** ± 0.53	-13.43 ** ± 0.52	-13.70 ** ± 0.52	-13.51 ** ± 0.43
(h)	3.03 ± 2.22	2.12 ± 2.21	1.98 ± 2.24	2.57 ± 1.56	2.50 ± 1.58	2.05 ± 1.57	2.37 ± 1.28
(i)	3.59 ± 2.17	2.49 ± 2.17	2.35 ± 2.19	3.04 * ± 1.53	2.97 ± 1.54	2.42 ± 1.54	2.81 * ± 1.25
(j)	0.73 ± 0.78	-0.40 ± 0.79	-0.18 ± 0.78	0.17 ± 0.56	0.28 ± 0.55	-0.29 ± 0.55	0.05 ± 0.45
(l)	3.00 ± 3.48	4.00 ± 3.51	2.81 ± 3.48	3.50 ± 2.47	2.90 ± 2.46	3.40 ± 2.47	3.27 ± 2.01
$(h/d)^{1/2}$	0.48	0.39	0.38	0.44	0.43	0.39	0.42

*, ** significance at 5% and 1% level, respectively

Table 18. Estimates of the genetic components using six-parameter model for panicle diameter in cross 1.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Rainy 2006							
m	29.38 ± 0.37	29.66 ± 0.37	30.09 ± 0.38	29.52 ± 0.26	29.74 ± 0.26	29.87 ± 0.27	29.71 ± 0.21
(d)	-16.56 ** ± 0.80	-15.41 ** ± 0.82	-15.88 ** ± 0.81	-15.99 ** ± 0.57	-16.22 ** ± 0.57	-15.65 ** ± 0.58	-15.95 ** ± 0.47
(h)	6.07 ** ± 2.23	4.72 * ± 2.27	5.56 * ± 2.28	5.40 ** ± 1.59	5.82 ** ± 1.60	5.14 ** ± 1.61	5.45 ** ± 1.31
(i)	4.53 ** ± 2.17	2.64 ± 2.20	4.21 ± 2.23	3.58 * ± 1.54	4.37 ** ± 1.55	3.43 * ± 1.57	3.79 ** ± 1.27
(j)	-0.80 ± 0.86	-0.12 ± 0.87	-0.27 ± 0.86	-0.46 ± 0.61	-0.53 ± 0.61	-0.20 ± 0.61	-0.40 ± 0.50
(l)	10.72 ** ± 3.68	14.84 ** ± 3.75	6.96 ± 3.72	12.78 ** ± 2.62	8.84 ** ± 2.61	10.90 ** ± 2.64	10.84 ** ± 2.15
$(h/d)^{1/2}$	0.61	0.55	0.59	0.58	0.60	0.57	0.58
Summer 2007							
m	29.05 ± 0.35	29.42 ± 0.37	27.46 ± 0.37	29.24 ± 0.25	28.26 ± 0.26	28.44 ± 0.26	28.65 ± 0.21
(d)	-16.19 ** ± 0.79	-14.78 ** ± 0.79	-12.93 ** ± 0.80	-15.49 ** ± 0.56	-14.56 ** ± 0.57	-13.85 ** ± 0.56	-14.63 ** ± 0.46
(h)	6.22 ** ± 2.15	4.82 * ± 2.19	9.29 ** ± 2.20	5.52 ** ± 1.53	7.75 ** ± 1.55	7.04 ** ± 1.56	6.77 ** ± 1.27
(i)	4.99 * ± 2.12	2.73 ± 2.16	8.23 ** ± 2.17	3.86 * ± 1.51	6.61 ** ± 1.53	5.47 ** ± 1.54	5.31 ** ± 1.25
(j)	-1.50 ± 0.83	0.27 ± 0.83	2.03 * ± 0.83	-0.61 ± 0.59	0.27 ± 0.59	1.15 * ± 0.59	0.27 ± 0.48
(l)	10.30 ** ± 3.54	13.43 ** ± 3.56	10.46 ** ± 3.59	11.86 ** ± 2.51	10.38 ** ± 2.54	11.96 ** ± 2.54	11.40 ** ± 2.07
$(h/d)^{1/2}$	0.62	0.57	0.85	0.60	0.73	0.71	0.68

*, ** significance at 5% and 1% level, respectively

Table 19. Estimates of the genetic components using six-parameter model for panicle diameter in cross 2.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Rainy 2006							
m	23.61 ± 0.29	24.02 ± 0.30	25.27 ± 0.28	23.81 ± 0.21	24.44 ± 0.20	24.65 ± 0.21	24.30 ± 0.17
(d)	-13.74 ** ± 0.58	-12.70 ** ± 0.58	-13.45 ** ± 0.55	-13.22 ** ± 0.41	-13.60 ** ± 0.40	-13.08 ** ± 0.40	-13.30 ** ± 0.33
(h)	8.39 ** ± 1.70	1.92 ± 1.73	1.46 ± 1.64	5.16 ** ± 1.22	4.92 ** ± 1.19	1.69 ± 1.20	3.92 ** ± 0.99
(i)	8.27 ** ± 1.64	3.64 * ± 1.68	3.49 * ± 1.58	5.95 ** ± 1.18	5.88 ** ± 1.14	3.57 ** ± 1.16	5.13 ** ± 0.95
(j)	-0.39 ± 0.65	1.02 ± 0.64	-0.08 ± 0.61	0.32 ± 0.46	-0.23 ± 0.45	0.47 ± 0.45	0.19 ± 0.37
(l)	-1.35 ± 2.75	4.23 ± 2.73	2.06 ± 2.64	1.44 ± 1.96	0.35 ± 1.92	3.14 ± 1.92	1.65 ± 1.58
$(h/d)^{1/2}$	0.78	0.39	0.33	0.62	0.60	0.36	0.54
Summer 2007							
m	24.93 ± 0.29	23.97 ± 0.30	24.64 ± 0.30	24.45 ± 0.21	24.78 ± 0.21	24.31 ± 0.21	24.51 ± 0.17
(d)	-12.09 ** ± 0.57	-14.10 ** ± 0.58	-14.18 ** ± 0.54	-13.09 ** ± 0.41	-13.14 ** ± 0.39	-14.14 ** ± 0.40	-13.46 ** ± 0.33
(h)	3.68 * ± 1.71	4.87 ** ± 1.73	5.46 ** ± 1.66	4.28 ** ± 1.22	4.57 ** ± 1.19	5.17 ** ± 1.20	4.67 ** ± 0.98
(i)	4.48 ** ± 1.64	5.28 ** ± 1.66	5.84 ** ± 1.60	4.88 ** ± 1.17	5.16 ** ± 1.15	5.56 ** ± 1.15	5.20 ** ± 0.95
(j)	0.36 ± 0.63	-1.83 ** ± 0.65	-2.26 ** ± 0.58	-0.73 ± 0.45	-0.95 * ± 0.43	-2.04 ** ± 0.44	-1.24 ** ± 0.36
(l)	1.84 ± 2.74	3.48 ± 2.79	0.32 ± 2.62	2.66 ± 1.96	1.08 ± 1.90	1.90 ± 1.91	1.88 ± 1.57
$(h/d)^{1/2}$	0.55	0.59	0.62	0.57	0.59	0.60	0.59

*, ** significance at 5% and 1% level, respectively

Table 20. Estimates of the genetic components using six-parameter model for grain size in cross 1.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Rainy 2006							
m	7.97 ± 0.14	7.87 ± 0.14	7.84 ± 0.14	7.92 ± 0.10	7.90 ± 0.10	7.85 ± 0.10	7.89 ± 0.08
(d)	-2.62 ** ± 0.19	-2.75 ** ± 0.20	-2.79 ** ± 0.20	-2.68 ** ± 0.14	-2.70 ** ± 0.14	-2.77 ** ± 0.14	-2.72 ** ± 0.11
(h)	-1.65 * ± 0.69	-1.64 * ± 0.71	-1.26 ± 0.71	-1.64 ** ± 0.49	-1.45 ** ± 0.49	-1.45 ** ± 0.50	-1.52 ** ± 0.41
(i)	-1.31 * ± 0.67	-1.03 ± 0.67	-0.86 ± 0.68	-1.17 * ± 0.47	-1.08 * ± 0.47	-0.94 ± 0.48	-1.07 ** ± 0.39
(j)	1.69 ** ± 0.21	1.70 ** ± 0.22	1.56 ** ± 0.23	1.69 ** ± 0.15	1.63 ** ± 0.16	1.63 ** ± 0.16	1.65 ** ± 0.13
(l)	5.10 ** ± 1.01	4.52 ** ± 1.06	4.64 ** ± 1.06	4.81 ** ± 0.73	4.87 ** ± 0.73	4.58 ** ± 0.75	4.75 ** ± 0.60
$(h/d)^{1/2}$	0.79	0.77	0.67	0.78	0.73	0.72	0.75
Summer 2007							
m	8.53 ± 0.13	8.63 ± 0.12	8.69 ± 0.12	8.58 ± 0.09	8.61 ± 0.09	8.66 ± 0.09	8.62 ± 0.07
(d)	-2.95 ** ± 0.23	-3.16 ** ± 0.25	-2.91 ** ± 0.21	-3.06 ** ± 0.17	-2.93 ** ± 0.16	-3.04 ** ± 0.17	-3.01 ** ± 0.14
(h)	-1.17 ± 0.71	-2.44 ** ± 0.75	-1.51 * ± 0.70	-1.80 ** ± 0.52	-1.34 ** ± 0.50	-1.97 ** ± 0.51	-1.71 ** ± 0.42
(i)	-0.38 ± 0.70	-2.04 ** ± 0.71	-1.04 ± 0.64	-1.21 * ± 0.50	-0.71 ± 0.47	-1.54 ** ± 0.48	-1.15 ** ± 0.39
(j)	1.29 ** ± 0.25	1.10 ** ± 0.28	1.31 ** ± 0.25	1.20 ** ± 0.19	1.30 ** ± 0.18	1.20 ** ± 0.19	1.23 ** ± 0.15
(l)	2.17 * ± 1.11	5.34 ** ± 1.23	4.02 ** ± 1.12	3.75 ** ± 0.83	3.09 ** ± 0.79	4.68 ** ± 0.84	3.84 ** ± 0.67
$(h/d)^{1/2}$	0.63	0.88	0.72	0.77	0.68	0.81	0.75

*, ** significance at 5% and 1% level, respectively

Table 21. Estimates of the genetic components using six-parameter model for grain size in cross 2.

Season /	Set 1			Set 2			Set 3
Parameter	B_1	B_2	B_3	B_{12}	B_{13}	B_{23}	B_{123}
Rainy 2006							
m	8.39 ± 0.13	8.51 ± 0.13	8.47 ± 0.12	8.45 ± 0.09	8.43 ± 0.09	8.49 ± 0.09	8.46 ± 0.07
(d)	-2.79 ** ± 0.25	-2.39 ** ± 0.23	-2.65 ** ± 0.24	-2.59 ** ± 0.17	-2.72 ** ± 0.17	-2.52 ** ± 0.17	-2.61 ** ± 0.14
(h)	-1.07 ± 0.73	-1.82 ** ± 0.70	-1.51 * ± 0.69	-1.44 ** ± 0.51	-1.29 * ± 0.50	-1.67 ** ± 0.49	-1.47 ** ± 0.41
(i)	-0.02 ± 0.72	-0.91 ± 0.69	-0.72 ± 0.68	-0.46 ± 0.50	-0.37 ± 0.49	-0.81 ± 0.48	-0.55 ± 0.40
(j)	1.53 ** ± 0.27	1.89 ** ± 0.26	1.59 ** ± 0.27	1.71 ** ± 0.19	1.56 ** ± 0.19	1.74 ** ± 0.19	1.67 ** ± 0.16
(l)	2.91 * ± 1.14	4.02 ** ± 1.10	3.44 ** ± 1.12	3.47 ** ± 0.79	3.18 ** ± 0.80	3.73 ** ± 0.78	3.46 ** ± 0.65
$(h/d)^{1/2}$	0.62	0.87	0.76	0.75	0.69	0.81	0.75
Summer 2007							
m	9.33 ± 0.15	9.52 ± 0.12	9.51 ± 0.14	9.42 ± 0.10	9.42 ± 0.10	9.52 ± 0.09	9.45 ± 0.08
(d)	-3.71 ** ± 0.23	-3.93 ** ± 0.26	-3.98 ** ± 0.24	-3.82 ** ± 0.18	-3.84 ** ± 0.17	-3.96 ** ± 0.18	-3.87 ** ± 0.14
(h)	-0.93 ± 0.76	-1.96 ** ± 0.73	-0.85 ± 0.75	-1.44 ** ± 0.53	-0.89 ± 0.53	-1.41 ** ± 0.52	-1.25 ** ± 0.43
(i)	-0.15 ± 0.75	-1.21 ± 0.72	-0.32 ± 0.74	-0.68 ± 0.52	-0.23 ± 0.53	-0.76 ± 0.52	-0.56 * ± 0.43
(j)	0.85 ** ± 0.25	0.67 * ± 0.29	0.32 ± 0.26	0.76 ** ± 0.19	0.58 ** ± 0.18	0.50 * ± 0.20	0.61 ** ± 0.16
(l)	2.61 * ± 1.13	4.97 ** ± 1.19	2.58 * ± 1.14	3.79 ** ± 0.82	2.60 * ± 0.80	3.78 ** ± 0.82	3.39 ** ± 0.67
$(h/d)^{1/2}$	0.50	0.71	0.46	0.61	0.48	0.60	0.57

*, ** significance at 5% and 1% level, respectively

Table 22. Triple test cross analysis for panicle length.

ANOVA for testing epistatic model ($L_{1i} + L_{2i} - 2L_{3i}$)

Source	DF	SS	MS	F ratio
[i] type epistasis	1	53.93	53.93 **	12.19
[j+l] type epistasis	59	13212.74	223.94 **	50.63
Total epistasis	60	13266.68	221.11 **	49.99
[i] x block	2	2.81	1.40	0.31
[j+l] x block	118	528.00	4.47	
Total epistasis x block	120	530.80	4.42	

ANOVA for testing additive model ($L_{1i} + L_{2i}$)

Source	DF	SS	MS	F ratio
Replication	2	0.64	0.32	0.29
Lines (sums)	59	16509.56	279.82 **	251.84
Error	118	131.11	1.11	

Additive component (D) = 371.62

ANOVA for testing dominance model ($L_{1i} - L_{2i}$)

Source	DF	SS	MS	F ratio
Replication	2	6.07	3.04	2.70
Lines (differences)	59	20648.68	349.98 **	311.08
Error	118	132.76	1.13	

Dominance component (H) = 465.14

Degree of dominance = 1.12

Direction of dominance (r) = -0.59**

Table 23. Triple test cross analysis for panicle diameter.

ANOVA for testing epistatic model ($L_{1i} + L_{2i} - 2L_{3i}$)

Source	DF	SS	MS	F ratio
[i] type epistasis	1	127.67	127.67 **	39.26
[j+l] type epistasis	59	8802.63	149.20 **	45.88
Total epistasis	60	8930.29	148.84 **	45.77
[i] x block	2	1.03	0.51	0.16
[j+l] x block	118	389.19	3.30	
Total epistasis x block	120	390.21	3.25	

ANOVA for testing additive model ($L_{1i} + L_{2i}$)

Source	DF	SS	MS	F ratio
Replication	2	0.95	0.48	0.47
Lines (sums)	59	7024.43	119.06 **	118.24
Error	118	118.82	1.01	

Additive component (D) = 157.40

ANOVA for testing dominance model ($L_{1i} - L_{2i}$)

Source	DF	SS	MS	F ratio
Replication	2	4.06	2.03	2.76
Lines (differences)	59	6034.30	102.28 **	138.72
Error	118	87.00	0.74	

Dominance component (H) = 135.39

Degree of dominance = 0.93

Direction of dominance (r) = -0.67**

Table 24. Triple test cross analysis for grain size.

ANOVA for testing epistatic model ($L_{1i} + L_{2i} - 2L_{3i}$)

Source	DF	SS	MS	F ratio
[i] type epistasis	1	5.21	5.21	2.59
[j+l] type epistasis	59	1279.03	21.68 **	10.80
Total epistasis	60	1284.24	21.40 **	10.66
[i] x block	2	3.09	1.55	0.77
[j+l] x block	118	237.74	2.01	
Total epistasis x block	120	240.83	2.01	

ANOVA for testing additive model ($L_{1i} + L_{2i}$)

Source	DF	SS	MS	F ratio
Replication	2	3.14	1.57	2.74
Lines (sums)	59	470.46	7.97 **	13.96
Error	118	67.42	0.57	

Additive component (D) = 9.87

ANOVA for testing dominance model ($L_{1i} - L_{2i}$)

Source	DF	SS	MS	F ratio
Replication	2	3.49	1.74	2.59
Lines (differences)	59	585.87	9.93 **	14.77
Error	118	79.33	0.67	

Dominance component (H) = 12.34

Degree of dominance = 1.12

Direction of dominance (r) = -0.09

Table 25. Components of variances and heritabilities estimated for panicle length, panicle diameter and grain size.

Parameters	Panicle length				Panicle diameter				Grain size			
	Cross 1		Cross 2		Cross 1		Cross 2		Cross 1		Cross 2	
	R 06	S 07	R 06	S 07	R 06	S 07	R 06	S 07	R 06	S 07	R 06	S 07
Genotypic variance	97.44	99.29	47.95	58.97	44.79	44.78	27.59	27.81	5.97	4.72	5.16	6.28
Additive variance	83.21	90.28	43.10	55.80	31.00	29.94	27.69	29.46	9.10	5.20	5.19	7.05
Dominance variance	14.23	9.02	4.85	3.18	13.79	14.84	-0.10	-1.65	-3.14	-0.49	-0.03	-0.77
Environmental variance	1.90	2.11	2.58	2.70	3.67	1.91	2.85	2.91	0.52	0.65	0.33	0.28
Dominance ratio	0.41	0.32	0.34	0.24	0.67	0.70	0.06	0.24	0.59	0.31	0.08	0.33
Broad-sense heritability	98.08	97.92	94.89	95.62	92.43	95.90	90.64	90.52	92.02	87.95	94.07	95.77
Narrow-sense heritability	83.76	89.03	85.29	90.47	63.97	64.12	90.67	91.00	94.62	88.95	94.11	96.13

R 06 - Rainy 2006 S 07 - Summer 2007

Table 26. Correlation coefficient (r) among the observed traits in F_2 population of trait-specific crosses.

Panicle length crosses	Cross 1						Cross 2					
	PL	PD	GS	DF	PH	PT	PL	PD	GS	DF	PH	PT
Panicle length (PL)	1.000						1.000					
Panicle diameter (PD)	0.105	1.000					0.228 *	1.000				
Grain size (GS)	0.167 *	0.202 *	1.000				0.118	0.258 **	1.000			
Days to 50% flowering (DF)	0.024	-0.089	0.003	1.000			0.360 **	-0.176	-0.065	1.000		
Plant height (PH)	0.250 **	0.084	-0.073	0.143	1.000		0.384 **	0.267 **	0.091	0.120	1.000	
Productive tiller number (PT)	0.012	0.047	0.230 *	-0.178 *	-0.066	1.000	0.088	0.197 *	0.122	-0.239 **	0.250 **	1.000
Single plant yield (SPY)	0.224 *	0.313 **	0.352 **	-0.157	0.131	0.744 **	0.320 **	0.335 **	0.339 **	-0.091	0.421 **	0.712 **

Panicle diameter crosses	Cross 1						Cross 2					
	PD	PL	GS	DF	PH	PT	PD	PL	GS	DF	PH	PT
Panicle diameter (PD)	1.000						1.000					
Panicle length (PL)	0.194 *	1.000					0.480 **	1.000				
Grain size (GS)	0.281 **	-0.016	1.000				0.267 **	0.298 **	1.000			
Days to 50% flowering (DF)	-0.278 **	-0.197 *	0.000	1.000			0.200 *	0.123	0.147	1.000		
Plant height (PH)	0.035	0.259 **	0.015	0.109	1.000		0.220 *	0.360 **	0.016	0.215 *	1.000	
Productive tiller number (PT)	0.140	0.166 *	0.026	0.082	-0.010	1.000	0.079	0.055	-0.079	-0.103	0.188 *	1.000
Single plant yield (SPY)	0.428 **	0.406 **	0.325 **	-0.024	0.223 *	0.432 **	0.352 **	0.333 **	0.115	-0.076	0.297 **	0.824 **

Grain size crosses	Cross 1						Cross 2					
	GS	PL	PD	DF	PH	PT	GS	PL	PD	DF	PH	PT
Grain size (GS)	1.000						1.000					
Panicle length (PL)	0.048	1.000					0.052	1.000				
Panicle diameter (PD)	0.488 **	-0.002	1.000				0.671 **	0.155	1.000			
Days to 50% flowering (DF)	0.094	0.249 **	0.061	1.000			-0.158	0.002	-0.125	1.000		
Plant height (PH)	-0.007	0.448 **	0.082	0.389 **	1.000		-0.015	0.151	0.135	0.225 *	1.000	
Productive tiller number (PT)	-0.025	0.314 **	0.158	0.205 *	0.152	1.000	-0.108	0.327 **	0.021	0.079	0.081	1.000
Single plant yield (SPY)	0.323 **	0.404 **	0.531 **	0.120	0.171 *	0.732 **	0.313 **	0.266 **	0.311 **	0.075	0.185 *	0.764 **

Table 27. Descriptive statistics and heritability estimates for the observed traits in the F_2 and $F_{2:3}$ mapping populations.

Parameters	Panicle length (cm)	Panicle diameter (mm)	Grain size (g)	Plant height (cm)	Productive tiller number	Panicle grain weight (g)	Panicle weight (g)	Panicle harvest index (%)
Mean								
P_1	28.59	18.35	5.41	101.21	2.70	15.08	21.15	63.25
P_2	24.64	35.07	12.02	108.60	1.33	22.75	37.20	65.77
F_2	27.78	26.38	8.08	114.21	2.39	-	-	-
$F_{2:3}$	27.44	25.97	8.73	108.84	1.74	21.73	33.61	64.77
Standard error								
F_2	1.27	1.33	1.14	1.99	1.08	-	-	-
$F_{2:3}$	0.81	1.12	0.76	2.05	0.24	1.60	1.58	4.01
F ratio								
$F_{2:3}$	8.49 **	11.20 **	5.25 **	20.89 **	2.65 *	6.68 **	12.71 **	2.28
Heritability								
F_2	0.71	0.72	0.59	0.87	0.35	-	-	-
$F_{2:3}$	0.88	0.91	0.81	0.95	0.62	0.85	0.92	0.56

Table 28. Correlation coefficient (r) among the observed traits in $F_{2:3}$ mapping population.

Traits	Panicle length (cm)	Panicle diameter (mm)	Grain size (g)	Panicle grain weight (g)	Panicle weight (g)	Panicle harvest index (%)	Plant height (cm)	Productive tiller number
Panicle length (cm)	1.000							
Panicle diameter (mm)	(-0.300)**	1.000						
Grain size (g)	(-0.140)	0.553**	1.000					
Panicle grain weight (g)	0.221*	0.276**	0.200*	1.000				
Panicle weight (g)	0.162*	0.438**	0.271**	0.867**	1.000			
Panicle harvest index (%)	0.142	(-0.225)*	(-0.114)	0.406**	(-0.093)	1.000		
Plant height (cm)	0.427**	(-0.226)*	(-0.054)	0.119	0.143	(-0.043)	1.000	
Productive tillers number	0.240*	(-0.302)**	(-0.239)**	0.244**	0.096	0.267**	0.096	1.000

*, ** significance at 5% and 1% level, respectively

Table 29. Polymorphic information in parental lines of mapping population for different marker types.

Markers type	No. of markers survyed	No. of polymorphic markers	Per cent polymorphism	No. of selected polymorphic markers
SNP	96	40	41.7	24
SSR	96	36	37.5	10
EST-SSR	96	28	29.2	6
STS	43	5	11.6	4
Total	331	109	32.9	44

Table 30. Linkage group-wise marker position and chi-square values.

Linkage group	Marker	Position (cM)	Marker interval (cM)	Chi square value	Probablity
LG1	Xpsms80	0.0	–	4.90	0.10
	Xpsms86	10.6	10.6	1.73	0.50
	Xpsms39	20.6	10.0	4.94	0.10
	Xpsmp2069	41.7	21.1	4.52	0.10
	Xpsmp669	55.4	13.7	3.85	0.20
	Xicmp3017	58.7	3.3	4.31	0.20
	Xpsms58	65.9	7.2	5.47	0.10
	Xpsms29	110.0	44.1	1.47	0.50
LG2	Xpsms92	0.0	–	5.44	0.10
	Xicmp3063	17.6	17.6	12.84 **	0.01
	Xpsmp2237	45.9	28.3	4.92	0.10
	Xpsms89	58.2	12.3	4.87	0.10
	Xpsms78	73.4	15.2	4.02	0.10
	Xpsmp592	84.8	11.4	4.10	0.10
	Xpsms75	99.7	14.9	5.27	0.10
	Xpsms73	195.0	95.3	5.16	0.10
LG3	Xpsmp37	0.0	–	4.73	0.10
	Xicmp3073	15.9	15.9	0.06	0.95
	Xpsms68	81.3	65.4	5.25	0.10
	Xpsmp2222	109.1	27.8	4.58	0.10
	Xpsms17	119.0	9.9	3.12	0.20
	Xpsms32	128.6	9.6	3.47	0.20
	Xpsms61	133.5	4.9	4.27	0.10
	Xpsms31	148.9	15.4	6.96 *	0.05
	Xpsmp2068	180.1	31.2	1.78	0.50
LG4	Xpsms77	0.0	–	2.10	0.50
	Xpsmp2084	37.7	37.7	5.09	0.10
LG5	Xicmp3027	0.0	–	4.50	0.20
	Xpsmp2064	44.5	44.5	3.42	0.20
	Xpsms74	56.6	12.1	3.38	0.20
	Xpsms2	63.5	6.9	2.81	0.50
	Xpsmp345	97.1	33.6	4.30	0.20
	Xpsms18	150.4	53.3	10.25 **	0.01
	Xpsmp2078	172.0	21.6	5.26	0.10
LG6	Xicmp3081	0.0	–	1.46	0.50
	Xpsms88	98.7	98.7	4.29	0.05
	Xpsmp2270	104.6	5.9	3.89	0.05
	Xicmp3086	105.7	1.1	4.34	0.05
	Xpsms59	107.3	1.6	6.40	0.05
	Xpsms41	227.9	120.6	4.98	0.10
LG7	Xpsms76	0.0	–	4.62	0.10
	Xpsms6	38.7	38.7	4.88	0.10
	Xpsmp2203	52.8	14.1	3.76	0.20
	Xpsmp2027	96.0	43.2	10.38 **	0.01

*, ** significance at 5% and 1% level, respectively

Table 31. Distribution and distance coverage of markers across seven linkage groups.

Linkage group	No. of markers	% of total markers	Total distance (cM)	Average distance (cM)
LG1	8	18	110.0	13.8
LG2	8	18	195.1	24.4
LG3	9	20	180.1	20.0
LG4	2	5	37.7	18.9
LG5	7	16	172.0	24.6
LG6	6	14	227.8	38.0
LG7	4	9	96.0	24.0
Total	44	100	1018.7	23.4

Table 32. QTLs identified for panicle length in the $F_{2:3}$ progenies and F_2 mapping population.

Model		LG	Position (cM)	Flanking Markers	LOD	R^2 (%)	Additive effects (R^2_{par})	Dominance effects (R^2_{par})	Interaction between loci	Epistatic effects (R^2_{par})	R^2_{adj} (%)
Additive											
	$F_{2:3}$	1	18	Xpsms86 - Xpsms39	4.1	9.4	-0.8 (8.9)		-	-	
		2	48	Xpsmp2237 - Xpsms89	5.9	13.5	-1.6 (25.9)		-	-	
		3	104	Xpsms68 - Xpsmp2222	4.9	11.3	1.0 (9.3)		-	-	
		4	26	Xpsms77 - Xpsmp2084	2.9	7.0	0.8 (6.8)		-	-	
		6	4	Xicmp3081 - Xpsms88	3.3	8.1	-0.1 (8.2)		-	-	
		6	104	Xpsms88 - Xpsmp2270	8.0	17.9	-1.3 (17.6)		-	-	
		7	42	Xpsms6 - Xpsmp2203	3.5	8.2	-0.8 (8.0)		-	-	40.7
	F_2	QTL not detected									
Additive + Dominance	$F_{2:3}$	1	18	Xpsms86 - Xpsms39	4.6	10.7	-0.8 (8.7)	0.4 (1.4)	-	-	
		2	48	Xpsmp2237 - Xpsms89	6.1	13.9	-1.6 (26.9)	-0.1 (0.1)	-	-	
		3	104	Xpsms68 - Xpsmp2222	5.6	12.8	1.9 (11.5)	0.0 (0)	-	-	
		3	130	Xpsms32 - Xpsms61	2.6	6.1	-1.0 (4.5)	0.7 (0.6)	-	-	
		4	28	Xpsms77 - Xpsmp2084	3.2	7.6	0.7 (5.4)	-0.3 (0.4)	-	-	
		6	22	Xicmp3081 - Xpsms88	3.4	8.3	-1.1 (9.2)	0.4 (0.3)	-	-	
		6	104	Xpsms88 - Xpsmp2270	8.2	18.2	-1.1 (12.8)	0.2 (0.3)	-	-	
		7	44	Xpsms6 - Xpsmp2203	3.7	8.5	-0.7 (6.8)	-0.3 (0.9)	-	-	42.7
	F_2	2	96	Xpsm592 - Xpsms75	3.3	7.8	-1.7 (0.5)	0.7 (0.7)	-	-	
		6	64	Xicmp3081 - Xpsms88	3.7	8.9	-2.0 (3.4)	4.9 (3.4)	-	-	13.1

Contd..

Table 32 (Contd..). QTLs identified for panicle length in the $F_{2:3}$ progenies and F_2 mapping population.

Model		LG	Position (cM)	Flanking Markers	LOD	R^2 (%)	Additive effects (R^2_{par})	Dominance effects (R^2_{par})	Interaction between loci	Epistatic effects (R^2_{par})	R^2_{adj} (%)
Epistatic											
	$F_{2:3}$	1	18	Xpsms86 - Xpsms39	4.6	10.7	-1.6 (1.4)	-0.4 (0.0)	D1*D6	-4.8 (6.8)	
		2	48	Xpsmp2237 - Xpsms89	6.1	13.9	-3.3 (4.3)	3.3 (1.8)	A3*A7	6.6 (12.3)	
		3	104	Xpsms68 - Xpsmp2222	5.6	12.8	-9.1 (1.3)	8.0 (0.4)	D3*D8	7.1 (6.5)	
		3	130	Xpsms32 - Xpsms61	2.6	6.1	8.9 (1.3)	16.5 (2.8)	A4*A7	-4.7 (11.2)	
		4	28	Xpsms77 - Xpsmp2084	3.2	7.6	1.0 (0.2)	-2.8 (1.4)			
		6	22	Xicmp3081 - Xpsms88	3.4	8.3	0.8 (0.2)	5.0 (1.0)			
		6	104	Xpsms88 - Xpsmp2270	8.2	18.2	2.8 (0.6)	6.2 (2.2)			
		7	44	Xpsms6 - Xpsmp2203	3.7	8.5	-1.8 (2.6)	0.1 (0.0)			40.1
	F_2	2	96	Xpsm592 - Xpsms75	3.3	7.8	-0.5 (0.0)	2.1 (0.4)			
		6	64	Xicmp3081 - Xpsms88	3.7	8.9	-0.4 (0.0)	6.3 (1.7)			12.5

Table 33. QTLs identified for panicle diameter in the $F_{2:3}$ progenies and F_2 mapping population.

Model		LG	Position (cM)	Flanking Markers	LOD	R^2 (%)	Additive effects (R^2_{par})	Dominance effects (R^2_{par})	Interaction between loci	Epistatic effects (R^2_{par})	R^2_{adj} (%)
Additive											
	$F_{2:3}$	2	80	Xpsms78 - Xpsmp592	14.7	30.2	1.9 (24.4)		-	-	
		3	116	Xpsmp2222 - Xpsms17	14.0	29.0	2.0 (28.6)		-	-	
		6	104	Xpsms88 - Xpsmp2270	5.4	12.4	1.0 (8.9)		-	-	
		7	50	Xpsms6 - Xpsmp2203	3.6		1.1 (10.2)		-	-	44.3
	F_2	QTL not detected									
Additive + Dominance											
	$F_{2:3}$	2	80	Xpsms78 - Xpsmp592	14.7	30.2	1.9 (26.4)	0.1 (0.1)	-	-	
		3	116	Xpsmp2222 - Xpsms17	14.5	29.9	2.0 (29.1)	0.3 (0.5)	-	-	
		5	58	Xpsms74 - Xpsms2	2.6	6.3	0.6 (3.6)	-0.6 (2.1)	-	-	
		6	104	Xpsms88 - Xpsmp2270	5.4	12.5	1.1 (9.7)	0.0 (0.0)	-	-	
		7	48	Xpsms6 - Xpsmp2203	4.0	9.2	1.1 (9.7)	0.4 (0.8)	-	-	45.8
	F_2	QTL not detected									
Epistatic											
	$F_{2:3}$	2	80	Xpsms78 - Xpsmp592	14.7	30.2	1.1 (0.9)	-0.1 (0.0)	A1*D2	1.4 (2.8)	
		3	116	Xpsmp2222 - Xpsms17	14.5	29.9	0.8 (0.5)	1.0 (0.4)			
		5	58	Xpsms74 - Xpsms2	2.6	6.3	1.4 (1.7)	-2.3 (2.8)			
		6	104	Xpsms88 - Xpsmp2270	5.4	12.5	2.5 (1.0)	0.0 (0.0)			
		7	48	Xpsms6 - Xpsmp2203	4.0	9.2	1.3 (1.3)	1.0 (0.4)			41.0
	F_2	QTL not detected									

Table 34. QTLs identified for grain size in the $F_{2:3}$ progenies and F_2 mapping population.

Model		LG	Position (cM)	Flanking Markers	LOD	R^2 (%)	Additive effects (R^2_{par})	Dominance effects (R^2_{par})	Interaction between loci	Epistatic effects (R^2_{par})	R^2_{adj} (%)
Additive											
	$F_{2:3}$	1	28	Xpsms39 - Xpsmp2069	2.8	6.7	0.6 (6.4)		-	-	
		3	0	Xpsmp37 - Xicmp3073	3.1	7.5	0.6 (10.4)		-	-	13.3
									-	-	
	F_2	3	6	Xpsmp37 - Xicmp3073	8.8	19.0	1.5 (22.9)		-	-	
		6	100	Xpsms88 - Xpsmp2270	4.8	11.0	0.9 (9.9)		-	-	32.2
Additive + Dominance											
	$F_{2:3}$	1	28	Xpsms39 - Xpsmp2069	2.8	6.7	0.5 (6.4)	0.1 (0.1)	-	-	
		3	2	Xpsmp37 - Xicmp3073	3.7	8.9	0.6 (9.7)	0.4 (2.6)	-	-	
		5	16	Xicmp3027 - Xpsmp2064	2.5	6.4	0.1 (0.3)	-0.8 (4.2)	-	-	
		6	106	Xicmp3086 - Xpsms59	3.3	7.7	0.5 (6.4)	0.3 (1.7)	-	-	
		7	32	Xpsms76 - Xpsms6	2.6	6.1	0.4 (5.6)	-0.2 (0.4)	-	-	23.6
	F_2	3	6	Xpsmp37 - Xicmp3073	9.4	21.2	1.5 (24)	0.6 (2.3)	-	-	
		6	102	Xpsms88 - Xpsmp2270	6.6	14.9	1.1 (13.2)	0.7 (3.9)	-	-	35.6

Contd..

Table 34 (Contd..). QTLs identified for grain size in the $F_{2:3}$ progenies and F_2 mapping population.

Model		LG	Position (cM)	Flanking Markers	LOD	R^2 (%)	Additive effects (R^2_{par})	Dominance effects (R^2_{par})	Interaction between loci	Epistatic effects (R^2_{par})	R^2_{adj} (%)
Epistatic											
	$F_{2:3}$	1	28	Xpsms39 - Xpsmp2069	2.8	6.7	1.2 (2.2)	-2.0 (4.4)	D1*D4	1.3 (3.4)	
		3	2	Xpsmp37 - Xicmp3073	3.7	8.9	0.9 (0.6)	1.6 (1.6)	D1*A5	1.0 (4.3)	
		5	16	Xicmp3027 - Xpsmp2064	2.5	6.4	-1.2 (0.5)	-2.8 (1.5)	A2*D3	-1.3 (3.1)	
		6	106	Xicmp3086 - Xpsms59	3.3	7.7	-1.4 (2.0)	-2.3 (4.2)	D2*D3	-1.7 (3.7)	
		7	32	Xpsms76 - Xpsms6	2.6	6.1	0.0 (0.0)	0.2 (0.0)	D3*D4	3.8 (3.4)	29.6
	F_2	3	6	Xpsmp37 - Xicmp3073	9.4	21.2	2.1 (12.1)	-1.1 (2.2)	D1*D2	2.6 (7.9)	
		6	102	Xpsms88 - Xpsmp2270	6.6	14.9	0.6 (1.0)	-0.8 (1.3)			41.1

Table 35. QTLs identified for panicle weight and panicle harvest index in $F_{2:3}$ mapping population.

Trait	Model	LG	Position (cM)	Flanking Markers	LOD	R^2 (%)	Additive effects (R^2_{par})	Dominance effects (R^2_{par})	R^2_{adj} (%)
Panicle weight (g)									
	Additive	QTL not detected							
	Additive + Dominance	3	118	Xpsmp2222 -Xpsms17	2.6	6.1	1.6 (3.8)	-0.6 (0.3)	2.8
Panicle harvest index (%)									
	Additive	3	50	Xicmp3073 - Xpsms68	3.0	7.2	1.6 (3.7)		2.7
	Additive + Dominance	3	54	Xicmp3073 - Xpsms68	3.5	8.3	1.8 (4.7)	-2.2 (1.6)	3.7

Table 36. QTLs identified for plant height in the $F_{2:3}$ progenies and F_2 mapping population.

Model		LG	Position (cM)	Flanking Markers	LOD	R^2 (%)	Additive effects (R^2_{par})	Dominance effects (R^2_{par})	Interaction between loci	Epistatic effects (R^2_{par})	R^2_{adj} (%)
Additive											
	$F_{2:3}$	1	56	Xpsmp669 - Xicmp3017	8.3	18.3	-6.7 (26.7)		-	-	
		2	48	Xpsmp2237 - Xpsms89	3.4	8.0	-3.4 (7.4)		-	-	
		3	128	Xpsms17 - Xpsms32	8.1	18.1	-5.2 (17.2)		-	-	
		4	24	Xpsms77 - Xpsmp2084	3.1	7.3	2.7 (4.1)		-	-	
		5	46	Xpsmp2064 - Xpsms74	7.6	17.1	5.0 (17.4)		-	-	42.1
	F_2	1	52	Xpsmp2069 - Xpsmp669	6.5	14.7	-8.6 (13.6)		-	-	
		5	56	Xpsmp2064 - Xpsms74	4.9	11.3	6.0 (9.1)		-	-	18.2
Additive + Dominance											
	$F_{2:3}$	1	12	Xpsms86 - Xpsms39	4.1	9.6	-2.6 (5.1)	2.4 (2.6)	-	-	
		1	56	Xpsmp669 - Xicmp3017	11.0	23.6	-5.8 (18.8)	3.0 (4.4)	-	-	
		2	48	Xpsmp2237 - Xpsms89	3.4	8.0	-3.6 (9.1)	0.0 (0.0)	-	-	
		3	128	Xpsms17 - Xpsms32	9.6	21.0	-5.4 (21)	2.7 (4.0)	-	-	
		4	30	Xpsms77 - Xpsmp2084	3.3	7.8	2.5 (4.2)	-1.4 (0.7)	-	-	
		5	46	Xpsmp2064 - Xpsms74	7.7	17.1	5.4 (22.3)	0.5 (0.1)	-	-	49.3
	F_2	1	56	Xpsmp669 - Xicmp3017	8.0	17.8	-8.0 (14.1)	5.1 (3.9)	-	-	
		5	48	Xpsmp2064 - Xpsms74	5.3	12.1	6.5 (9.9)	2.7 (0.9)	-	-	20.1

Contd..

Table 36 (Contd..). QTLs identified for plant height in the $F_{2:3}$ progenies and F_2 mapping population.

Model		LG	Position (cM)	Flanking Markers	LOD	R^2 (%)	Additive effects (R^2_{par})	Dominance effects (R^2_{par})	Interaction between loci	Epistatic effects (R^2_{par})	R^2_{adj} (%)
Epistatic											
	$F_{2:3}$	1	12	Xpsms86 - Xpsms39	4.1	9.6	-2.4 (0.1)	14.7 (4.7)	NIL		
		1	56	Xpsmp669 - Xicmp3017	11.0	23.6	-2.0 (0.1)	-1.0 (0.0)			
		2	48	Xpsmp2237 - Xpsms89	3.4	8.0	4.1 (0.6)	1.3 (0.0)			
		3	128	Xpsms17 - Xpsms32	9.6	21.0	-9.3 (4.9)	-3.8 (0.5)			
		4	30	Xpsms77 - Xpsmp2084	3.3	7.8	2.0 (0.1)	3.2 (0.1)			
		5	46	Xpsmp2064 - Xpsms74	7.7	17.1	5.8 (1.6)	-1.1 (0.0)			45.4
	F_2	1	56	Xpsmp669 - Xicmp3017	8.0	17.8	-8.6 (5.8)	4.3 (1.0)	NIL		
		5	48	Xpsmp2064 - Xpsms74	5.3	12.1	7.4 (4.3)	1.8 (0.2)			18.7

Table 37. QTLs identified for productive tiller number in the $F_{2:3}$ progenies and F_2 mapping population.

Model		LG	Position (cM)	Flanking Markers	LOD	R^2 (%)	Additive effects (R^2_{par})	Dominance effects (R^2_{par})	R^2_{adj} (%)
Additive									
	$F_{2:3}$	QTL not detected							
	F_2	QTL not detected							
Additive + Dominance									
	$F_{2:3}$	7	44	Xpsms6 - Xpsmp2203	3.5	8.1	-0.1 (5.5)	-0.1 (3.3)	6.5
	F_2	QTL not detected							

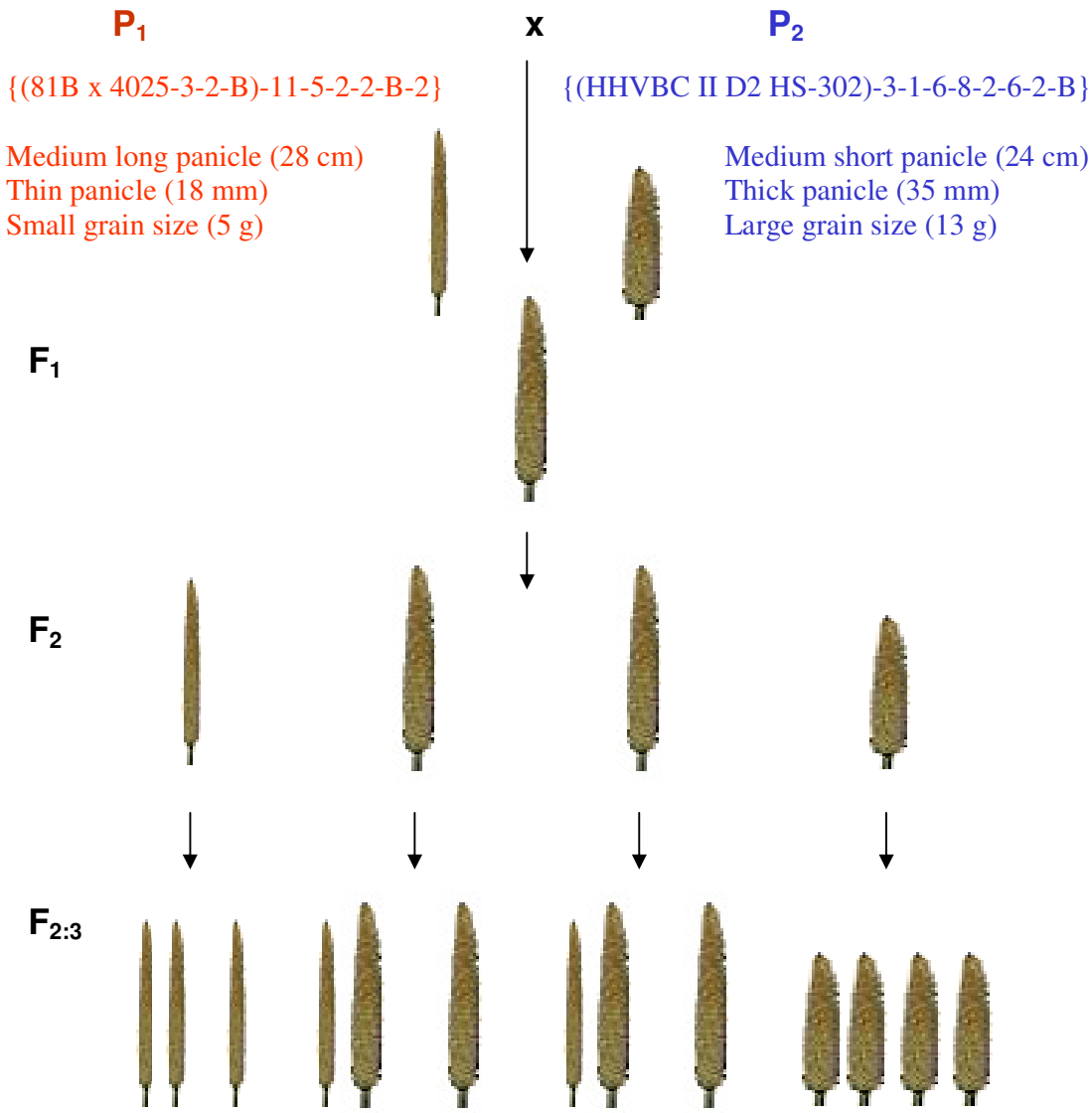


Figure 1: Schematic representation of the development of $F_{2:3}$ mapping progenies.

- Two pearl millet inbred lines showing substantial variation for sink size traits as well for molecular markers as parental lines
- Plant-to-plant cross was made to produce F_1 seeds.
- F_1 panicles were selfed to generate F_2 seeds
- Around 400 F_2 seeds from a single F_1 panicle were sown to generate $F_{2:3}$ progenies.
- A set of 188 $F_{2:3}$ progenies were derived from a single F_2 population by selfing individual plants.

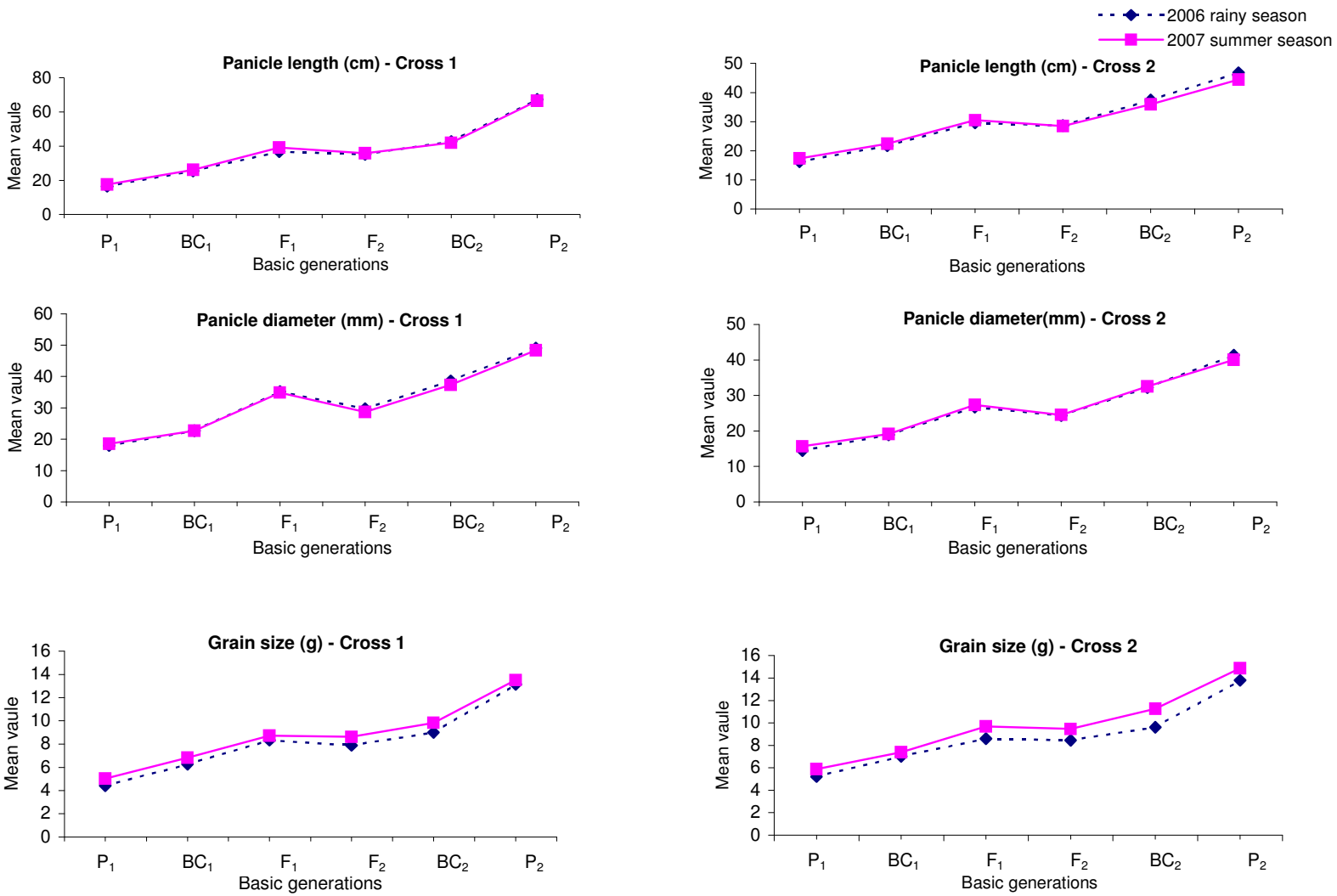
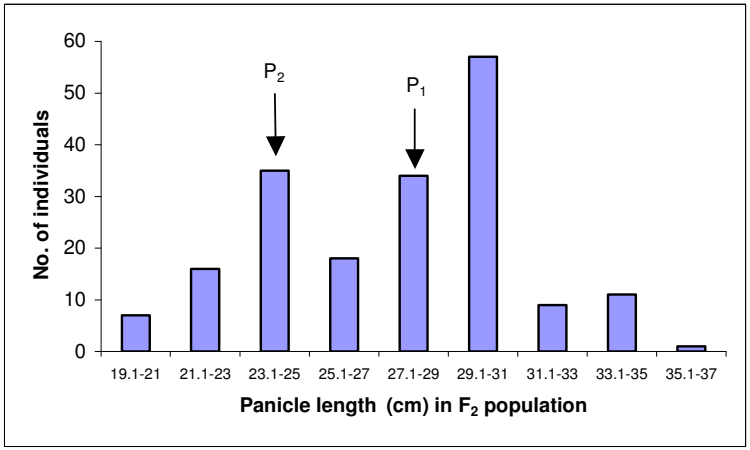
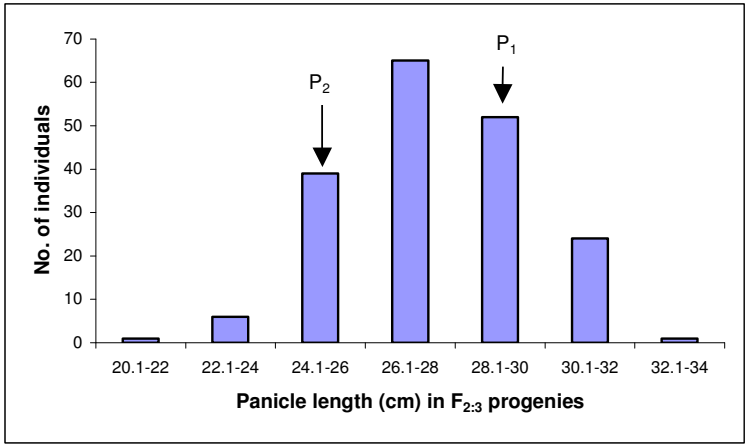
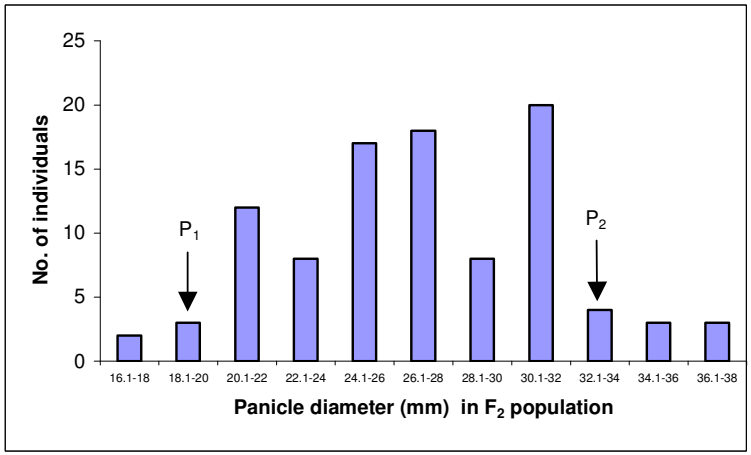


Figure 2. Mean performance of parents, F_1, F_2 and backcross generations for sink size traits during 2006 rainy and 2007 summer seasons.







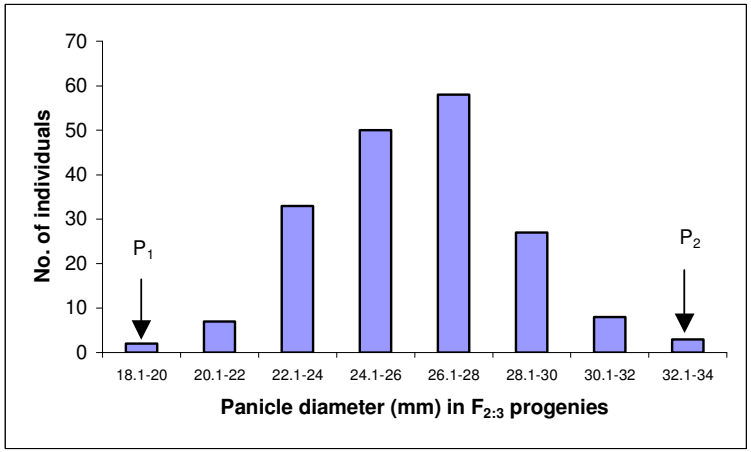
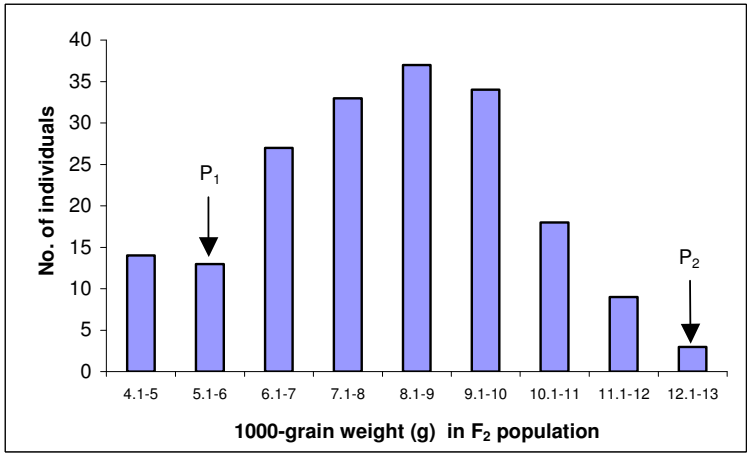
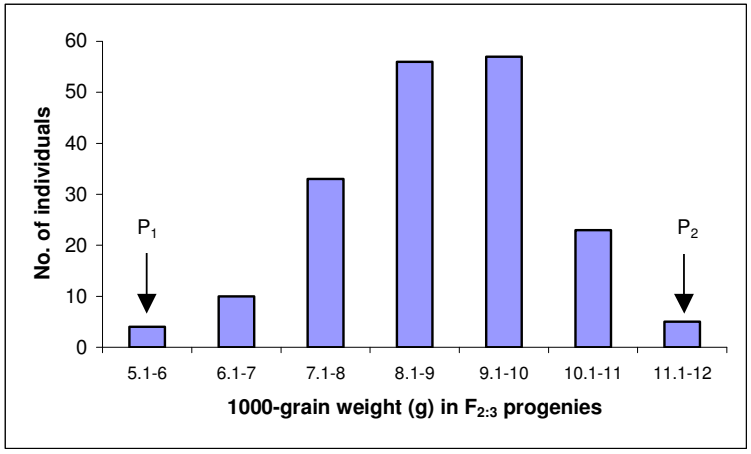
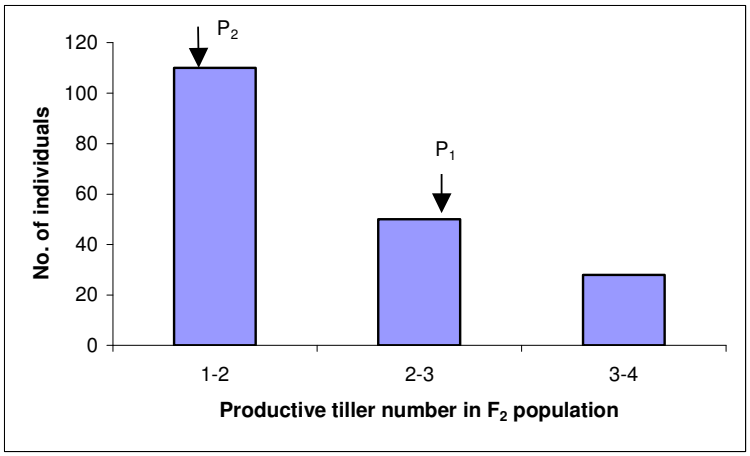


Figure 3. Frequency distribution for the panicle length and panicle diameter in the F_2 and $F_{2:3}$ mappping populations.







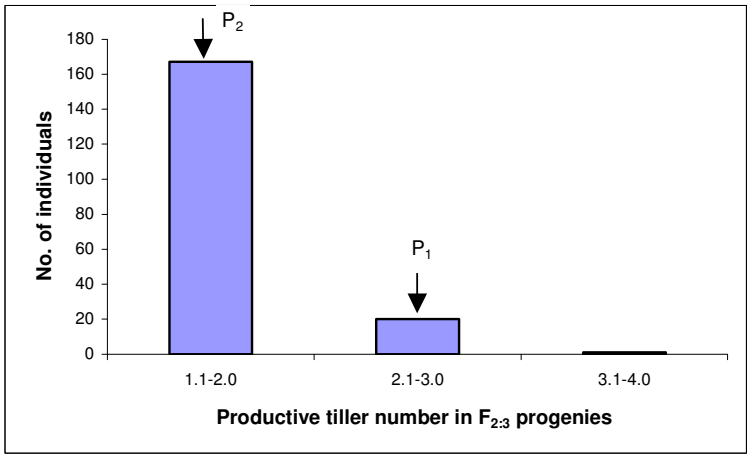
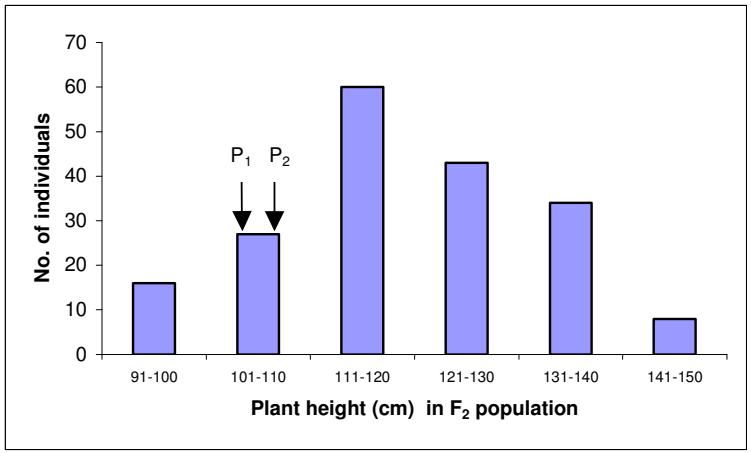


Figure 4. Frequency distribution for the 1000-grain weight and productive tiller number in the F_2 and $F_{2:3}$ mappping populations.



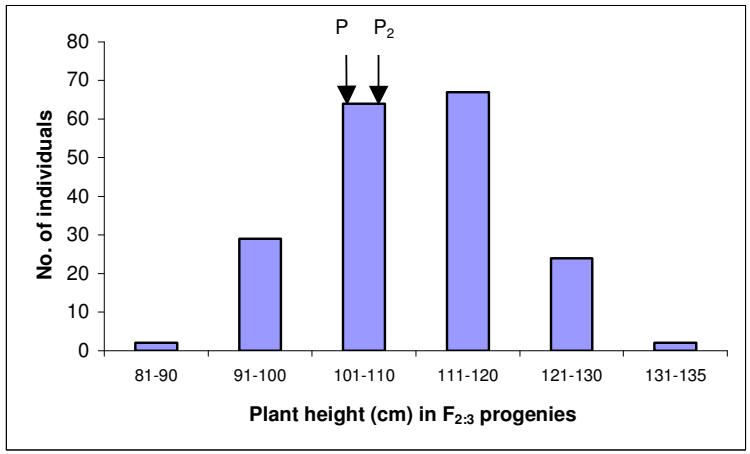
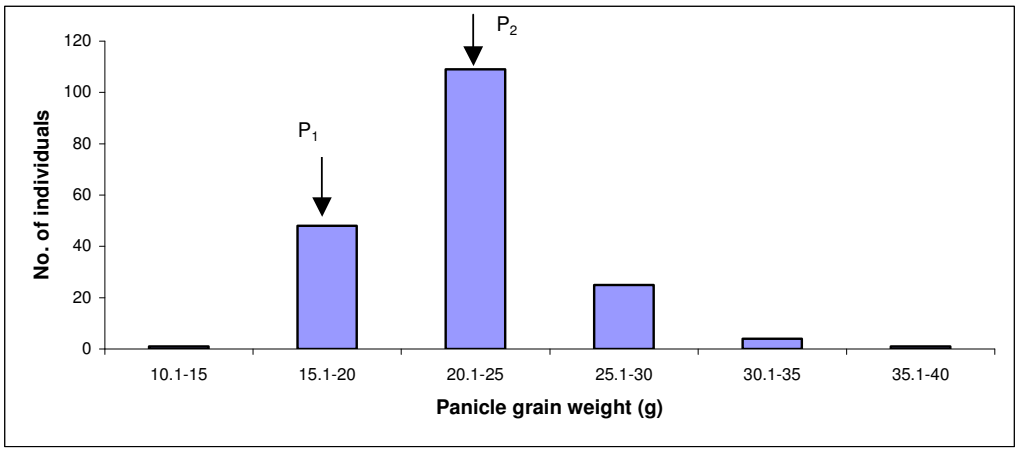
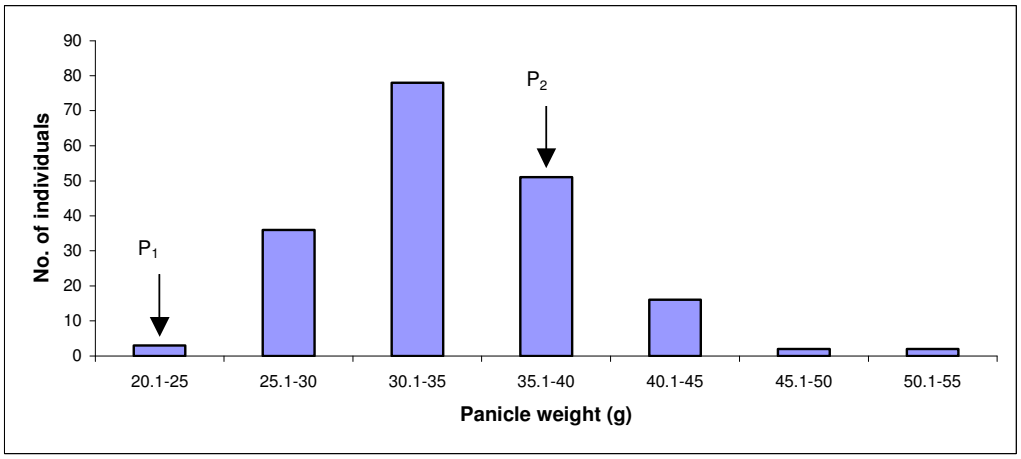


Figure 5. Frequency distribution for the plant height in the F_2 and $F_{2:3}$ mappping populations.





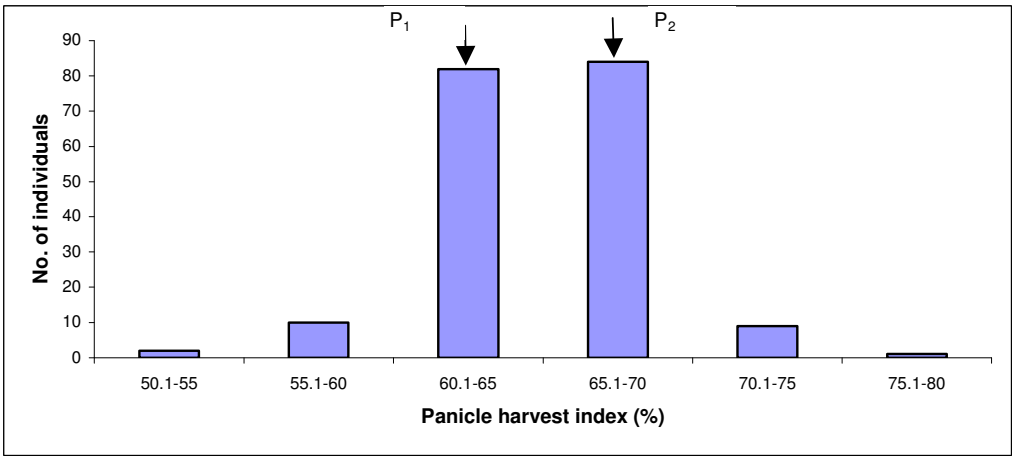


Figure 6. Frequency distribution for the panicle grain weight, panicle weight and panicle harvest index in the $F_{2:3}$ progenies.

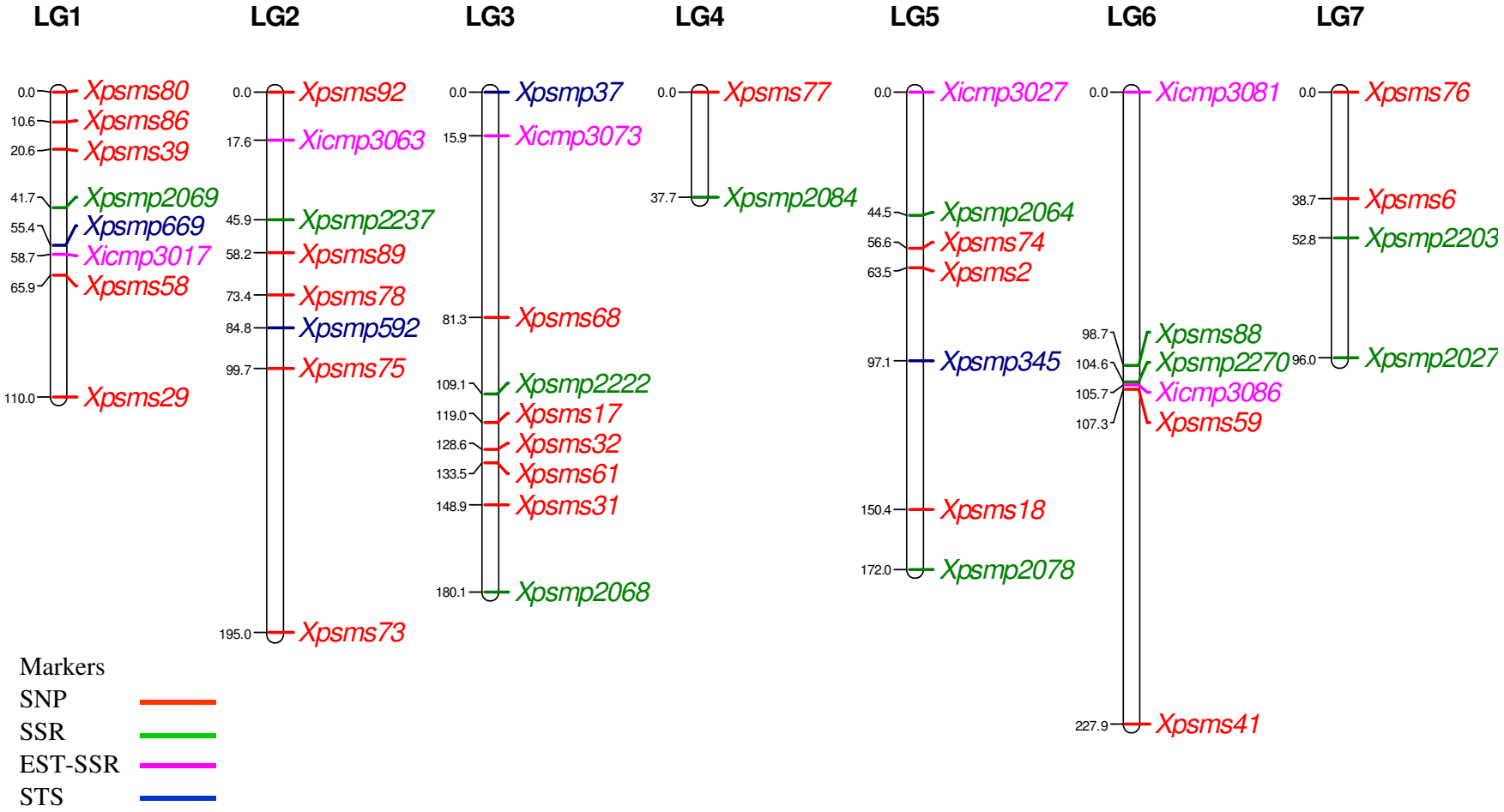


Figure 7. Linkage map constructed using 44 markers in $F_{2:3}$ pearl millet mapping population.

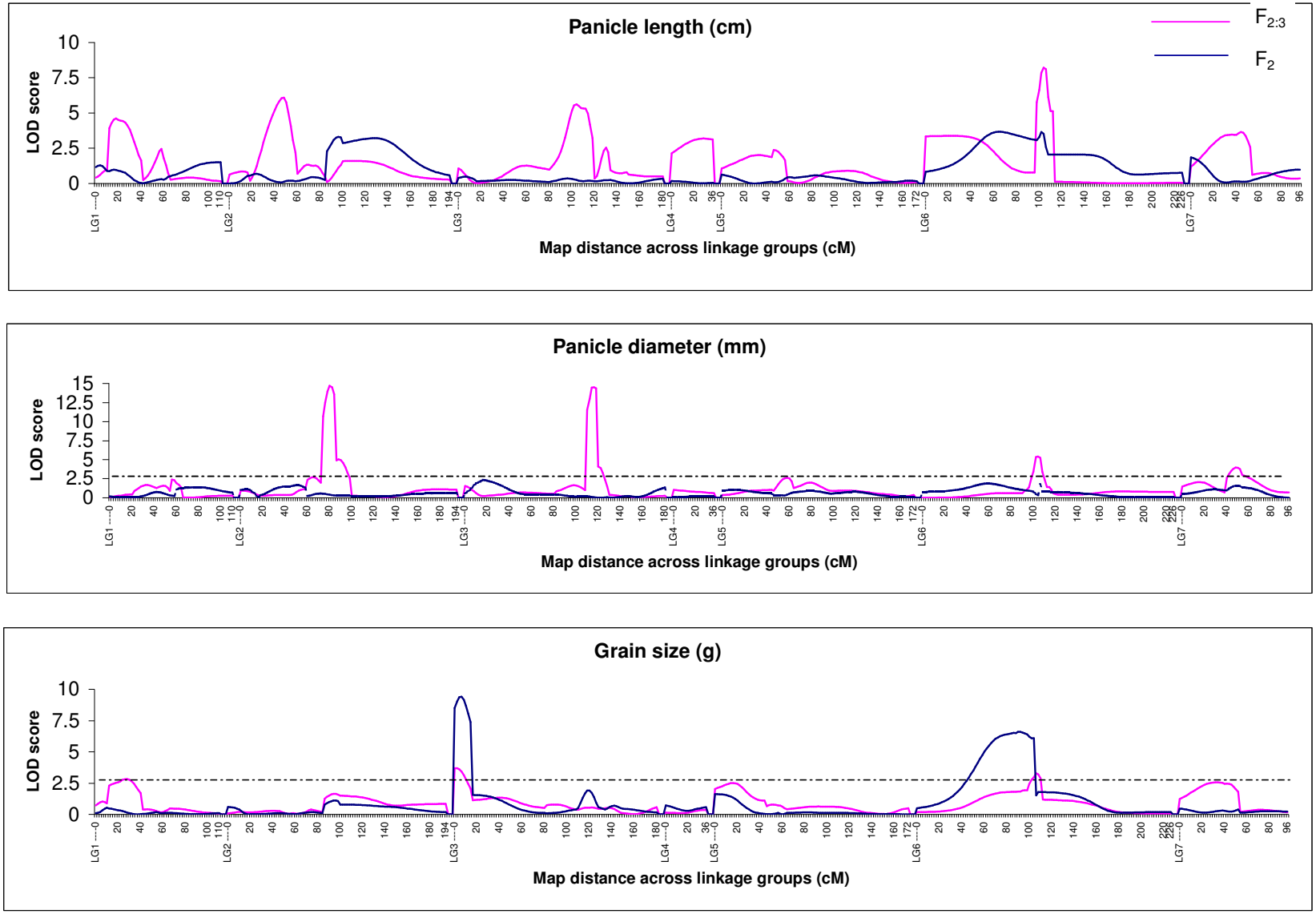


Figure 8. QTL LOD peaks detected for panicle length, panicle diameter and grain size in the F_2 and $F_{2:3}$ mapping populations.

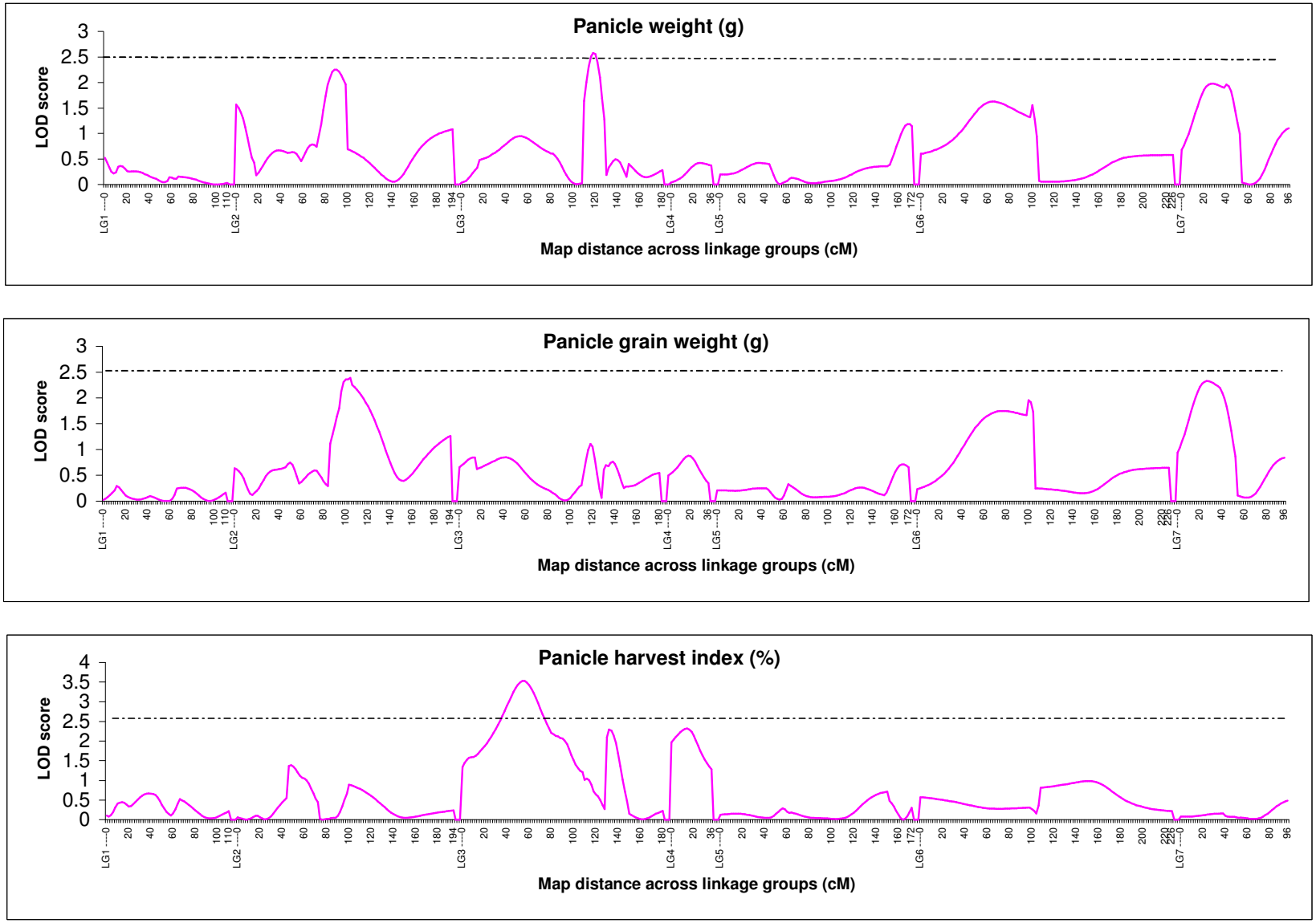


Figure 9. QTL LOD peaks detected for panicle weight, panicle grain weight and panicle harvest index in the $F_{2:3}$ progenies.

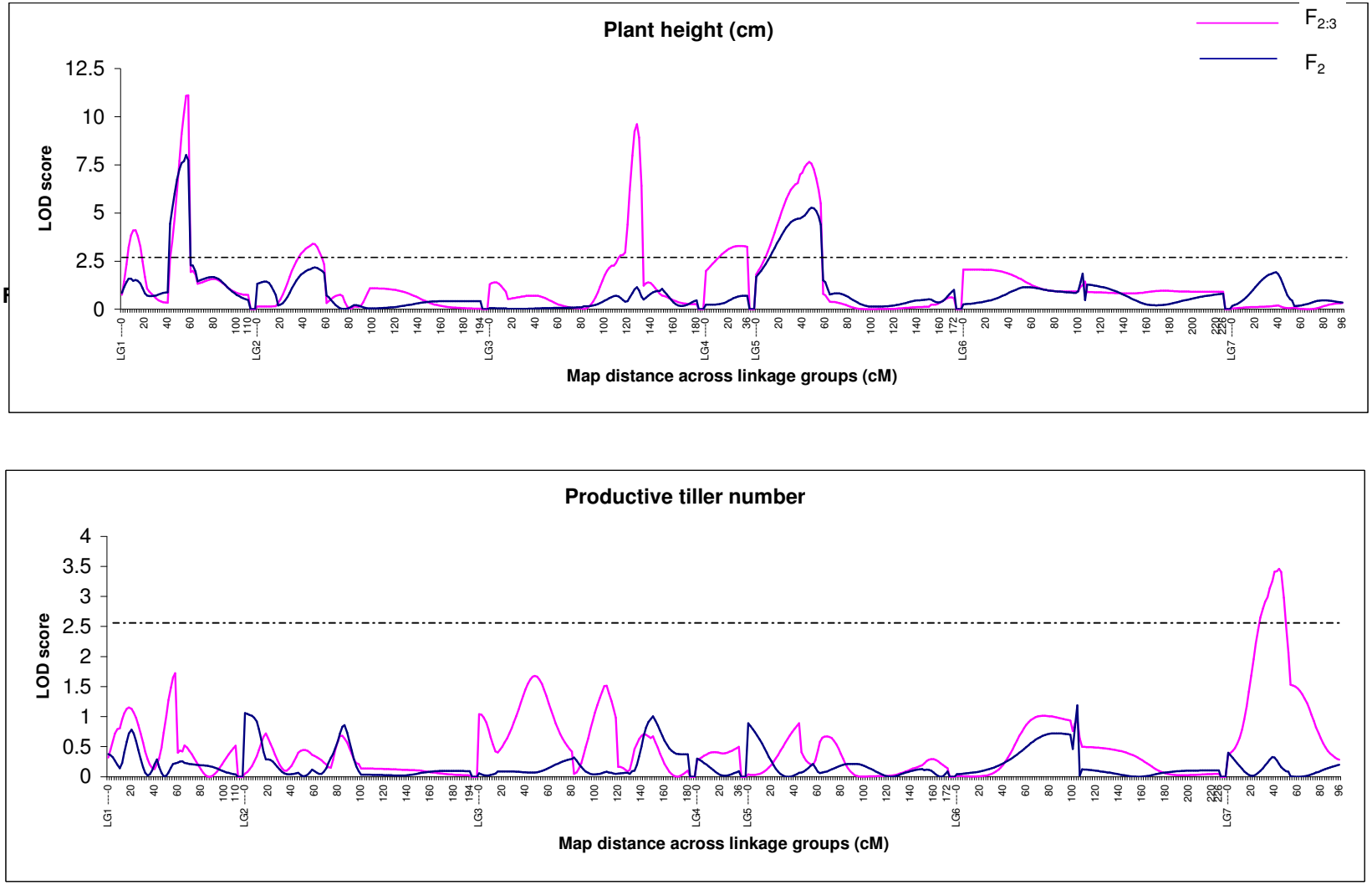


Figure 10. QTL LOD peaks detected for plant height and productive tiller number in the F_2 and $F_{2:3}$ mapping populations.

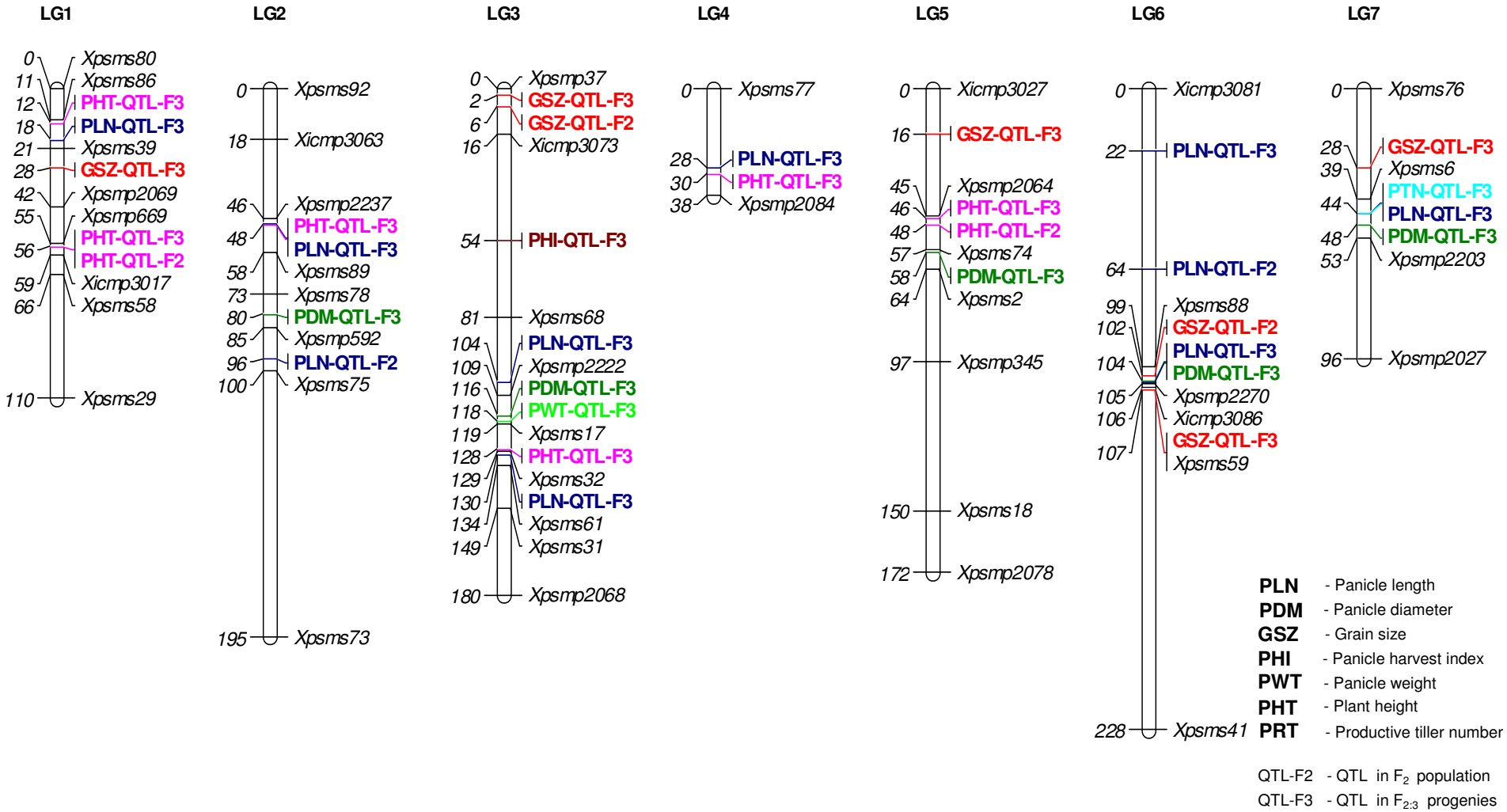
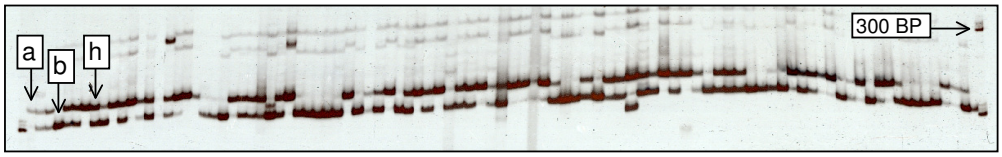
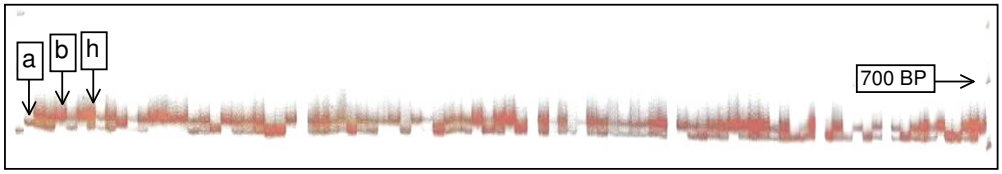


Figure 11. Linkage map showing the position of detected QTLs across the F_2 and $F_{2:3}$ mapping populations.

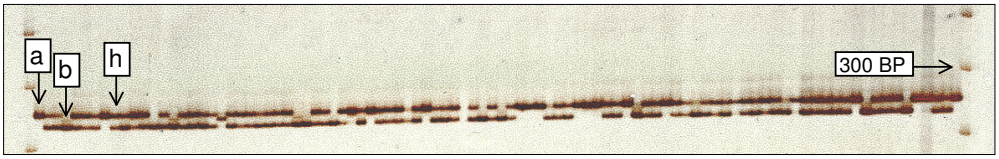
a) EST-SSR (*Xicmp* 3081) marker screened using PAGE gel.



b) STS (*Xpsmp* 592) marker screened using PAGE gel.



c) SSCP- SNP (*Xpsms* 31) marker screened using MDE gel.



d) Genomic SSR (*Xpsmp* 2064) marker screened using ABI Prism 3700.

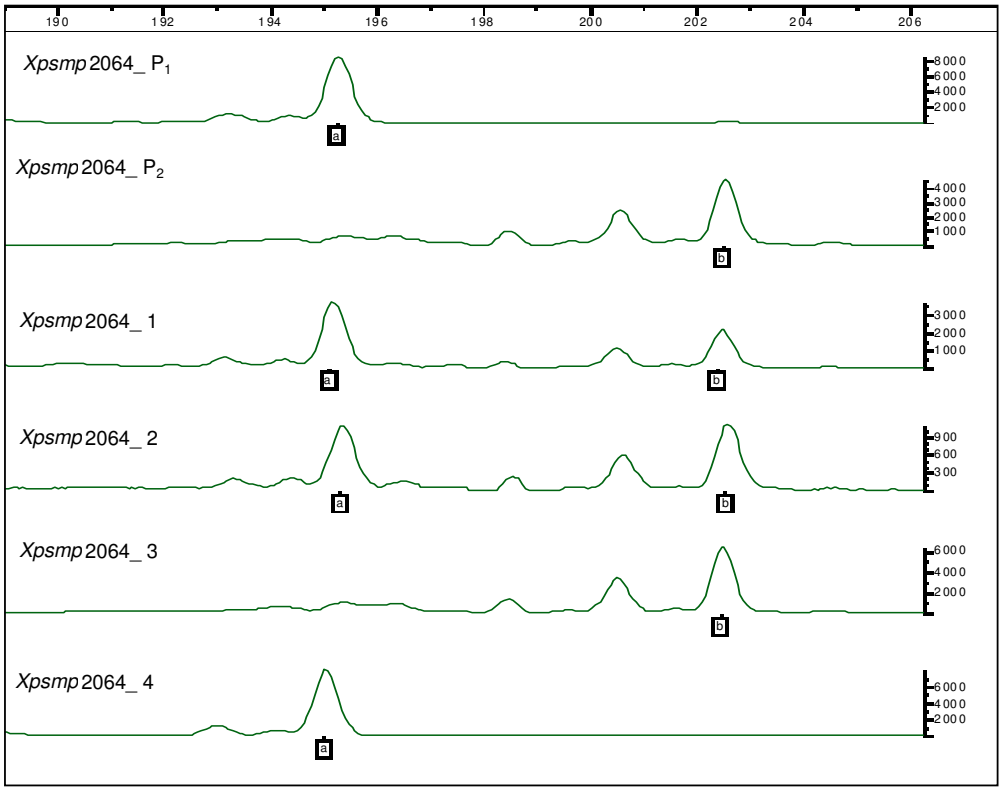


Plate 1 : Images of PCR products of EST-SSR (a) and STS (b), SSCP-SNP (a) and genomic SSR (d) markers screened on $F_{2:3}$ mapping population.

Appendix 1. Reagents required for DNA extraction.

S.No.	Chemicals / Reagents	Chemical composition / Remark
1	3% CTAB (Cetyl Trimethyl Ammonium Bromide) buffer	10 mM Tris 1.21 g 1.4 M NaCl 8.18 g 20 mM EDTA 0.745 g 3% CTAB 3.0 g Distilled water 100.0 ml Adjust to pH 8.0 using HCl. Add 0.17 ml mercaptoethanol only at the time of keeping the buffer in boiling water.
2	Chloroform:Isoamyl Alcohol (24:1)	Chloroform 96 ml Isoamyl alcohol (IAA) 4 ml Store in dark at room temperature.
3	Isopropanol	Keep isopropanol at –20°C. Use only ice cold isopropanol.
4	RNase A (10 mg/ml)	Dissolve 100 mg of pancreatic RNase A in 100 ml of 10 mM Tris (pH 7.5) and 15 mM NaCl. Heat in boiling water bath for 15 minutes and allow to cool slowly to room temperature. Dispense into aliquots and store at –20°C. Working stocks may be stored at 4°C.
5	Phenol:Chloroform:Isoamyl Alcohol (25:24:1)	Phenol (equilibrated) 50.0 ml Chloroform:IAA (24:1) 50.0 ml Store at 4°C.
6	Sodium Acetate (3 M, pH 5.2)	Dissolve 40.824 g of sodium acetate in 60 ml distilled water and adjust to pH 5.2 using glacial acetic acid. Make the volume up to 100 ml with distilled water and autoclave.
7	Absolute Ethanol	Store at –20°C
8	70% Ethanol	Absolute ethanol 70 ml Distilled water 30 ml
9	$T_1E_{0.1}$ Buffer	10 mM Tris 121.0 g 1mM EDTA 0.0372 g Distilled water 100.0 ml
10	$T_{10}E_1$ Buffer	0.5 M Tris 6.050 g 0.5 M EDTA 9.306 g 2 M NaCl 11.688 g Distilled water 100.0 ml

Appendix 2. Reagents required for DNA quantification.

S.No.	Chemicals / Reagents	Chemical composition / Remark
1	0.8% agarose	0.8 g agarose was dissolved in 100 ml of 1X TBE buffer.
2	10X TBE buffer	Dissolve 109 g of Tris and 55 g of boric acid one by one in 800 ml distilled water and add 40 ml of 0.5 M EDTA (pH 8.0). Bring volume to 1 litre with distilled water. Sterilize by autoclaving and store at 4°C.
3	Ethidium bromide (10mg/ml)	Dissolve 100 mg of ethidium bromide in 10 ml distilled water. Wrap the tube in aluminium foil and store at 4°C.
4	Orange loading dye	0.5 M EDTA (pH-8.0) 10 ml 5 M NaCl 1 ml Glycerol 50 ml Distilled water 39 ml Add orange dye powder till the colour becomes sufficiently dark.

Appendix 3. Reagents required for polyacrylamide gel electrophoresis.

S.No.	Chemicals / Reagents	Chemical composition / Remark
1	0.5 M EDTA (pH 8.0)	Dissolve 186.12 g of EDTA (MW 372.24) in 750 ml of distilled water. Add NaOH pellets to raise the pH to 8.0. Make up the volume to 1000 ml with distilled water and autoclave.
2	1 M NaOH	Dissolve 40 g of NaOH (MW 40) in distilled water to a final volume of 1000 ml and autoclave.
3	Acrylamide/Bisacrylamide (29:1 V/V)	40% Acrylamide 145 ml 2% bisacrylamide 5 ml Make up the volume to 200 ml with distilled water. Solution can be stored up to one month at 4℃.
4	Ammonium persulphate (APS)	10% (w/v) APS in distilled water. Store at 4℃.
5	Bind Silane buffer	Bind Silane 1.5 ml Acetic acid 5.0 ml Ethanol 993.5 ml Store at 4℃.
6	Repel silane	Commercially available (Amersham Biosciences AB Lippsata, Sweden).
7	10X TBE (Tris Borate EDTA buffer)	Dissolve 109 g of Tris and 55 g of boric acid one by one in 800 ml distilled water. Add 40 ml of 0.5 M EDTA, adjust solution to pH 8.0, and make volume to 1000 ml with distilled water. Sterilize by autoclaving and store at 4℃.
8	Orange loading dye	0.5 M EDTA (pH 8.0) 10 ml 5 M NaCl 1 ml Glycerol 50 ml Distilled water 39 ml Add orange dye powder until colour is sufficiently dark.
9	100 bp ladder	Mix 50 µl 100 bp ladder (stock concentration is 1 µg/µl, Amersham), 165 µl Bromophenol blue dye (6X), and 785 µl $T_{10}E_1$ (marker concentration is 50 ng/µl, 2 µl is sufficient for loading on polyacrylamide gels and 3.5 µl on agarose gels)
10	6X Bromophenol blue Dye	Bromophenol blue 200 mg Xylene cyanol FF 200 mg Glycerol 24 ml Distilled water 56 ml

Appendix 4. Reagents required for silver staining.

S.No.	Chemicals / Reagents	Chemical composition / Remark
1	Distilled Water	2 litres de-ionized water
2	0.1% CTAB	2 g of CTAB in 2 litres of distilled water
3	0.3% Ammonia solution	26 ml of 25% ammonia solution in 2 litres of distilled water
4	0.1% Silver Nitrate solution	Dissolve 2 g silver nitrate in 2 litres of distilled water and add 8 ml of 1 M NaOH. Stir well and add ammonia solution till the solution becomes colourless
5	Developer	Dissolve 30 g of sodium carbonate and 400 µl of formaldehyde in 2 litres of distilled water
6	Fixing solution	30 ml of glycerol in 2 litres of distilled water