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Epistasis is an important genetic basis of grain size in pearl millet

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Abstract
Epistatic interactions contribute significantly to the genetic basis of variation present in grain size of pearl millet. This is demonstrated with the classical genetic experiment involving generation means and triple test cross (TTC) analyses and also through the QTL analysis involving $F_{2:3}$ progenies of a single cross between inbreeds of contrasting grain sizes. The data on grain size were obtained from six basic generations (P_1, P_2, F_1, F_2, BC_1 and BC_2), 180 TTC families and 188 $F_{2:3}$ progenies for generation means, TTC and QTL analysis, respectively. The results of classical genetic analysis revealed that a large part of the genetic variation of grain size was under the epistasis control, particularly interactions of the dominance x dominance and dominance x additive, which were largely significant in both the generation means and TTC analysis. The presence of epistasis must imply multiple QTL for grain size. The QTL analysis located five significant main effects QTLs in the regions of chromosome 1, 3, 5, 6 and 7. Furthermore, significant epistatic effects, additive x dominance and dominance x dominance were also observed among the detected QTLs. The results of QTL analysis are consistent with the classical genetic approaches for the nature of epistatic interactions. The presence of epistatic interactions through classical genetic analysis and among the detected QTLs for grain size suggested that the marginal effects could be severally biased. Epistasis therefore needs to be considered, as the nature of interactions can guide the researcher to the selection of appropriate genetic background to obtain maximal gain.

Keywords: Epistasis; grain size; pearl millet; generation means; triple test cross; QTL analysis

1. Introduction
Grain yield in pearl millet was positively related to grain size. Large grain size is also advantageous in crop establishment, conferring improved rates of seedling emergence and plant stands, faster initial seedling growth, and faster early growth. In addition, large grain size improves processing quality of the grain. A large grain size can also bring a higher market price and is often cited as preferred characteristic in new pearl millet cultivars in farmer surveys (Bidinger and Raju 2000) [1]. Thus, lot of emphasis is being given to incorporate large grain size in the hybrids and parental lines in pearl millet breeding programmes. However, selection for grain size in pearl millet breeding programmes has not always produced the desired gains desired. For instance, Khadr and Qyinlye (1978) [18] reported that three cycles of mass selection for increased grain size and grain yield of pearl millet gave inconsistent responses. The extent to which mass selection can change a particular trait, however is expected to correspond with its heritability. The heritability estimate for grain size is likely to be low because of effects of gain mass of seed number or percentage seed set, inbreeding depression, and inter – intra plant and panicle competition for assimilates (Hash 1986) [14]. From these studies we could infer that grain size is a complex quantitative trait, and might controlled by polygenes that have a relatively small effects. There is great possibility that epistatic interaction accounts for a significant proportion of the genetic variation of quantitative traits. The prevalence and nature of epistasis are central to a range of questions in quantitative genetics.
Epistasis is the interaction between different genetic loci in determining phenotype. Evidence for the presence of epistasis has been established by comparing the average phenotype of various generations (the F_1, F_2 or backcross progenies) to corresponding values of the

parental lines. The mean phenotype of F_2 individuals should be exactly intermediate between the F_1 and midparent means in the absence of epistasis. The often observed "F_2 breakdown" where the average F_2 phenotype is substantially lower than expected, is thus an example of epistasis. The estimation of epistatic effects from first order statistic (i.e., generation means) reflect sums of effects at individual loci may cancel each other. An elegant extension of the design III proposed by Kearsey and Jinks (1968) [17], named the triple test cross (TTC) design, provides a powerful test of significance for the presence of epistasis. In TTC deign, testcross are produced not only with the two parental lines but also with the F_1 derived from them. The mean analysis may detect epistatic interaction nearly fixation, while the variance (TTC) analysis resulting from the same allele may be small. Either analysis taken alone may produce an ambiguous indication of the epistasis, but together provide a clear picture on the epistasis of the trait. With both the methods, the inability to detect epistasis cannot be taken as evidence for the absence of epistasis because of the canceling of epistatic effects among loci. New approaches and methods for estimating epistasis for quantitative traits needed to be considered.

New insights from molecular approaches have also demonstrated the importance of epistatic interaction in the inheritance of complex traits. Importance of epistasis as the genetic basis of heterosis has been reported in rice (Yu *et al*. 1997; Xing *et al*. 2002; Mei *et al*. 2003) [44, 40, 28], maize (Doebley *et al*. 1995) [9] and *Arabidopsis thaliana* (Syed and Chend 2005) [36]. Additional studies have reported epistasis among QTL for various traits including grain weight in rice (Gao *et al*. 2004) [11], grain yield and its components in maize (Ma *et al*. 2007) [22], fruit quality in tomato (Causse *et al*. 2007) [6], oil content in rapeseed (Zhoa *et al*. 2005) [45], pollen sterility in sunflower (Kim and Rieserberg 2001) [19] and tolerance to barely yellow dwarf virus in Oat (Zhu *et al*. 2003) [46]. Amount and type of epistasis present in crop species can have major consequence on both the reliability of predictions and the design of breeding programmes. Its presence may also have important consequence on the success of detection, introgression and characterization of the genes controlling quantitative traits. There is a risk that the estimated effects of detected QTLs could be severely biased in cases in which epistasis is ignored. Overestimation of individual QTL effects leads to erroneous interpretations of the relative importance of detected QTLs, but also to problems with confirming QTL effects in further crosses and to lower economic gain if attempts are made to use the QTLs. Therefore, epistasis needs to be considered if choosing the validation strategy for the detected QTLs, as the nature of the interaction can guide the researcher to the appropriate genetic background to obtain maximal power for replication (Carlborg and Haley 2004) [4].

The aims of present study were to determine the extent of epistasis variation resident for grain size in pearl millet. A pair of large contrasting lines was used to synthesize a set of line-cross derivatives for generation means, TTC and QTL analysis. The progenies produced from this array were evaluated for grain size in a single experiment. This study addresses two basic questions: Do the grain size exhibit significant epistasis? If so, are epistatic effects consistent in their direction across different sets of analysis?

2. Materials and Methods

2.1 Plant materials and development of genetic populations

A pair of contrasting inbred lines for grain size with diverse pedigree having similar flowering period were selected from the trait-specific breeding lines during 2005 rainy season for the genetic basis of grain size. The inbred line (81B x 4025-3-2-B)-11-5-2-2-B-2-B had small grain size (1000-grain weight is 5g) and the inbred line HHVBC II D2 HS-302-3-1-6-8-2-6-2-B had large grain size (1000-grain weight is 13g). These inbred lines were used as source material for generating three groups of genetic populations.

The first group included the basic genetic populations for generation mean analysis, the parental lines were sown in 4 m four-row plots in a crossing block to generate F_1s during post-rainy season of 2005-06. The crosses were made between line with low trait value as seed parent and line with high trait value as pollen parent during January and February 2006. Crossed (F_1s) and selfed panicles were harvested separately. Panicles were threshed after proper drying to the optimum moisture content. The seeds of parental lines and their F_1's were planted under greenhouse condition for generating F_2 and back cross seeds. Parental lines were sown in 5 pots and their F_1s 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_1s were selfed to generate the F_2 seeds and also backcrossed with their female and male parent to generate BC_1 and BC_2 seeds, respectively during May-June 2006. Thus, seeds of six generations (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 second group of material (TTC families), the parental lines and the F_1s were sown in 4 m two-row plots at three staggered sowing with one-week interval to synchronize with the flowering period of the F_2 population, which were planted in 4 m twenty row-plot during 2006 rainy season. More than 200 F_2 plants were selfed and pollens collected from 100 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}), but sufficient seeds were obtained for all three familes from only 60 of F_2 individuals.

The F_2 populations were also utilized to generate F_2-derived F_3 ($F_{2:3}$) mapping progenies. A set of 188 $F_{2:3}$ progenies were derived from the F_2 population constituted third group of material, which were subjected to both genotyping and phenotypic observation.

2.2 Field experiments and phenotypic observations

Six basic generations, triple test cross families and $F_{2:3}$ mapping progenies were evaluated as one trial during 2007 summer season. Six generations (P_1, P_2, F_1, F_2, BC_1 and BC_1) were planted in a randomized complete block design with 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. The TTC families were evaluated along with the generation means in a randomized complete block design with three replications during 2007 summer season. This consisted of 180 TTC families (60 each of L_{1i}, L_{2i} and L_{3i}) for each trait specific groups, which were planted in single-row plots. In the same experimental plot, 188 $F_{2:3}$ mapping populations were also evaluated for phenotypic observations on grain size. 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. In this experiment, 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.

The grain size was measured as index from weight of 1000 grains (measured in grams), taken from main panicle of each entry. For generation mean 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 the TTC analysis, observations were recorded on 10 competitive plants from each of the 180 TTC families in each replication. In the mapping population, observation on grain size was recorded from 10 random plants in each of 188 $F_{2:3}$ progenies and parents.

2.3 Statistical analysis

2.3.1 Generation mean analysis: The basic generations data obtained were subjected to scaling test for 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) [23]. Joint scaling test of Cavalli (1952) [7] was also performed to estimate the three-parameter mid-parental value (m), dominance (h) and additive (d) gene effects following the least square method (Mather and Jinks 1971) [26]. 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. In case of inadequacy of three-parameter model revealed through the scaling or join scaling test, 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.

2.3.2 Triple test cross analysis: Triple test cross (TTC) analysis has been carried out using the model proposed by Kearsey and Jinks (1968) [17]. 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.

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

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.

Before testing individual epistasis, the homogeneity of the interaction was first tested. 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. If it is significant, each of three types of epistasis can be tested against their respective interaction with blocks. On the assumption of no epistasis, an additive-dominance model was also fitted for the observed data as outlined by Kearsey and Jinks (1968) [17].

2.3.3 Linkage map and QTL analysis

The genotyping of the $F_{2:3}$ mapping population and the construction of the linkage map were described in detailed in a paper by Vengadessan *et al*. (2013) [38]. In this linkage map, collinear markers were removed from the analysis before the map was constructed using Mapmaker/Exp 3.0 (Lander *et al*. 1987). The map obtained spans a total length of 1018 cM and comprises 44 loci including 24 single-strand conformational polymorphism - single nucleotide polymorphism (SSCP-SNP), 10 SSR, 6 EST-SSR and 4 STS markers. This map covered a substantially larger proportion of the pearl millet nuclear genome and is comparable with earlier reported linkage maps for this species.

The grain size data sets of 188 F_2 plants and the predicted means of $F_{2:3}$ progenies and their genotyping data from 44 markers were used to identify genomic regions associated with grain size using composite interval mapping (CIM) analysis. Computations were performed using the software package PLABQTL ver 1.1 (Utz and Melchinger 1995) [37], which performs CIM using a regression approach (Haley and Knott 1992) with selected markers as cofactors. Since the mapping population used in the present study constituted of $F_{2:3}$ progenies, along with the additive (A) model, additive-dominance (A+D) and all three type of epistatic interaction (AA+AD+DD) were also estimated between the detected QTLs. 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 allele from female parent was contributed to the trait of interest and negative if allele from male parent contributed towards the trait of interest.

3. Results

3.1 Generation means analysis

Analysis of variance performed for the non-segregating generations (P_1, P_2 and F_1) revealed non-significant variation between blocks for grain size, and hence the data in each block of a season were pooled for generation mean analysis. The mean performance of six basic generations (P_1, P_2, F_1, F_2, $BC_1$and BC_2) for panicle length, panicle girth and grain size are presented in Table 1. Small grain size parent (P_1) had 5.0 g of 1000-grain weight, whereas in the large grain size parent (P_2) it had13.50 g. The mean of F_1 was lower than the mid parental value. The F_2 mean was substantially differed with other generation and varied drastically for grain size. The means of BC1 and BC2 progenies resembled their respective recurrent parents.

The results of scaling tests for grain size revealed that scale A showed non-significance, however scale B and C significantly differed from zero. The three parameters (m, d and h) obtained using joint scaling test were also found to be highly significant for this trait. The additive effect (–4.0) was higher in magnitude than the dominance effects (–0.9). The estimated chi-square values were also found to be highly significant, suggesting the presence of epistatic interactions. Since both scaling and joint scaling test results revealed inadequacy of simple additive-dominance model, six-parameter model, which included all possible digenic interactions, was adopted and the results are presented in Table 1. Both the additive and dominance effects were highly significant. The magnitude of additive effect (–3.0) was higher than dominance effect (–1.7). All three types of digenic interactions were highly significant. The dominance x dominance interaction (3.8) was higher in magnitude than the additive x additive (–1.1) and additive x dominance (1.2) interaction components, respectively. The presence of duplicate epistasis was inferred from the negative sign of dominance component and positive sign of dominance x dominance component.

3.2 Triple test cross analysis

TTC analysis was carried as per the method given by Kearsey and Jinks (1968) [17], and the results are presented in Table 1. Analysis of variance for grain size showed that the interaction of blocks with additive x additive (i) as well as additive x dominance and dominance x dominance (j + l) epistatic components were 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 for grain size. When the overall epistasis was partitioned, the results showed non-significant additive x additive (i) epistasis and highly significant additive x dominance and dominance x dominance (j + l) type of epistasis for grain size. 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 lower than the dominance component (12.34). The degree of dominance being more than unity (1.12) revealed overdominance for this trait. The value for correlation coefficient (r_{sd}) was non-significant, indicating symmetrical distribution of dominant alleles among parents.

Table 1: Generation means and triple test cross analysis for grain size.

GMA parameters with standard error						**TTC parameters**	**DF**	**MSS**	
Means						**Additive model (L_{1i} + L_{2i})**			
P_1	5.03	±	0.08			Replication	2	1.57	
P_2	13.52	±	0.09			Lines (sums)	59	7.97	**
F_1	8.73	±	0.12			Error	118	0.57	
F_2	8.62	±	0.07						
BC_1	6.82	±	0.09						
BC_2	9.83	±	0.10						
Scaling test						**Dominance model (L_{1i} - L_{2i})**			
A	-0.11	±	0.24			Replication	2	1.74	
B	-2.58	±	0.25	**		Lines (differences)	59	9.93	**
C	-1.53	±	0.39	**		Error	118	0.67	
Joint scaling test						**Genetic components**			
m	9.11	±	0.06	**		Additive component (D)		9.87	
(d)	-4.02	±	0.06	**		Dominance component (H)		12.34	
(h)	-0.90	±	0.12	**		Degree of dominance		1.12	
χ^2 value	114.16	**				Direction of dominance (r)		-0.09	
Six-parameters						**Epistatic model (L_{1i} +L_{2i} - 2L_{3i})**			
m	8.62	±	0.07	**		[i] type epistasis	1	5.21	
(d)	-3.01	±	0.14	**		[j+l] type epistasis	59	21.68	**
(h)	-1.71	±	0.42	**		Total epistasis	60	21.40	**
(i)	-1.15	±	0.39	**		[i] x block	2	1.55	
(j)	1.23	±	0.15	**		[j+l] x block	118	2.01	
(l)	3.84	±	0.67	**		Total epistasis x block	120	2.01	
$(h/d)^{1/2}$	0.75								

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

3.3 QTL analysis

3.3.1 Phenotypic analysis: The analysis of variance for the replicated phenotypic data set from the $F_{2:3}$ trial showed that variances due to mapping progenies were highly significant ($P \geq 0.01$) for grain size. The mean performance for grain size (1000-grain weight) in P_1 was 5 .3 g, while in P_2 it was 11.7 g, whereas in $F_{2:3}$ progenies it was 8.1 g. The heritability estimate for grain size was was high (0.81). The frequency distribution for grain size represented a continuous symmetrical distribution (Figure 1).

3.3.2 QTL analysis for grain size: The additive model detected two QTLs for grain size using the $F_{2:3}$ progeny data set (Table 2). 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. The favourable alleles for all these QTLs were from P_2 parent. This model explained a total of 13.3% of observed variation for grain size. 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. 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. 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%. 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%. This model explained observed variation of 29.6%. In general, across three genetic models, a total of 5 QTLs were identified. These QTLs were distributed across LG 1, 3, 5, 6 and 7 (Figure 1). Individual QTLs explained 6.1 to 8.9% of the observed phenotypic variation. The epistatic model detected significant interactions among all the detected QTLs. The observed variation for grain size was best explained through the epistatic model (29.6%).

Table 2: QTLs identified for grain size in the $F_{2:3}$ mapping population

Genetic 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													
	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
Additive + Dominance													
	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
Epistatic													
	1	28	Xpsms39 - Xpsmp2069	2.8	6.7	1.2	(2.2)	-2.0	(4.4)	D1*D6	1.3	(3.4)	
	3	2	Xpsmp37 - Xicmp3073	3.7	8.9	0.9	(0.6)	1.6	(1.6)	D1*A7	1.0	(4.3)	
	5	16	Xicmp3027 - Xpsmp2064	2.5	6.4	-1.2	(0.5)	-2.8	(1.5)	A3*D5	-1.3	(3.1)	
	6	106	Xicmp3086 - Xpsms59	3.3	7.7	-1.4	(2.0)	-2.3	(4.2)	D3*D5	-1.7	(3.7)	
	7	32	Xpsms76 - Xpsms6	2.6	6.1	0.0	(0.0)	0.2	(0.0)	D5*D6	3.8	(3.4)	29.6

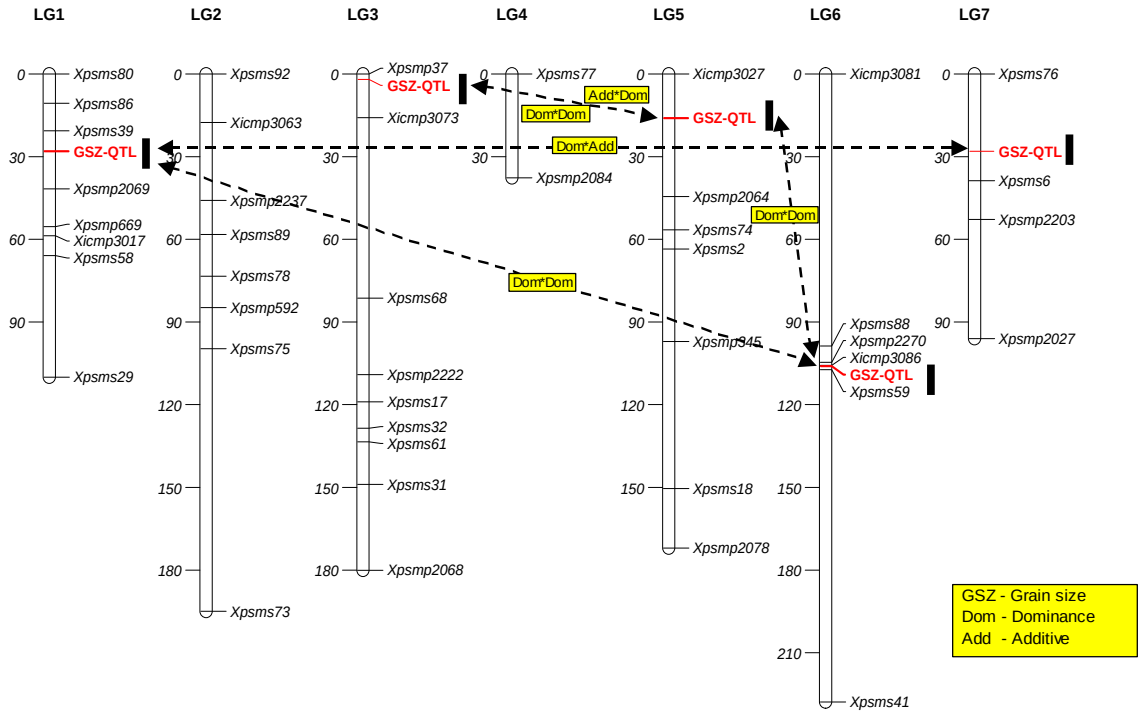

Fig 1: Linkage map showing the position and epistatic interactions between the detected QTLs of grain size in $F_{2:3}$ mapping population

4. Discussion

Epistasis seems inevitable when one considers the bio-chemical pathways that culminate in the expression of quantitative traits, which comprises of gene networks that interact at the genetical and molecular levels. The extent to which epistasis is involved in regulating complex traits is not known, and so we cannot assume that epistasis will be found for every trait in every population. However, epistasis has been overlooked for too long and needs to be routinely explored in complex trait studies since its presence had important consequence in the design of breeding programmes. In the present study, we explored the presence of epistasis for grain size in pearl millet by considering epistatic models of conventional genetic (generation means and triple test cross analysis) and recent QTL analyses.

Considering the results of first and second-degree statistics, generation means analysis (first degree) provides strong evidence for the presence of epistatic interactions for grain size since the estimates of scales B and C are highly significant, and 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 chi-square test showed highly significant values, and thus warranted the use of the six-parameter model to estimate the epistatic effects. The TTC analysis of variance (second degree) also reveals the presence of total epistasis. Six-parameter model and TTC analysis revealed that both additive and dominance effects were significant for grain size. The role of both additive and dominance gene effects for the inheritance of grain size confirmed the earlier reports of Gotmare and Govila (1999) [13], Chand *et al*. (1973) [8] and Phul and Athwal (1969) [31]. The generation means revealed higher magnitude of additive component than dominance component 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. However, the estimates of additive and dominance components for grain size may not be free from bias, since both the generation means and TTC analyses signified 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 canceling out 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) [27]. 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 that the dominance allele have increasing effects on the grain size.

All three types of epistatic interaction are found to be highly significant for the inheritance of grain size in generation means, however the dominance x dominance epistasis was higher in magnitude than additive x dominance and additive x additive epistasis. Whereas in TTC analysis, the partitioning of total epistatic variation 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. These finding corresponds to Chand *et al*. (1973) [8] who reported the significance of both additive x dominance and dominance x dominance interactions for grain size. Whereas, Gill *et al*. (1974) [12] reported only additive x dominance (j) interaction for this trait. However, Phul and Athwal (1969) [31] 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. The observed large significant dominance component of interaction for grain size is a recognizable pattern underlying genetic parameters for fitness traits that might be the result of directional selection (Mather 1966; Mather 1983) [24, 25]. Willis and Orr (1993) [39] 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 epistasis. Earlier studies made by Singh *et al*. (1972) [34] also reported duplicate epistasis for this trait, however Phul and Athwal (1969) [31] indicated the presence of complementary epistasis for grain size.

The most frequently used statistical methods for QTL analysis of experimental crosses only model the marginal genetic effects (additive / dominance) of individual loci, thus ignoring interactions between QTL (epistasis). The identification of epistatic loci is an important step toward resolution of discrepancy between QTL mapping and classical genetic dogma, contributes to better understanding of the persistence of quantitative genetic variation in populations, and implies reconsiderations of optimal mapping methodology and marker-assisted breeding strategies for improvement of complex traits (Li *et al*. 1997) [21]. According to Carlborg and Haley (2004) [4], epistatic model is necessary for validating the importance of the detected QTLs, the knowledge on the type of interactions can guide a researcher to choose the appropriate genetic background of recipient lines in MAS to obtain maximal gain. There are several methods for mapping epistatic QTL in experimental population (Kao *et al*. 1999; Sen *et al*. 2001; Carlborg and Anderson, 2002; Yi *et al*. 2003) [16, 33, 5, 43]. Recently, Xu *et al*. (2007) [41] addressed a Bayesian - based modeling approach that avoids model selection problems and fits all main effects and all interactions simultaneously, but shrinks the estimates of effects with little statistical support to zero. The use of these methodologies poses more technical challenges and demands more from the data than individual QTL mapping. For these reasons, epistatic QTL mapping is not yet a standard tool in complex trait studies. However, Holland (2007) opinion that one can test for epistatic effects between QTL that have significant main effects, which greatly eases the model selection problems.

In the present study, epistatic interactions were tested between the QTL have significant main effects by applying epistasis model using PlabQTL software. A total of five putative QTLs detected for grain size on LG 1, 3, 5, 6 and 7 using $F_{2:3}$ data set across different genetic models. The QTLs mapped on LG 1 and LG 3 appear to be comparable to those reported by Bidinger *et al*. (2007) [2] 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) [42]. 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. The lower level 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 to the hypothesis that polygenes controlling important metric traits such as grain size are usually distributed among several identified QTLs that may not be linked to one another (Fatokun *et al*. 1992) [10]. Furthermore, significant epistatic interactions, additive x dominance and dominance x dominance were observed among the detected QTLs. The presence of epistatic interactions among the detected QTLs suggested that the marginal effects of these QTLs could be biased. Marker-assisted transfer of single QTL effecting grain size based on marginal effect may be a fruitless endeavor since all the detected QTLs show strong epistasis with one another and so fail to interact with target genome in the same way as with donor genome. Before attempting for marker-assisted transfer of QTLs from the donor to receipt parent, breeder should have profound information of the genetic background of recipient parent to obtain maximal gain. With a more profound understanding of epistasis, breeders may be empowered to utilize new allele from genetic resources that exhibit favorable epistasis with the genetic background. The analysis of epistatic interactions will be of increasing importance in future molecular quantitative genetic research and for marker-assisted breeding.

The most noticeable finding of the present study based on classical genetic and QTL analysis is the prevalence and importance of epistasis particularly dominance interactions for grain size in pearl millet as it explained a much greater portion of the total variation for grain that the main effects. 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 models are defined with reference to different types of population (Yang 2004). In both generation means and TTC analysis, the parameter captures 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 also confirmed the above interpretation to a large extent, except for additive x additive interaction which was found to be significant in generation means, however its magnitude was lower than additive x dominance and dominance x dominance interactions. Nanda *et al*. (1990) [30] 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 conventional genetic analysis on grain size reveal 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 5 QTLs were detected for grain size in this study, and also consistent with classical genetic approaches for the type and direction of epistatic interactions. Thus, the dominance x dominance and additive x dominance epistasis were predominantly observed among the detected QTL are in great agreement with generation means and TTC analyses. However, there are also inconsistencies. The generation means indicated significant additive x additive epistasis, although TTC and QTL analysis 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 had much larger population size.

In summary, as noted by Melchinger *et al*. (2008) [29] we are still at the beginning of understanding the complex interactions of individual genes and gene networks even with

extensive genomic tools at hand. Knowledge about epistasis will facilitate the assessment of gene action and function and will help to elucidate the quantitative genetic basis of complex traits. Furthermore, the combination of information from many type of analysis or data sources has improved the range and quality of conclusions that can be drawn, for example, our results provide strong evidence for the presences of pronounced epistasis for grain size in pearl millet, which has been concluded from the results of both classical genetic and QTL analyses.

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