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Research Article

GENETIC ANALYSIS FOR GRAIN WEIGHT AND GRAIN NUMBER IN WHEAT (*Triticum aestivum* L. Em. Thell)

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Abstract- There are three cordial components in wheat which determines the grain yield: tillers per plant, number of grains and grain weight per spike. All the three components are mutually exclusive and it has been witnessed that the higher number of grain normally correlates with lower grain weight. This negative linkage is serious unhampress selection gain. It is to be emphasized these both traits are polygenic and hence finding their recombinants of positive type's i.e. higher number of grains/spike along with higher grain weight is feasible. This would be achieved if there is adequate variability in segregating population and selection pressure is exerted in right direction with appropriate selection intensity. Keeping this hypothesis in mind we investigated the variability and gene effects in five wheat crosses involving discrete and contrasting parents over two crop seasons for these two traits. The F_2 population revealed the presence of adequate genetic variability over and above the parental range with various combinations i.e. higher number of grain-lower grain weight, medium number of grains-medium grain weight and higher number of grains with higher grain weight. However, there frequency varied over the both cropping seasons. The higher grain weight is invariably attributed to higher amount of deposition of carbohydrates (starch). Thus, the transgressive segregants for grain number and grain weight could be recovered in all five cross *viz*. Rm-Ts17 x PBW502; HS27 x PBW502; HS67 x PBW502; HJP81 x PBW502; HG2 x $HD2009_M$. Pedigree selection in such crosses is therefore expected to yield the dividends. The SSR marker analysis also indicated that some quantitative trait Loci QTLs) could be associated with either higher number of grains or higher grain weight or both the traits. Marker assisted selection, therefore should be feasible, our study suggest that both genotyping and phenotyping should be resorted to identify the desired transgressive segregants for high grain weight and higher grain number, so that wheat yield can be enhanced.

Keywords- Grain weight, Transgressive segregants, Gene effects, Grain number, Quantitative trait Loci.

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Introduction

World demand for wheat by 2050 is expected to rise by 60%, whereas the climate change is negatively affect the wheat production by 29% in the same vicinities [1] So, there is an urgent need to develop higher productivity wheat genotypes through agronomical, genetic and physiological interventions along with resource conservation technologies [2]. Yield potential and yield gains are essential to meet this demand. Accomplishing this goal, the systematic attempts for wheat improvements are needed through genetic and molecular manipulation of various yield components: spike number per plant, grain number per spike and grain weight [3-5]. Genetically, wheat yield or yield components are controlled by numerous genes with additive, non-additive, dominance, epistatic effects and genotype by environment interactions. Grain yield depends on several yield components including grain number which is strongly correlte with yield, grain size and grain weight usually measured as 1000 grains weight [6-10]. The higher grain weight is invariably attributed to higher amount of deposition of carbohydrates (starch) [4]. Therefore, grain yield is perhaps the most commonly studied but poorly understood trait related to agronomic performance of wheat. Therefore, it is of utmost interest to obtain more information about QTL governing those traits. In the past decade, the significant advances have been made in the field of genetic dissection of yield and yield related traits in bread wheat by applying the high-density linkage maps and QTL methodologies. Some recent studies include identification of a number of genes and QTLs for yield contributing traits in bread wheat [10-13]. In view of the importance of grain number and grain weight in wheat, as explained above, the present investigation studied the gene effects and molecular analysis for these two traits.

Material & Methods

The present investigations were conducted during two successive winter growing seasons (2008-2009 and 2009-2010) at the experimental area of the Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, India. Seven genetically diverse, homozygous lines of wheat were utilized in the present investigations for creating different generations namely, F_1, F_2, BC_1 BC_2 forming set of six generations in five crosses *viz.*, HJP81 x Rm-Ts17 (C-I), HS27 x PBW502 (C-II), HJP81 x PBW502 (C-III), HS67 x PBW502 (C-IV), HG2 x $HD2009_M$ (C-V). These generations along with parents were grown in a randomized block design with three replications for each cross. Data had been recorded on individual plant basis for all five crosses in respect of characters *viz.* grain weight per spike (g), number of kernels per spike. The data were subjected to generation mean analyses which included scaling tests [14], joint scaling test [15,16], estimation of gene effects by epistatic model of Hayman (1958). Total genomic DNA was isolated from 2-4 week old young leaf of both parents (HS67 and PBW502) and the 100 F_2 plants using CTAB procedure. The SSR primers were procured from *http://wheat.pw.usda.gov/GG2/index.shtml*. For SSR primers, PCR reaction was carried out in 25µl of reaction mix containing 50 ng of template

DNA, 200 µM of each dNTP, 10X PCR buffer, 1.5 mM $MgCl_2$, 0.2 µM each forward and reverse primer and 1.2 U Taq DNA polymerase. The following protocol was used for PCR amplification for SSR primers, initial denaturation 94°C for 4 min followed by 42 cycle of denaturation 94°C for 1 min, annealing 50°C-65°C for 1 min, extension 72°C for 2 min and with final extension for 14 min.PCR Amplified products were resolved on 6% polyacrylamide gels and DNA bands were then visualized using the Silver staining method (Promega Technical Manual, Part # TM023). SSR amplification profiles were scored visually, based on presence (taken as 1) or absence (taken as 0) of bands for each wheat genotype. NTSYS-PC software was used for molecular diversity analysis. The QTL analysis in F_2 population of HS27x PBW502(C-IV) for all polymorphic markers was performed with the computer program WinQTLCart 2.5. Kosambi function was used to convert the recombination frequency to genetic distances in centimorgans (cM). Data was put in as 2, 0 and 1 to mark genotypes of parent A; parent B, and heterozygotes, respectively, for co-dominant marker. Other situations were coded by 12 = not 2; i.e. 1 or 0 (for dominant markers), 10 = not 0; i.e. 1 or 2 (for dominant markers), '-' = missing data for the individual at a locus.

Result & Discussion

Grain yield is a complex trait and usually controlled by a number of component traits such as number of plant per unit area, number of spike per plant, number of grain per spike, 1000-grain weight (TGW) and other related traits such as spike length (SL) and spikelet number per spike (SNS) with minor effects [5]. Among these, the grain weight is a complex quantitative trait under polygenic control which is influenced by various genetic interactions at all stages of growth which make it difficult to be manipulated for improvement in breeding programs [2]. Wheat grain (caryopsis), a single-seeded fruit, has been a major target for selection since doestication of cereals. Grain is a very stable yield component which is weight is positively associated with grain yield, with relatively high heritability [3]. Quantitative trait loci (QTL) affecting grain weight, grain size, and grain shape have been reported on most wheat chromosomes [3,9,21]. The phenotypic variation in grain weight and size is also affected by environmental factors such as water availability and extreme temperatures, which affect the rate and duration of the grain-filling process. Thus, the present study planned to dissect the genetic basis of grain number and number of grains per spike in the six basic generations of four selected single cross under two sowing seasons.

Genetic analysis

Analysis of variance revealed that mean squares due to generations were significant for all characters in all crosses in both seasons [Table-1]. So, the further genetic analyses were performed to calculate the gene effects for grain weight and grain number [Table-2, 3]. The joint scaling test indicated the fitness of the additive-dominance model for the cross HS67×PBW502 in WS_1 and HS67×PBW502 (WS_1) which is supported by non-significant χ^2 value. Significant positive additive gene effects were observed in the cross-III under WS_1 and WS_2; C-IV under WS_2 for number of grains per spike (NGS) and the crosses namely, C-I (WS_1, WS_2); C-III (WS_1, WS_2) for GWS [Table-2]. However, the crosses C-I under WS_1 and WS_2; C-II under WS_1 and WS_2 recorded significant negative additive gene effects for NGS whereas, negative additive gene effects for GWS were exhibited by the crosses *viz.* C-IV (S_2) and C-V (WS_1, WS_2) [Table-3]. Significant Negative dominance gene effects for NGS were recorded in the crosses C-III (WS_2), C-IV (WS_1) and C-V (WS_1, WS_2) while the cross C-II (WS_1) exhibited this type of gene effect for GWS . Positive dominance gene effects for GWS were observed for the crosses *viz.* C-I (WS_1, WS_2) C-III (WS_1, WS_2); C-V (WS_1, WS_2). Among digenic interaction, positive additive x additive (i) type was significant in the cross-V under WS_1 only, while the crosses, C-II under WS_1 and WS_2; C-IV (WS_2) exhibited negative additive x additive type of interactions for the trait NGS. For GWS, the significant positive additive x additive (i) non-allelic interaction was observed in the cross C-III (WS_2) and C-V (WS_2), while these effects were negative in the cross C-II (WS_1) only. Significant positive additive x dominance (j) interaction was observed in the crosses C-II (WS_1) and C-I (WS_1, WS_2), whereas it was negative in the cross-V (WS_1) for NGS. The grain weight per spike was showed positive dominance x dominance (l) interaction for the cross C-II (WS_1) whereas the cross C-III (WS_2) exhibited the negative dominance x dominance (l) interaction. Positive additive x dominance (j) type of interaction was observed in the C-V under WS_2, while the crosses C-I (WS_1); C-IV (WS_1) exhibited significant negative additive x dominance (j) interaction for the number of grains per spike. Positive dominance x dominance (l) type of interaction was observed in all the crosses under both WS_1 and WS_2 seasons except C-V under WS_1 where it showed negative dominance x dominance (l) interaction. Duplicate type of epistasis was present in the crosses *viz.* C-III (WS_1) and C-V (WS_1), while the complementary type of interaction was observed in the crosses *viz.*, C-III (WS_2); C-IV (WS_1) and C-V (WS_2). The cross C-II (WS_1), C-III (WS_2) for this trait indicating that duplicate type of epistasis is operating for grain weight per spike.

Table-1 *Mean performances of six basic generations for grain number per spike and weight of kernel/spike (g), in the five wheat crosses for two growing seasons: 2008-09 (WS_1) and 2009-10 (WS_2)*

Number of Kernels/Spike							
Cross-I HJP81 x Rm-Ts 17		P_1	P_2	F_1	F_2	BC_1	BC_2
		Mean ± SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
	WS_1	59.56±0.378	69.3±0.558	68.42±0.636	59.55±1.732	55.55±1.957	63.80±1.610
	WS_2	55.83±0.381	65.9±0.303	68.97±0.645	58.88±1.415	56.57±1.771	60.17±2.325
Cross- II	WS_1	52.76±0.307	41.43±0.404	56.53±0.342	50.54±1.254	49.42±1.932	46.91±2.049
HS 27 x PBW502	WS_2	50.87±0.66	39.77±0.46	57.49± 0.27	53.05±1.40	49.42±1.93	46.91.049
Cross-III	WS_1	52.53±0.396	40.46±0.523	60.51±0.384	55.38±1.659	53.53±1.289	49.93±2.403
HJP81xPBW502	WS_2	56.03±0.44	40.97±0.56	59.84±0.60	47.27±1.42	50.98±1.72	45.82±2.25
Cross-IV	WS_1	55.900±0.254	43.63±0.357	67.75±0.378	55.67±1.468	53.46±2.091	54.26±2.556
HS67 x PBW 502	WS_2	51.17±0.48	40.33±0.41	67.82±0.57	53.53±2.06	51.80±2.25	48.04±2.40
Cross -V	WS_1	52.66±0.434	66.80±0.435	66.75±0.405	52.01±1.781	57.64±2.001	61.53±2.164
(HG2 x $HD2009_M$)	WS_2	50.07±0.48	67.90±0.41	68.49±0.57	56.23±2.06	54.98±2.25	59.22±2.40
Weight of Kernel/Spike(g)							
Cross-I	WS_1	2.40±0.043	3.33±0.090	3.96±0.091	3.41±0.129	3.78±0.186	3.08±0.171
HJP81 x Rm-Ts17	WS_2	2.63±0.047	3.47±0.074	3.63±0.070	3.40±0.131	3.87±0.191	3.12±0.178
Cross- II	WS_1	2.16±0.012	1.78±0.015	3.47±0.015	2.93±.145	2.20±0.147	2.17±0.222
HS 27 x PBW502	WS_2	1.86±0.01	1.55±0.01	2.01±0.03	1.77±0.08	1.90±0.09	1.97±0.521
Cross-III	WS_1	2.11±0.023	1.75±0.02	2.39±0.023	2.14±0.067	2.33±0.042	2.10±0.102
HJP81xPBW502	WS_2	2.27±0.01	1.51±0.02	1.99±0.02	1.38±0.06	2.01±0.08	1.72±0.09
Cross-IV	WS_1	2.28±0.009	1.66±0.011	2.94±0.012	2.52±0.098	2.66±0.140	2.18±0.139
HS67 x PBW 502	WS_2	1.01±0.01	1.54±0.01	1.45±0.01	1.71±0.09	1.37±0.10	1.53±0.15
Cross-V	WS_1	2.62±0.024	3.62±0.023	4.37±0.031	3.34±0.134	3.12±0.178	3.96±0.173
(HG2 x $HD2009_M$)	WS_2	2.59±0.01	3.24±0.01	3.28±0.01	2.17±0.09	2.49±0.10	2.93±0.15

Table-2 *Estimates of different scaling tests and genetic effects for number of grains/spike (NGS) in the five wheat crosses for two sowing years: 2008-09(WS_1) and 2009-10(WS_2)*

Crosses	Cross-I (HJP81 x Rm-Ts 17)		Cross-II HS 27 x PBW 502		Cross-III HJP81 x PBW 502		Cross-IV HS67 x PBW 502		Cross -V HG2 x HD 2009_M	
	WS_1	WS_2	WS_1	WS_2	WS_1	WS_2	WS_1	WS_2	WS_1	WS_2
Parameter	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ±SE	Estimate ± SE	Estimate ±SE
Scaling test										
A	16.87**±2.3	11.65**±2.1	10.45**±2.2	9.51**± 2.24	5.97**±1.52	13.92**± 2.03	16.72**±2.429	15.38**± 2.028	4.13±2.336	8.60**±2.6385
B	10.12**±1.9	14.52**±2.7	4.14±2.386	3.33± 2.3816	1.11±2.800	9.16**±2.641	2.86±2.966	12.06**± 3.061	10.48**±2.52	17.95**±2.795
C	27.49**±4.9	24.16**±3.3	5.10±2.938	-6.43*±3.2324	-7.52±3.874	27.62**±3.37	12.35**±3.427	13.03**± 3.35	44.92**±4.15	30.03**±4.806
D	-0.24±2.478	1.00±2.349	4.74*±2.178	9.64**± 2.281	7.3**±2.479	-2.26± 2.3133	3.61±2.551	7.20**± 2.453	-15.15**±2.67	-1.74±3.039
Joint scaling test(Three Parameter)										
m	64.11**±0.2	60.7**±0.1	47.02**±0.1	45.24**± 0.16	46.46**±0.2	48.14**±0.20	49.69**±0.12	45.57**±0.165	59.50**±0.17	58.79**± 0.18
d	4.76**±0.19	5.1**±0.14	-5.66**±0.1	-5.56**±0.16	-5.96**±0.2	-7.53**± 0.2	-6.1**±0.126	-5.40**±0.166	7.03**±0.176	8.91**±0.182
h	3.14**±0.41	7.12**±0.3	9.35**±0.24	12.22**±0.22	13.96**±0.3	10.62**±0.39	17.81**±0.25	21.8**±0.2964	6.85**±0.293	9.04**±0.375
χ^2 (df=3)	118.462**	104.014**	27.22**	24.08**	19.74**	118.38**	60.47**	85.83**	135.10**	87.79**
Six Parameter										
m	59.55**±1.0	58.8**±0.8	50.54**±0.7	52.98**± 0.80	55.3**±0.95	47.26**±0.82	55.67**±0.84	53.52**± 0.82	52.01**±1.02	56.22**±1.19
d	-8.2**±1.46	-3.6*±1.68	2.51±1.626	2.51±1.6260	3.60*±1.574	5.15**± 1.63	-0.80±1.906	3.75*±1.8201	-3.89**±1.7	-4.25*±1.898
h	4.48±4.974	6.10±4.716	-0.06±4.362	-7.05±4.569	-0.60±4.967	15.87**±4.65	10.76*±5.108	7.65±4.9158	37.32**±5.34	12.99*±6.090
i	0.49±4.957	-2.0±4.699	-9.49*±4.35	-19.28**±4.56	-14.61±4.95	4.54±4.6267	-7.22±5.102	-14.4**±4.907	30.30**±5.33	3.49±6.0788
j	-6.75*±2.95	2.86±3.387	-6.31±3.265	-6.17±3.2675	-4.86±3.172	-4.75±3.2955	-13.86**±3.8	-3.33±3.6554	6.35±3.422	9.35*±3.8152
l	26.5**±7.14	28.2**±7.5	24.09**±7.1	32.1**±7.26	21.7**±7.39	18.55*±7.357	26.80**±8.36	41.87**±8.011	-15.68**±7.9	23.05*±8.988
Type of gene interac-tion	--		--	----	Duplicate	Complementary	Complementary	----	Duplicate	Complementary

df = degrees of freedom, calculated as the number of generations minus the number of estimated genetic parameters
(*, **) indicates that the value was significant by the t-test at the 5% and 1% probability level respectively. N.S. = Non-significant

Table-3 *Estimates of different scaling tests and genetic effects for grain weight/spike (GWS) in the five wheat crosses for two sowing years: 2008-09(WS_1) and 2009-10(WS_2)*

Crosses	Cross -I (HJP81 x Rm-Ts 17)		Cross -II HS 27 x PBW 502		Cross- III HJP81 x PBW 502		Cross -IV HS67 x PBW 502		Cross -V HG2 x HD 2009_M	
	WS_1	WS_2	WS_1	WS_2	WS_1	WS_2	WS_1	WS_2	WS_1	WS_2
Parameter	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ±SE	Estimate ± SE	Estimate ±SE
Scaling test										
A	-1.21**±0.2	-1.48*±0.23	1.2**±0.17	0.08± 0.1005	-0.16**±0.0	0.24**±0.093	-0.1±0.16	-0.28**± 0.1	0.74**±0.2	0.88**±0.11
B	1.13**±0.2	0.85*±0.21	0.91**±0.25	-0.37± 0.602	-0.06±0.119	0.07± 0.1067	0.23±0.161	-0.08± 0.132	0.06±0.201	0.65**±0.168
C	-0.01±0.321	-0.23±0.317	-0.87**±0.3	0.37± 0.291	0.10±0.158	2.24**±0.144	-0.27±0.228	-1.37**0.531	1.61**±0.313	3.70**± 0.20
D	-0.03±0.208	-0.19±0.214	1.50**±0.22	-0.33±0.319	-0.16±0.100	-0.96**±0.09	0.20±0.161	0.50± 0.277	-0.40±0.211	-1.08**±0.14
Joint scaling test(Three Parameter)										
m	2.8**±0.03	3.04**±0.02	1.97**±0.01	1.7**±0.004	1.9**±0.01	1.88**±0.006	1.97**±0.004	1.28**± 0.01	3.11**±0.010	2.91**±0.004
d	0.4**±0.03	0.39**±0.02	-0.19**±0.01	-0.15**±0.0	-0.2**±0.01	-0.4**±0.006	-0.3**±0.004	0.26**±0.0064	0.50**±0.010	0.32**±0.004
h	1.1**±0.06	0.61**±0.04	1.49**±0.01	0.30**±0.02	0.46**±0.02	0.07**±0.012	0.96**±0.008	0.18**±0.0166	1.23**±0.020	0.35**±0.009
χ^2 (df=3)	62.32**	61.7**	71.17**	N.S.	11.38**	246.85**	N.S.	14.76**	39.06**	404.12**
Six Parameter										
m	3.4**±0.07	3.40**±0.07	2.93**±0.08	1.76*± 0.047	2.14**±0.03	1.38**± 0.035	2.52**±0.057	1.70**± 0.132	3.34**±0.078	2.17**± 0.05
d	0.7**±0.15	0.75**±0.15	0.03±0.153	-0.07±0.305	0.23**±0.06	0.29**±0.070	0.48**±0.114	-0.16**±0.082	-0.84**±0.14	-0.43**±0.10
h	1.17**±0.4	0.97*±0.43	-1.5**±0.454	0.98±0.6386	0.79**±0.20	2.02**±0.19	0.55**±0.222	-0.84±0.554	2.05**±0.423	2.52**±0.286
i	0.07±0.417	0.39±0.427	-3.03**±0.45	0.66±0.6384	0.33±0.201	1.92**± 0.20	-0.41±0.322	-1.01±0.554	0.80±0.422	2.16**±0.286
j	2.3**±0.29	2.3**±0.305	-0.30±0.307	-0.45± 0.61	0.10±0.129	-0.16±0.1407	0.33±0.228	0.19±0.164	-0.68**±0.29	-.22±0.201
l	-0.15±0.666	-1.02±0.681	5.13**±0.70	-0.96±1.235	-0.56±0.301	-1.6**±0.315	0.54±0.510	0.65±0.623	0.01±0.653	-0.61±0.45
Type of gene interaction	--	--	Duplicate	---	------	Duplicate	-----	------	---	--

df = degrees of freedom, calculated as the number of generations minus the number of estimated genetic parameters
(*, **) indicates that the value was significant by the t-test at the 5% and 1% probability level respectively. N.S. = Non-significant

There was no evidence of epistatic effects in crosses, C-III (WS_1) C-IV (WS_2), although, the scaling tests were significant in these crosses which indicated the involvement of complex genetic interactions for grain weight per spike. Several workers have also reported the importance of non-allelic interactions in self-pollinated crops like wheat [5,9]. These above results are in accordance with reports published by other researchers [7,18,22-24].The dominance effect (h) is higher in magnitude than additive effect (d) as observed for kernel weight in cross HS27 x PBW502 under season 2009-10 (WS_2) and kernel weight in cross HS67 x PBW502 under season 2008-09 (WS_1) reveal the possibility of gene dispersion among the parents for these traits. In such situation, transgressive segregants are expected to be derived in later segregating generations and until then the populations need to be advanced followed single seed descend approach. In the present investigation, the presence of epistatsis was indicated on the basis of individual scales for most of the characters of studied i.e. number of kernels per spike

Molecular diversity analysis

Forty four SSR primers were used for this cross, out of which only 42 primers showed amplification. Using a total of 39 polymorphic SSR primers, 102 amplified bands were obtained of which 75 bands were polymorphic [Table-4]. The alleic polymorphism for SSR primer barc45; xgwm219 and wmc149 among F_2 population of wheat cross HS67xPBW502. The DNA amplification and

polymorphism generated among various individuals of F_2 population of Cross-IV (HS67 x PBW502) using these SSR primers are presented in [Table-4]. The total number of bands observed for every primer was recorded separately and polymorphic bands percentage was calculated subsequently. The total number of amplified bands varied between 1 (WMC500.1, CFD233 and WMC421) and 6 (primer Xgwm 149) with an average of 2.83 bands per primer. The polymorphism percentage ranged from as low as 33.3% to as high as 100%. Average polymorphism across all the 100 wheat genotypes was found to be 75.0%. Overall size of PCR amplified products ranged between 90bp and 320bp. For, cross HS67 x PBW502 (C-IV) the F_2 individuals exhibited similarity indices between them ranged from 0.396 to 0.789. Highest similarity of value was reported between individual 10th and 15th. Genetically most diverse individuals were 13th and 73rd with similarity value of 0.396. The average similarity across all the genotypes was found out to be 0.594 indicating a high level of genetic similarity among the F_2 individuals of this cross. The hierarchical cluster analysis revealed that the F_2 populations along with their parents were mainly divided into two major clusters at a similarity coefficient of 0.51, I and II. The cluster I comprised of single genotype, PBW502 (Parent II) whereas Cluster-II subdivided into two sub-clusters A and B at similarity coefficient of 0.53, sub-cluster A comprised of single genotype HS67 (parent I) and subcluster-B further subdivided into two groups C and D at similarity coefficient of 0.61. The group C further bifurcated at similarity coefficient 0.67 into two major sub-groups E and F which comprised of 12 and 31 individuals, respectively. The group 'C' bifurcated into two major sub-groups at similarity coefficient of 0.67 into G and H which consists of 32 and 25 F_2 individuals, respectively. The principal component analysis on SSR data in F_2 population of variation can be explained by three principal components based on first, second & third eigenvector, which accounted for 65.80, 5.39, and 3.30% variation respectively. The grouping of the 100 individual plants of F_2 population are shown in the 3-D and 2 D scaling along with their two parents [Fig-1 and 2]. It was evident form this analysis that all of the groups followed the same pattern as depicted in the dendrogram. Similar reports were recorded by using microsatellites markers for assessment of genetic diversity among cultivars and their wild relatives of wheat [10,12,22,27].

Table-4 *DNA fingerprint profile in 100 individuals of F_2 population of cross-IV (HS67 x PBW502) with 44 SSR primer sets*

Sr. No.	Primer	Size of of bands	Total No. of bands	Number of polymorphic bands	Number of monomorphic bands	Percentage polymorphism
1	BARC19	120-145	2	2	0	100
2	BARC26	110-210	2	2	0	100
3	BARC28	165-200	3	3	0	100
4	BARC45	130-200	4	2	2	50
5	BARC48	130-240	2	2	0	100
6	BARC113	140-220	3	2	1	66.66
7	BARC133	130-250	5	3	2	40.00
8	BARC187	100-190	3	2	1	66.66
9	BARC236	110-220	2	2	0	50
10	BARC263	130-180	2	2	0	50
11	BARC275	110-140	2	2	0	50
12	BARC288	95-160	2	2	0	50
13	BARC297	No amplification				
14	BARC344	No amplification				
15	CFA2104	120-220	3	2	1	66.66
16	BARC359	No amplification				
17	CFA2262	90-110	3	1	2	33.33
18	CFA2292	120-210	2	2	0	100
19	CFD239	105-190	2	2	0	100
20	WMC41	140-175	2	1	1	50
21	WMC110	100-200	2	2	0	100
22	WMC149	135-180	5	3	2	20
23	WMC232	185-230	3	2	1	66.66
24	WMC254	105-200	5	2	3	40.00
25	WMC134	No amplification				
26	WMC296	115-210	2	2	0	100
27	**WMC349**	**110-190**	**3**	1	2	33.33
28	WMC407	100-150	2	2	0	100
29	WMC413	100-140	4	3	1	20.50
30	WMC416	90-320	5	4	1	40.00
31	WMC475	185-220	2	2	0	100
32	WMC601	160-200	2	2	0	100
33	WMC727	120-320	2	2	0	100
34	WMC758	105-145	2	2	0	5031
35	XGWM219	180-250	5	3	2	60
36	XGWM261	165-200	2	2	0	100
37	XGWM443	150-230	4	2	2	50
38	XGWM515	170-210	2	2	0	50
39	WMC719	No amplification				
40	*XGWM642*	165-225	3	2	1	66.66
41	*XCFD223*	130-240	3	1	2	33.33
42	WMC766	No amplification				
43	WMC819	No amplification				
44	Wmc827	No amplification				
Average			102/36= 2.83	75	27	71.34139

QTL Mapping

In the present analysis, the two F_2 populations were selected as mapping population which was developed from the cross: HS67 x PBW502. The size of the mapping population was 100 individuals and the 36 polymorphic SSR primer sets were selected for QTL mapping on the basis of parental screening for polymorphism.

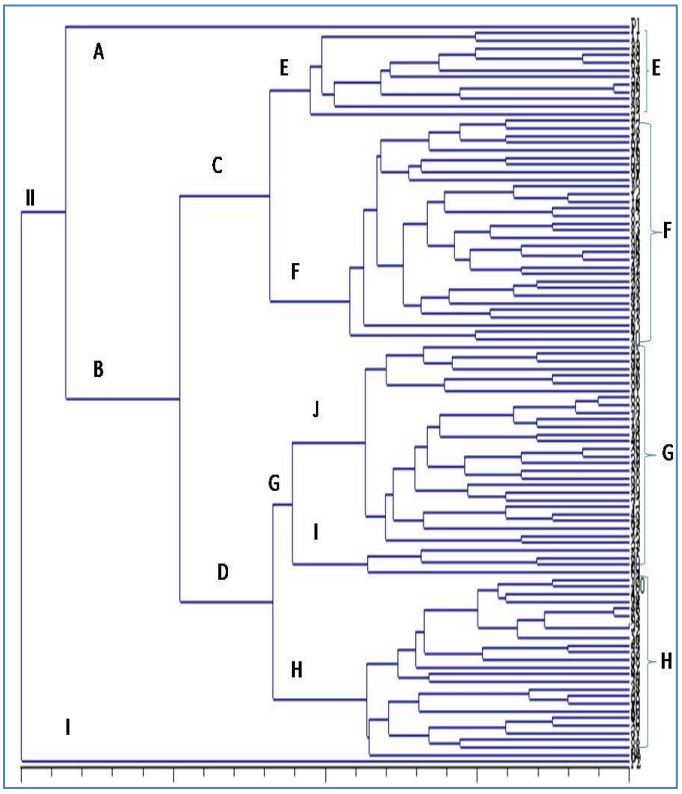

Fig-1 Dendrogram showing relationship among 100 individual plants of F_2 population of HS67 x PBW502 along with both parents generated by UPGMA analysis based on single primers using polymorphic SSR primer pairs

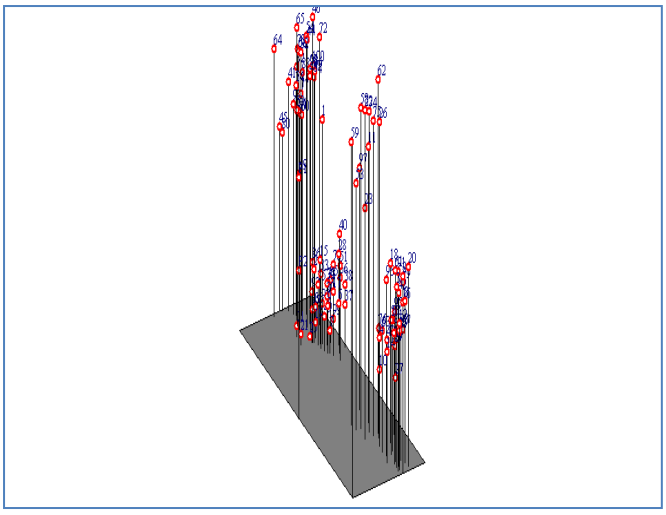

Fig-2 Three dimensional PCA (Principal component analysis) scaling of 100 F_2 individuals of HS67 x PBW502 along with both parents using SSR markers

The present study discovers four QTL located on chromosome 1A, 1B and 5A controlling grain weight per spike in bread wheat [Table-5, Fig-3 and 4]. Chromosome 5A is known to carry a number of major genes affecting productivity and adaptability and several QTL studies have reported Some QTLs on main chromosomes (1A, 3A, 4B, 5A, and 6A) a similar position to the QTLs identified in present study [1,5,19,25-27]. In order to exploit additive as well as non-additive gene effects simultaneously breeding methods like reciprocal recurrent selection and bi-parental mating approach should be followed. Improvement in all these characters can be made by simple pedigree selected based on progeny performance. Quick response to selection for the traits controlled by additive x additive gene effects is expected. Interestingly, the extent of polymorphism depicted by majority of primers and separation of DNA fragment on electrophoretic field revealed the similar trend of variation. When the association mapping was done using cartographic analysis, 4 QLTs figured important for grain weight/spike. Therefore, the approaches pursued in present studies are in agreement with each other, hence QTLs of interest may be identified which can relate to the grain yield [26,27].

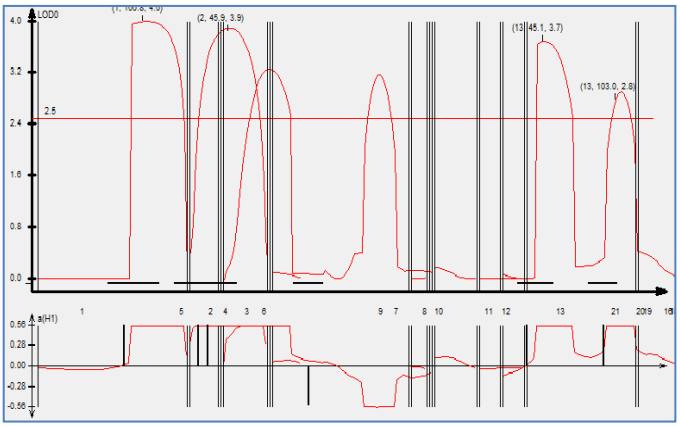

Fig-3 Cartograph showing the combined mapping of 4 QTLs combined for Grain weight per spike in cross HS67 x PBW502

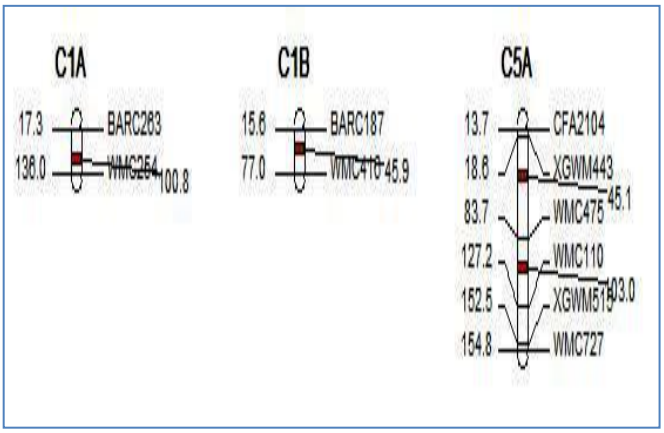

Fig-4 Linkage maps showing the location of 4 QTLs on different chromosomes for Grain weight per spike in cross HS67 x PBW502.

Table-5 *QTLs for Grain weight per spike and Spike Biomass*

Trait	Population	Chrom-osome	Nearest Marker	QTL Position (cM)	LOD Score
	F_2 of HS27x PBW502	1A	WMC254	100.8	4.0
		1B	WMC416	45.9	3.9
		5A	XGWM443	45.1	3.7
		5A	WMC110	103.0	2.8

Elucidating the genetic basis of variation in grain size and shape in wheat is instrumental to the effort to improve yield potential and processing performance, especially in the current climate where food security is at the epicenter of crop research worldwide. The present studies can be regarded as stepping stone and beginning for major supplementary efforts to exploit variation for grain number and grain weight and molecular diversity for it in pinpointing the candidate. Cluster of gene in QTLs for developing a coherent strategy based on genotyping and phenotyping for marker assisted selection (MAS). Pyramiding alleles of markers positively associated with grain size could result in wheat varieties with increased TGW [13]. Hence, the present study can be safely regarded as an effort in right direction.

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Conflict of Interest: None declared

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