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Generation mean analysis of gluten strength in bread wheat (*Triticum aestivum*): An effective utilization of micro-sedimentation test in early generation progenies

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ABSTRACT

Better bread-making quality is one of the most important targets in the quality improvement programmes of wheat (*Triticum aestivum* L.). Gluten strength is a major indicator of bread making quality and is measured by various methods such as the SDS-sedimentation test. However, the nature of gene action for gluten strength has not been studied so far. In the present study, generation mean analysis was undertaken to estimate the nature and magnitude of gene effects for gluten strength in two sets of crosses involving contrasting parents using a less known micro-sedimentation test. Scaling test indicated the involvement of epistasis on the expression of the trait and inadequacy of additive-dominance model. Therefore, both six and five parameter models were used to understand the nature and direction of gene action further. Similar types of gene-action for gluten strength were recorded, albeit with some differences in the direction and magnitude of the effects, in the two sets of crosses. Among the different epistatic components, dominance × dominance effect had the maximum contribution and was acting in the negative direction in both the crosses. Analysis of both the cross combinations using Castle-Wright's equation for 'number of effective genes/blocks' revealed that at least one major gene block besides several minor genes could be involved for the micro-sedimentation volume.

Key words: Epistasis, Generation means, Gluten strength, Micro-sedimentation, Wheat

Breeding for better bread-making quality is one of the most important objectives in the quality improvement programmes of wheat (*Triticum aestivum* L.). However, quality breeding is complex due to the polygenic inheritance of bread making quality and its component traits. Furthermore, negative correlations among different charecters and genotype × environmental interactions for many traits make progress using conventional breeding procedures even more difficult (Barnard *et al*. 2002). In addition, specific tests for early-generation selection that could speed up breeding and precision are limited (Mittelmann *et al.* 2000). Gluten strength has been reported to display a major influence on bread loaf volume (Blackman and Gill 1980, Preston *et al.* 1982, Pena 2009). It can be measured by early generation tests such as the sedimentation test or in later generations by farinograph indices, mixograph and alveograph test etc. Sodium dodecyl sulfate (SDS) sedimentation test developed by Axford *et al.* (1978) has gained wide acceptance as a useful, small-scale test in bread wheat breeding programs to predict gluten strength and baking quality. Higher SDS-sedimentation values correspond to higher gluten strength (Triboi and Martre 2004). However, this method requires relatively larger amounts of grain sample (six gram of whole meal per sample) and cannot be used for the evaluation of individual plants (Schuster *et al*. 1997). To carry out early generation selections and genetic studies, a reliable methodology requiring small amount of flour sample is required. A modified screening test for rapid estimation of gluten strength (also known as micro-sedimentation test) was proposed in durum wheat by Dick and Quick (1983). This test requires only one gram sample of whole meal as compared to six grams in case of SDS-sedimentation test. This also requires smaller amount of solutions, lesser equipments, is easy to perform, reliable and was suggested to be amenable to effectively evaluating gluten strength in the early segregating generations of a cross.

Estimation of gene action for a trait has a direct bearing upon the choice of breeding procedures to be followed in

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crop improvement programmes. The studies on gene action for gluten strength in bread wheat is lacking, though QTLs have been reported by a few researchers in durum wheat (Perreatant *et al.* 2000, Elouafi *et al.* 2000, Patil *et al.* 2009, Conti *et al.* 2011, Kumar *et al.* 2013). SDS- or Zeleny-sedimentation methods have been used in all these studies for phenotyping immortal mapping populations (recombinant inbred lines and doubled haploid lines). The present study was carried out using Generation Mean Analysis to understand the genetic components and their interactions influencing microsedimentation value in wheat as a measure of gluten strength.

MATERIALS AND METHODS

Four genetically diverse wheat genotypes, two with low gluten strength (HI 1500 and HD 2643), and the other two having high gluten strength (ADT 463 and ADT 432) were selected for this study and were obtained from Dr S S Singh, Professor, in the Division of Genetics (Table 1). ADT 463 and ADT 432 were also reported to be stable for SDS-sedimentation volume based upon the multilocation evaluation data (Kota 2008). HD 2643 and HI 1500 are released varieties of bread wheat developed at IARI, New Delhi and its Regional Station, Indore respectively. ADT 463 (P_1 of cross 1) was crossed with HI 1500 (P_2 of cross 1) and ADT 432 (P_1 of cross 2) with HD 2643 (P_2 of cross 2) representing high × low crosses to generate their respective F_1 generation at the IARI Research Farm, New Delhi during the winter (*rabi*) season 2008-09. Further, backcross [BC(P_1) and BC(P_2)] generations were developed by crossing the F_1s with their parents (P_1 and P_2) in the off-season nursery at IARI Regional Station, Wellington, Tamil Nadu during July-October 2009. The selfed seeds of the F_1 individuals were also harvested to obtain the F_2 generation simultaneously. A sample of F_2 seeds harvested from Wellington were grown at IARI Research Farm, New Delhi, during *rabi* season 2009-10 to obtain the seeds of F_3 generation.

All the generations [P_1, P_2, F_1, F_2, F_3, BC (P_1) and BC (P_2)] of each cross combination were evaluated in experimental trials during *rabi* 2010-11 at IARI experimental farm, New Delhi. After grain maturation, plants were uprooted, single plants separated and spikes of each single plant were hand harvested, threshed and stored in clean envelops. The threshed and cleaned seeds from each plant were milled (on Cyclotech Sample Mill 1093, Hooganas, Sweden) to obtain the whole meal from which 1g sample in replicate was evaluated with micro-sedimentation test.

Table 1 List of genotypes used in developing crosses

Genotype	Parentage	Developed at	Gluten strength[@]	Used in cross as parent
ADT 463 (DL 1144)	VEE/MJI//2*TUI/ 3/PASTOR	IARI, New Delhi	High (49.50ml)	Cross 1; P1
HI 1500	HW2002*2// STREMPALLI/ PNC-5	IARI Regional Station, Indore	Low (39.00 ml)	Cross 1; P2
ADT 432 (DL 1136)	CH 1B/A//PRL II/ CM 65531	IARI, New Delhi.	High (47.00ml)	Cross 2: P1
HD 2643	VEE'S'/HD2407// HD2329	IARI, New Delhi.	Low (36.50ml)	Cross 2; P2

[@]Based SDS-sedimentation volume of samples of two replications in 2009-10

The micro-sedimentation test for samples of individual plants was carried out as per the protocol suggested by Dick and Quick (1983) with minor modification of shaking time during vortexing (fifteen seconds in place of five seconds). The data for sedimentation volumes were obtained from 30 plants each of the P_1, P_2, and F_1 generations; 60 from each of BC (P_1) and BC (P_2) generations; 150 plants from F_2 and 100 plants from F_3 generation for each of the two crosses taken separately.

The generation means were estimated by considering means of micro-sedimentation volume of the grain samples of individual plants analyzed for that generation. The adequacy of the additive-dominance model was tested by the Scaling Test (Mather 1949) as well as the Joint Scaling Test (Cavalli 1952). Chi-square test was used to test the adequacy of the additive-dominance model, consisting of mean, additive, and dominance genetic effects. Where this model was found insufficient to explain variation among generation means, non-allelic interaction parameters, i e additive × additive, additive × dominance, and dominance × dominance, were included in the model and tested to identify the best fit model, based on the procedure suggested by Mather and Jinks (1982) in a sequential manner. Both six-parameter and five parameter models were tested and compared. The number of major gene blocks for the trait was calculated by Castle-Wright's equation (1921) as described by Sharma (1998). Statistical analysis was carried out using Windostat Version 8.0 software.

RESULTS AND DISCUSSION

Means of micro-sedimentation volumes of different generations were estimated (Table 2) and also tested for significance of genetic variation (Table 3) in segregating generations [F_2, F_3, BC (P_1) and BC (P_2)]. Genetic variability for microsedimentation was significant (at 5% level) in all the segregating generations for trait. Therefore, Generation Mean Analysis (GMA) could be carried out with both the crosses for this trait.

Scaling test and Joint scaling test for the three parameter model indicated the inadequacy of additive-dominance model and the involvement of non-allelic interactions on the expression of micro-sedimentation value (Table 4). Chi square test values were highly significant in case of both five and six parameter models for joint scaling test based on additive-dominance model. This suggested that epistatic interactions influence the trait which may be estimated through standard models. For micro-sedimentation value, estimates of six parameter model (Table 5) indicated highest

Table 2 Means of different generations in the two sets of crosses for microsedimentation volume (ml)

Generations	MS (Mean ± SE)	
	ADT 463 (P_1) × HI 1500 (P_2)	ADT 432 (P_1) × HD 2643 (P_2)
P_1	11.50±0.15	11.29±0.10
P_2	8.86±0.11	8.26±0.14
F_1	10.65±0.04	10.03±0.10
F_2	11.05±0.08	10.28±0.09
$BC(P_1)$	11.31±0.15	11.34±0.14
BC (P_2)	10.70±0.16	9.89±0.16
F_3	10.74±0.13	10.72±0.12

Table 3 Genetic variation test of the segregating generation of two crosses for flour micro-sedimentation volume

Genetic variation test for MS	Cross 1		Cross 2	
	Value	F Ratio	Value	F Ratio
F2	2.43	0.001***	2.78	0.000***
BC(P1)	3.80	0.000***	2.68	0.000***
BC (P2)	4.16	0.000***	3.83	0.000***
F3	4.84	0.000***	3.75	0.000***

***Significant at P=0.001

Table 4 Scaling test and joint scaling test for flour micro-sedimentation volume (ml)

Scaling test parameters	Six parameter model	
	Cross 1	Cross 2
A	0.46±0.34^{ns}	1.35±0.31**
B	1.89±0.33**	1.48±0.37**
C	2.53±0.37**	1.51±0.44**
D	0.09±0.27^{ns}	-0.66±0.28*
	Five parameter model	
C	2.53±0.337**	1.51±0.44**
D	0.49±0.58^{ns}	2.77±0.56**
Joint scaling test	*Six parameter model*	
m (Mean)	10.56±0.08	9.99±0.08
d (Additive)	1.32±0.08	1.47±0.08
h (Dominance)	0.18±0.09	0.34±0.13
χ^2	71.12**	34.83**
	Five parameter model	
m (Mean)	10.54±0.07	10.07±0.07
d (Additive)	1.43±0.09	1.42±0.09
h (Dominance)	0.18±0.09	0.15±0.13
χ^2	49.81**	42.37**

*Significant at P=0.05; ** Significant at P=0.01 and ^{ns}=Non-significant

influence of dominance × dominance type of epistasis which acted in negative direction in both the crosses. The result was similar for five parameter model in case of ADT 463 × HI 1500 (cross 1); whereas in ADT 432 × HD 2643(cross 2) it displayed non-significant positive value. The results obtained from the best fit model for micro-sedimentation value for six parameter model showed that in case of ADT 463 × HI 1500 (cross 1) dominance × dominance type of epistatic interaction played an important role, besides the significant influence of additive and dominance effects. While the estimate of additive and dominance component was in the positive direction, the dominance × dominance and additive × dominance interaction were found to act in the negative direction. Interestingly, the results from best fit model of five parameter model were almost of similar nature and magnitude. The only difference was that it could not estimate the influence of additive × dominance interaction which is an inherent limitation of the five parameter model (Hayman 1958). In case of best fit model for ADT 432 × HD 2643 (cross 2), significant additive × additive interaction was common for both five and six

Table 5 Estimation of different parameters for flour micro-sedimentation volume (ml)

Parameters	Six parameter model		Five parameter model	
	Cross 1	Cross 2	Cross 1	Cross 2
m (Mean)	11.05± 0.08**	10.28± 0.09**	11.05± 0.08**	10.28± 0.09**
d (Additive)	0.61± 0.21**	1.45± 0.21**	1.32± 0.09**	1.52± 0.09**
h (Dominance)	0.29± 0.54^{ns}	1.57± 0.57**	0.57± 0.38^{ns}	–1.33± 0.38**
i (Add. × Add.)	–0.19± 0.53^{ns}	1.32± 0.55*	2.74± 0.36**	1.44± 0.36**
j (Add. × Dom.)	–0.71± 0.23^{ns}	–0.06± 0.23^{ns}		
l (Dom. × Dom.)	–2.16± 0.94*	–4.14± 0.96**	–2.73± 0.93**	1.67± 1.01^{ns}
	Best fit model (Joint scaling test)			
m (Mean)	10.18± 0.09	8.48± 0.55	10.21± 0.08	10.78± 0.14
d (Additive)	1.32± 0.09	1.51± 0.08	1.33± 0.09	1.52± 0.09
h (Dominance)	2.95± 0.37	5.65± 1.46	3.00± 0.41	–0.79± 0.20
i (Add. × Add.)		1.30± 0.55		–1.01± 0.03
j (Add. × Dom.)	–1.43± 0.47			
l (Dom. × Dom.)	–2.47± 0.32	–4.10± 0.95	–2.55± 0.36	
χ^2	0.12^{ns}	0.08^{ns}	0.72^{ns}	0.10^{ns}

*Significant at P=0.05; ** Significant at P=0.01 and ^{ns}=Non significant. For Best Fit Model (Joint scaling test) only significant components are presented.

parameter models, besides additive and dominance effects. Although, a major contribution of dominance × dominance epistasis was estimated which acted in negative direction in six parameter model, it was found to be absent in five parameter model. However from the overall results, it may be concluded with a high confidence level that dominance × dominance epistasis acting in negative direction shows very high influence on micro-sedimentation value, which in turn explains the negative heterobeltiosis and low mid-parent heterosis value observed in the F_1s of the two crosses. The studies on gene action for gluten strength in bread wheat is lacking, though QTLs have been reported by a few researchers in durum wheat (Perreatant *et al.* 2000, Elouafi *et al.* 2000, Patil *et al.* 2009, Conti *et al.* 2011, Kumar *et al*. 2013). Even in the case where QTL for gluten strength in bread wheat (Zanetti 2001) was mapped, the phenotyping for this trait was carried out with immortalized mapping populations (recombinant inbred lines and doubled haploid lines) where large sample size can be drawn for SDS based macro-sedimentation test. The study is perhaps first of its kind where genetic analysis for gluten strength was carried out in early segregating generations of bread wheat based on microsedimentation test.

Analysis with Castle-Wright's equation for 'number of effective genes/blocks (represented as n)' revealed that at least one major gene block (n = 1.07 in case of ADT 463 × HI 1500 and n = 1.35 in case of ADT 432 × HD 2643) could be involved for the micro-sedimentation value in both the cross combinations besides some minor genes. Study on mapping of QTLs associated with grain protein and gluten strength in durum wheat population were carried out (Perretant *et al.* 2000, Patil *et al.* 2009). An important and stable QTL affecting gluten strength was mapped and located on chromosome 1BL (Glu-B1) consistently detected over six environments. Beside this, additional minor QTLs were found in three environments on chromosomes 6AL and in two environments on chromosomes 6BL, 7AS and 4BS. Some of these QTLs also displayed pleiotropic effects for grain yield, test weight, thousand-kernel-weight and days to heading. Kumar *et al.* (2013) mapped QTL for gluten strength using a doubled haploid population of durum wheat. They utilized phenotypic data on gluten strength measured by sedimentation volume and framework linkage map of 228 markers to conduct single- and two locus QTL analyses. They could identify only one consistent QTL (QGs.ndsu-1B) on chromosome 1BS contributing up to 90% of the phenotypic or 93% of the genotypic variation. Thus, results from this QTL analysis are quite similar with our result from generation mean analysis assessed by the micro-sedimentation protocol for measuring gluten strength in two different crosses. In another study (Conti *et al.* 2011), epistatic QTLs and QTL × environment interactions were also recorded for gluten strength. Overall, the present study revealed the significant contribution of non-allelic interactions or epistasis, besides the main effects, in determining the gene action for micro-sedimentation volume.

To summarize, the study is, perhaps, the first to analyze the gene action for gluten strength based on micro-sedimentation test in bread wheat using generation mean analysis. Estimation of gene action for a trait has a direct bearing upon the choice of breeding procedures to be followed in crop improvement programmes. The non-additive gene effect (dominance, additive × dominance and dominance × dominance epistasis) was observed to be predominant over the additive (additive and additive × additive epistasis) gene effect for micro-sedimentation value. Among the different epistatic components, dominance × dominance effect had the maximum contribution and was acting in the negative direction along with the additive × dominance effect, while the additive × additive effect was found to act in positive direction. Main effects (mean, additive and dominance) mostly acted in the positive direction. All these findings indicate that direct selection for micro-sedimentation/ gluten strength will not be effective in the early segregating generations of bread wheat. Rather, selection may be carried out only after fixation of the genotypes in the advanced (selfed) generations.

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