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GENETIC VARIATION AT LOCI CONTROLLING QUALITY TRAITS IN SPRING WHEAT

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Selection for quality traits in bread wheat (*Triticum aestivum* L.) during early breeding generations requires quick analytical methods that need small grain samples. Marker assisted selection can be useful for the improvement of quality traits in wheat. The present study was conducted to screen 117 Pakistani adapted spring wheat varieties with DNA markers linked with genes controlling composition of low and high molecular weight glutenin subunits (LMW-GS and HMW-GS, respectively), starch viscosity, Polyphenol oxidase (PPO) activity and grain hardness. DNA fragments associated with the presence/absence of quality related genes were amplified using Polymerase chain reaction (PCR) and detected using agarose gel electrophoresis. Positive allele of *ω-secalin*, which indicates presence of 1B.1R translocation, was found in 77 (66%) varieties. The marker *PPO05* was found in 30 (26%) varieties, indicating lower PPO activity. Grain hardness controlled by *Pinb-D1b* allele was present in 49 (42%) varieties. Allele *Wx-B1b* which confers superior noodle quality was found in 48 (41%) varieties. HMW-GS encoded by *Glu-D1d* allele that exerts a positive effect on dough strength was present in 115 (98%) varieties. LMW-GS alleles *Glu-A3d* and *Glu-B3* were observed in 21 (18%) and 76 (65%) varieties, respectively. Results of the present study may help wheat breeders in selecting parents for improving desirable quality attributes of future wheat varieties. The varieties, identified having desirable quality genes, in this study can be used in the wheat breeding programs aiming to improve quality traits. Early generation marker assisted selection can help to efficiently utilize resources of a breeding program.

Keywords: DNA markers; grain hardness; LMW-GS and HMW-GS; Marker Assisted Selection; PPOs activity; Wheat quality

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) provides more than 50% of the total calories and 60% of the total protein eaten by mankind (Sial *et al.,* 2005). One hundred grams of wheat seed consists of about 12.6-15.4 grams protein, 71-68 grams carbohydrate, 1.5-1.9 grams total fat, 3.2-3.6 mg iron and 12 grams of nutritional fiber (Kumar *et al.,* 2011). The primary determinant of wheat processing and industrial quality is the grain texture or hardness that affects many end-use properties of wheat (Feiz *et al.,* 2009). The hardness of wheat grain is chiefly controlled by Hardness locus (*Ha*), which is positioned on chromosome 5DS (Ram *et al.,* 2002). *Puroindoline a* and *Puroindoline b* (*Pin a* and *Pin b*, respectively) genes lie on *Ha* locus and are involved in grain texture (Wanjugi *et al.,* 2007). The wild types of *Pina-D1a* and *Pinb-D1a* genes give soft texture (*Ha*), whereas mutation in any of the two genes confers hard texture to wheat grain (Giroux and Moris, 1998). The soft textured and white grained wheat varieties have reduced particle-size distribution, relatively pale and starchy kernels and are preferred for manufacturing biscuits, piecrust and other breakfast foods (Devaux *et al.,* 1998; Knott *et al.,* 2009). The high quantity of gluten in flour from hard wheat is preferred for making bread, macaroni, spaghetti fine cakes and other pasta products (Morris, 2002). Durum (*T. durum*) is the hardest wheat (Morris, 2002).

Grain protein content determines the nutritional value of wheat grain as well as the rheological and technological properties of wheat flour (Zhao *et al.,* 2010). The bread making quality of wheat is associated with the absence or presence of specific proteins and their subunits (Snape *et al.,* 1993). The endosperm proteins have a key role in determination of wheat quality. The four major types of endosperm proteins in wheat include Prolamines, Albumins, Gliadins and Glutenins (Shewry *et al.,* 1992; Rogers, 2001). Gliadins and glutenins are wheat storage proteins and are the principal components of wheat gluten. Gluten proteins give unique visco-elastic properties to the wheat flour. Payne *et al.* (1987) reported that glutenin proteins content and compositions control most of the variation of wheat flour quality. Glutenin subunits are divided into LMW-GS and

HMW-GS subunits. LMW-GS and HMW-GS have molecular weight of 23 to 68kDa and 77 to 160kDa, respectively. These two subunits also differ from each other in their structure and amino acid composition (Branlard *et al.,* 1989).

Starch is an important constituent of wheat flour (Nakamura *et al.,* 2002). Starch is found in amyloplast in granules form and accounts for 65-70% of grain (Li *et al.,* 1999). Wheat starch consists of 70-80% amylopectin and 20-30% amylose (Li *et al.,* 1999). Waxy (*Wx*) genes produce waxy proteins that are also known as Granule-bound starch synthase (Vrinten and Nakamura, 2000). These proteins take part in amylose formation in kernel starch granules and amylopectin in non-storage tissue (Nakamura *et al.,* 2002). Waxy genes are found on the three homoeologous genomes of wheat in which *Wx-A1* is present on chromosome 7AS, *Wx-B1* on chromosome 4AL, and *Wx-D1* on chromosome 7DS (Miura *et al*., 1994).

Polyphenol oxidases (PPOs) are copper containing metallo enzymes, which are produced in nucleus, and then moved to plastids (Anderson and Morris, 2003). These enzymes oxidize phenols into quinones that produce black and brown pigments after reaction with amino acids and proteins (Wang *et al.,* 2009). The black and brown pigments result in the darkening and discoloration of wheat-based products such as steamed bread, pasta, pan bread and Asian noodles (He *et al.,* 2009). There is no negative effect of PPOs on the nutritional value of wheat except the darken color, which is disliked by consumers (Simeone *et al.,* 2002).

Global increase in the population and the demand for more food brought the attention of researchers to develop molecular marker techniques for the exploration and improvement of plant genome (Landjeva *et al.,* 2007). Molecular markers are valuable tools in improving the efficiency of selection in modern plant breeding. When found associated with a character of interest, DNA markers can be used as indirect selection criterion. This enables selection for quality traits in early segregating generations which is otherwise not possible due to the requirement of relatively large sample for various quality tests. Marker assisted selection also enables selection before the crop is mature. Gene specific markers have been developed for various quality traits in wheat. Details of these markers are given in Materials and Methods section.

Very few studies have been conducted on the DNA markers for determination of end-use quality in Pakistani adapted spring wheat varieties. Development of a marker assisted selection protocol for quality improvement is likely to speed up future wheat breeding in Pakistan. The present study was conducted to assess allelic variation at loci controlling important quality traits in Pakistani adapted spring wheat varieties.

MATERIALS AND METHODS

***Plant material*:** The seeds of 117 Pakistani adapted spring wheat varieties along with control varieties were kindly provided by Wheat Program and Crop Diseases Research Program (CDRP), National Agricultural Research Centre, Islamabad. For DNA extraction six to eight seeds of each variety were sown in pots and kept in the glass house. Leaf tissues from 2-4 weeks old plants of each variety were cut using sterilized scissors.

***DNA extraction and quantification*:** Genomic DNA was extracted from fresh leaf tissues using CTAB method (Doyle and Doyle, 1987) with minor modification. Small leaf pieces of each variety were ground in 2 % CTAB extraction buffer. About 750 µl of the emulsion was taken in a 1.5ml micro centrifuge tube and incubated at 65ºC for 30 min. After incubation, 750 µl Chloroform:Isoamyl Alcohol (24:1) was added and centrifuged at 12,000 rpm for 10 min. The supernatant was transferred to a new 1.5 ml tube and 0.8 volume chilled Isopropanol was added. After incubation at 4ºC for 10 min, the tubes were centrifuged at 12,000 rpm for 10 min at 4ºC to precipitate the DNA. DNA pellet was washed with 70 % Ethanol and subsequently air dried for 30 min, re-suspended in 100 µl of TE buffer and treated with 1µl of RNase A (10 mg/ml) to remove RNA. DNA was quantified using 1% agarose gel. The quantity of DNA in samples was estimated by comparing their band strengths with those of 25, 50 and 75ng/µl DNA standards. DNA samples were diluted to working concentration of 25ng/µl.

***Primers and Polymerase Chain Reaction (PCR)*:** Fourteen Gene-specific DNA markers previously developed for genes controlling quality traits were selected for this study (Table 1). Primers were synthesized from Gene Link, USA and Bio Basic, Canada. PCR reaction mixture of 20µl was prepared containing 1× PCR buffer with $(NH_4)_2SO_4$, 3mM $MgCl_2$, 0.2mM dNTPs mix,10pmol each of the reverse and forward primers, one unit of Taq DNA polymerase and 1µl of 25ng/µl DNA template. Amplifications were carried out in an automated thermal cycler (Applied Biosystems Veriti, 96 well). Annealing temperatures and PCR profile of all markers are given in Table 1. PCR amplified products were separated on 1-2% agarose gel, depending on the product size and subsequently visualized using gel documentation system. Results were confirmed using two independent PCR reactions.

***Quality tests*:** Already available data of three technological quality tests including particle size index (PSI), SDS Sedimentation and gluten consistency for some of the varieties studied were kindly provided by Wheat Program, NARC, Islamabad, Pakistan. PSI and SDS Sedimentation tests were carried out according to Williams *et al*. (1988). Gluten content was determined as standard method using "Glutamatic 2200 System".

Table 1. DNA Markers used for detecting quality related genes in spring wheat

S. No	Primer name	Sequence (5′ to 3′)	Product size (bp)	Linkage	Amplification profile	References
1	*ω-secalin-F*	ACCTTCCTCATCTTTGTCCT	1076	*1B·1R*	94◦C 7min (1cycle) 94◦C 1min, 58◦C 1min, 72◦C 1:30 min (35cycles) 72◦C 10min (1cycle)	Chai *et al*. 2006
2	*ω-secalin-R*	CCGATGCCTATACCACTACT				
3	*Glu-A3d-F*	ACCAGTTATTCATCCATCTGCTC	488	*Glu-A3*	94◦C 5min (1cycle) 94◦C 40s, 58◦C 40s, 72◦C 1min (35cycles) 72◦C 7min (1cycle)	Zhang *et al*. 2004
4	*Glu-A3d-R*	GTGGTTTCGTACAACGGCTCG				
5	*Glu-B3-F*	GGTACCAACAACAACAACCC	636	LMW-GS	94◦C 6min (1cycle) 94◦C 45s, 58◦C 45s, 72◦C 1min (35cycles) 72◦C 7min (1cycle)	Van Campenhout *et al*. 1995; de Froidmont, 1998
6	*Glu-B3-R*	GTTGCTGCTGAGGTTGGTTC				
7	*Pinb-D1b-F*	ATGAAGACCTTATTCCTCCTA	250	*Pin b*	94◦C 5min (1cycle) 94◦C 45s, 58◦C 45s, 72◦C 1min (35cycles) 72◦C 7min (1cycle)	Giroux and Morris, 1997
8	*Pinb-D1b-R*	CTCATGCTCACAGCCGCT				
9	*PPO05-F*	TCCGCAACTGCCAAACGC	<750	*PPO-2Aa/b*	94◦C 5min (1cycle) 94◦C 45s, 61◦C 45s, 72◦C 1min (35cycles) 72◦C 10min (1cycle)	Wang *et al*. 2009
10	*PPO05-R*	GGTCCCACTGGAGTCAAGGTC	>750			
11	*Glu-D1d-F*	GCCTAGCAACCTTCACAATC	450	*Glu-D1*	94◦C 6min (1cycle) 94◦C 1min, 58◦C 1min, 72◦C 1:30 min (35cycles) 72◦C 10 min (1cycle)	D'Ovidio and Anderson, 1994
12	*Glu-D1d-R*	GAAACCTGCTGCGGACAAG				
13	*Wx-B1a-F*	CTGGCCTGCTACCTCAAGAGCAACT	425	*Wx-B1*	94◦C 5min (1cycle) 94◦C 45s, 65◦C 45s, 72◦C 1min (35cycles) 72◦C 7 min (1cycle)	Nakamura *et al*. 2002
14	*Wx-B1a-R*	CTGACGTCCATGCCGTTGACGA				
15	*Glu-B1-2a-F*	TTAGCGCTAAGTGCCGTCT	527	*Glu-B1*	94◦C 7 min (1cycle) 94◦C 1 min, 55-63◦C 1 min, 72◦C 1:30 min (35cycles) 72◦C 7-10 min (1cycle)	Lei *et al*. 2006
16	*Glu-B1-2a-R*	TTGTCCTATTTGCTGCCCTT				
17	*LMW-1*	GCCTTTCTTGTTTACGGCTG	1700	*LMW-TD22*	94◦C 4min (1cycle) 94◦C 40s, 57-63◦C 45s, 72◦C 1min (35cycles) 72◦C 7-10 min (1cycle)	Colot *et al*. 1989; Cloutier *et al*. 2001
18	*LMW-2*	TCAGATTGACATCCACACAAT				
19	*Wx-B1-W-BDFL*	CTGGCCTGCTACCTCAAGAGCAACT	778	*Wx-B1*	94◦C 5min (1cycle) 94◦C 40s, 62◦C 40s, 72◦C 1min (15cycles) 94◦C 40s, 65◦C 40s, 72◦C 1min (35cycles) 72◦C 7-10 min (1cycle)	Saito *et al*. 2009
20	*Wx-B1-W-BRC1*	GGTTGCGGTTGGGGTCGATGAC				
21	*Wx-B1-N-BFC*	CGTAGTAAGGTGCAAAAAAGTGCCACG	668	*Wx-B1*	94◦C 5min (1cycle) 94◦C 40s, 62◦C 40s, 72◦C 1min (15cycles) 94◦C 40s, 65◦C 40s, 72◦C 1min (35cycles) 72◦C 7-10 min (1cycle)	Saito *et al*. 2009
22	*Wx-B1-N-BRC2*	ACAGCCTTATTGTACCAAGACCCATGTGTG				
23	*GBSS-4A-L*	AACCAGCAGCGCTTCAGCCT	400	*Wx-B1*	94◦C 5min (1cycle) 94◦C 45s, 62-67◦C 45s, 72◦C 1min (35cycles) 72◦C 7-10 min (1cycle)	Briney *et al*. 1998
24	*GBSS-4A-R*	TTGAGCTGCGCGAAGTCGTC				
25	*Ppo-D1a-F*	TGCTGACCGACCTTGACTCC	713	*PPO-D1a*	94◦C 5min (1cycle) 94◦C 45s, 55-63◦C 45s, 72◦C 1min (41cycles) 72◦C 7-10 min (1cycle)	He *et al*. 2007
26	*Ppo-D1a-R*	CTCGTCACCGTCACCCGTAT				
27	*Ppo-A1a/b-F*	ACATGCATGCCTACCTAATGG	685	*PPO-Aa/b*	94◦C 5min (1cycle) 94◦C 45s, 55-63◦C 45s, 72◦C 1min (41cycles) 72◦C 7-10 min (1cycle)	Sun *et al*. 2005
28	*Ppo-A1a/b-R*	ATCGCATGATGCACGTAGAG	876			

***Statistical Analysis*:** Association between marker data of *Glu-B3* with technological tests was determined using Pearson's correlation. Marker alleles were scored individually and indicated as 2 for presence of allele and 1

for absence. Gluten consistency was scored as 1 for weak (W), 2 for medium strong to weak medium-strong (MS/W-MS), whereas 3 for strong and strong to medium strong (S/S-MS). To find the correlation between marker data and quality data, Pearson's correlation coefficients were calculated using software 'Minitab 15'. Marker data were used to cluster the 117 wheat genotypes into different groups using SAS (v 9.3; SAS Institute Inc., Cary, NC).

RESULTS

In present study, 14 DNA markers were used to determine the presence/absence of genes controlling important quality traits in 117 Pakistani adapted spring wheat varieties. Seven markers including *ω-secalin*, *Glu-A3d*, *Glu-B3*, *Glu-D1d*, *Wx-B1a*, *Pinb-D1b* and *PPO05* showed expected results, whereas 7 markers did not show expected DNA fragments associated with quality related genes in the tested varieties.

***DNA marker analysis*:** Marker data of 7 primer pairs used on 117 Pakistani adapted spring wheat varieties are shown in supplementary Table 1. PCR analysis of 117 Pakistani wheat varieties with marker *ω-secalin* produced a 1076-bp fragment in 77 (66%) varieties that indicated the presence of 1B.1R translocation (Fig.1). The remaining 40 (34%) varieties did not amplify the desired fragment, suggesting the absence of 1B.1R translocation.

The co-dominant marker *PPO05* produced two bands of 685-bp and 876-bp in 30 varieties (Fig. 2). These two bands are associated with low polyphenol oxidase activity and 70% varieties showed only the 685-bp DNA fragment, which indicated the presence of higher polyphenol oxidase activity in these varieties. Five varieties including 'Hashim08', 'Ghaznavi', 'KT-2010' 'Raj', and 'Zam-04' did not amplify any product.

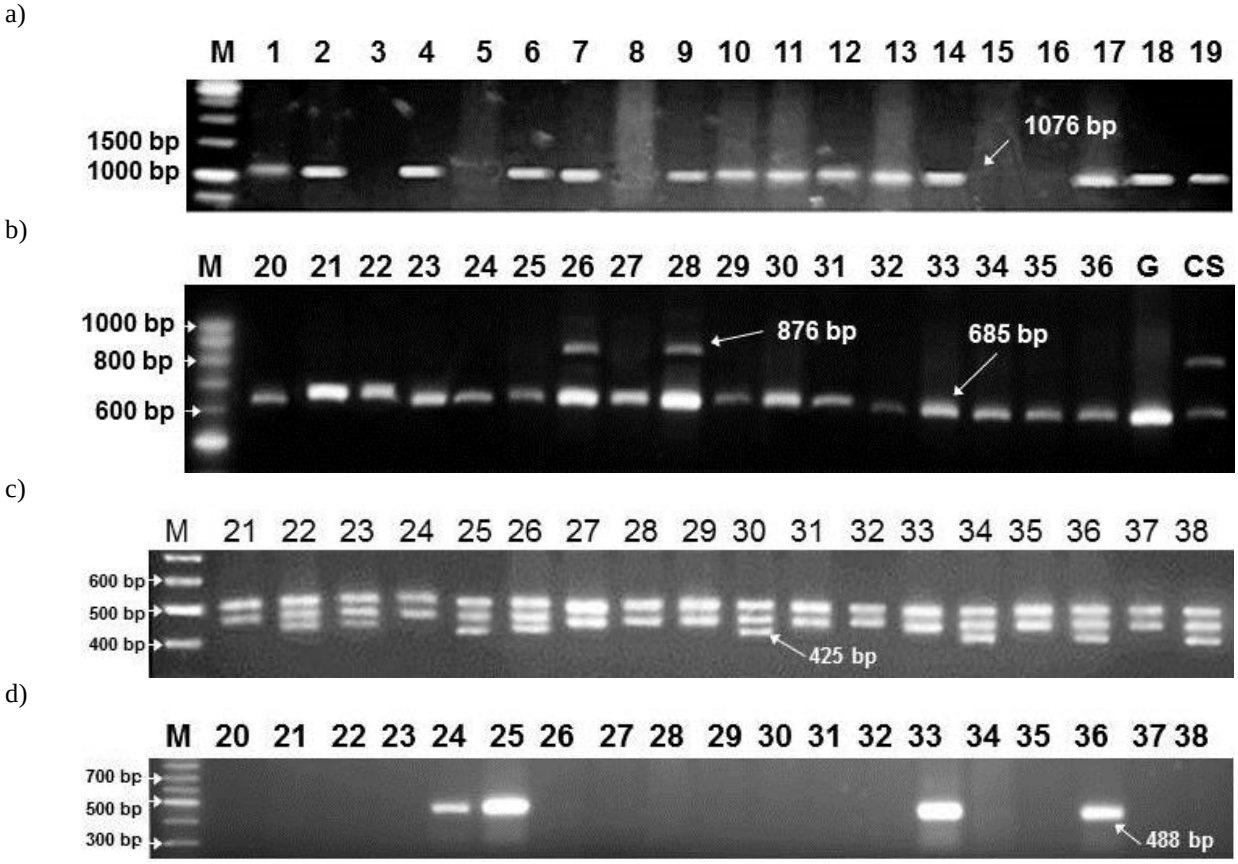

Figure 1. a. PCR amplified products of *ω-secalin* marker on 1% agarose gel (1kb DNA ladder). The arrow indicates the presence of 1076-bp DNA fragment associated with the presence of 1B.1R translocation. b. Banding pattern for co-dominant marker *PPO05* on 2% agarose gel (100-bp DNA ladder).The arrows indicate the presence of 685-bp and 876-bp DNA fragments associated with low PPO activity. c. Banding pattern of Pakistani wheat varieties with *Wx-B1a* marker (2% agarose, 100-bp DNA ladder).The arrow indicates the presence of *Wx-B1a*allele. d. PCR products of Pakistani wheat amplified with marker *Glu-A3d* (1.5% agarose, 100-bp DNA ladder).The arrow indicates the presence of *Glu-A3d* allele

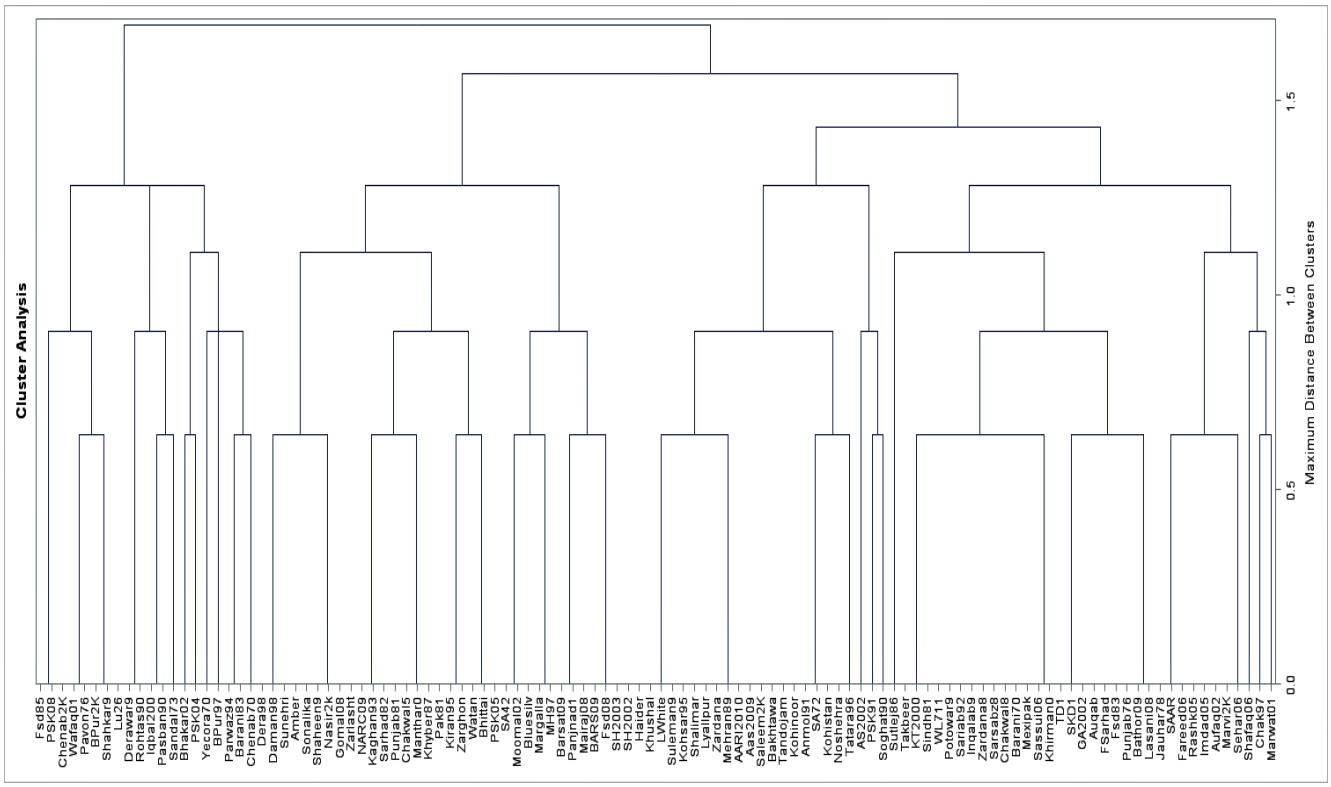

Figure 2. Dendogram showing grouping of 117 Pakistani wheat varieties based on 7 Marker data

Marker *Pinb-D1b* generated a 250-bp PCR product in 42% varieties. The 250-bp allele of *Pinb-D1b* is associated with hard grain texture. The remaining 68 varieties did not show the 250-bp fragment that revealed medium hard to soft grain texture (Supplementary Table 1).

PCR amplification of genomic DNA of 117 Pakistani adapted spring wheat varieties with marker *Wx-B1a* showed the presence of 425-bp fragment in 59% varieties, indicating the presence of *Wx-B1a* allele in these varieties (Fig. 3). The 425-bp fragment of *Wx-B1a* was found absent in the remaining 41% varieties, showing the presence of *Wx-B1b* allele. *Wx-B1b* allele is associated with good noodle quality.

Marker *Glu-D1d* produced a 450-bp fragment in 98% varieties (Supplementary Table 1). This fragment is associated with the presence of HMW-GS encoded by *Glu-D1d* allele. Allele *Glu-D1d* exerts a positive effect on gluten strength and plays a major role in noodle and bread making quality. Only two varieties, 'Sutlej-86' and 'Shafaq-2006' did not amplify the 450-bp PCR product, indicating the absence of *Glu-D1d* allele.

Glu-A3d marker produced a 488-bp fragment in 18% varieties, which indicated the presence of positive allele of *Glu-A3d* in 117 Pakistani wheat varieties (Fig. 4). This allele encodes a LMW-GS which is important for dough quality. The remaining 96 varieties did not amplify the 488-bp fragment, showing the presence of negative allele of *Glu-A3d* (Supplementary Table 1).

PCR analysis of 117 Pakistani wheat varieties with marker *Glu-B3* showed a 636-bp fragment in 65% varieties (Supplementary Table 1). This fragment is associated with the presence of *Glu-B3* allele and non-1B.1R translocation. *GluB3* is a LMW-GS allele responsible for better dough quality. The remaining 35% varieties did not show the 636-bp DNA fragment, indicating the absence of *Glu-B3* allele and presence of 1B.1R translocation. However 41 Varieties showed both 636-bp and 1076-bp fragments indicating the presence of *Glu-B3* and *ω-secalin* (1B.1R), respectively, whereas five varieties did not amplify any fragments of the above markers.

***Correlation analyses of Glu-B3 and Pinb-D1b with technological tests*:** In order to find the association of marker data with already available quality data for some of the varieties tested, Pearson's correlation coefficients were computed. We admit that the quality data we used for correlation analysis were not taken on the same seed source used for DNA extraction. However, we tried to find a rough estimate of the marker data with that of previously recorded quality data assuming that both seed source were of the same wheat variety. Marker data of *Glu-B3* of 26 varieties was positively correlated ($r=0.48$, $p<0.05$) with SDS sedimentation test data (Table 2). Similarly, *Glu-B3* marker

data of 48 varieties were compared with available data of gluten consistency. A weak positive correlation (r=0.21, p>0.05) was found between *Glu-B3* and gluten consistency (Table 3).

Marker data of marker *Pinb-D1b* of 39 varieties was compared with PSI (Particle Size Index) value. Twenty two varieties showed an association of PSI value with marker data. Nine varieties with 250-bp fragment had PSI value less than 40 (Table 4). Similarly, 13 varieties lacking 250-bp fragment had PSI value higher than 40. The remaining 17 varieties did not show any pattern (Table 4).

Cluster analysis grouped the 117 wheat varieties into 2 major and 8-9 small groups (Figure 2). We did not find any pattern of grouping of the genotypes either based on the year of release or on the breeding station of origin. Marwat-01, Chakwal-97 and Shafaq-06 clustered into a distinct group. Similarly, Soghat-90, Pirsabak-91 and AS-2002 also formed a separate group. Pirsabak-04 and Bhakkar-02 also grouped into a different cluster. The other varieties clustered into groups of more than 6 varieties.

Table 2. Comparison of marker *Glu-B3* data with SDS sedimentation value

No	Variety	*Glu-B3*	SDS sedimentation Value	Sr. No	Variety	*Glu-B3*	SDS sedimentation Value
1	Bakhtawar-92	-	22.8	14	Manthar-2003	+	15
2	Kaghan-93	+	29	15	SH-2003	+	22.5
3	Kohsar-95	+	29.3	16	Imdad-05	+	21.75
4	Shahkar-95	-	30.5	17	Rashkoh-05	+	24.5
5	B.Pur-97	-	27	18	Fareed-06	-	22.6
6	Haider-2000	+	25	19	Sehar-2006	+	26.2
7	Marvi-2000	-	30	20	Shafaq-2006	+	32.5
8	Saleem-2000	-	22.8	21	SKD1-2006	+	22.5
9	Marwat-2001	-	15	22	Mairaj-2008	-	28.6
10	Wafaq-2001	+	22	23	Chakwal-50	+	24.5
11	Bhakkar-02	+	20	24	NIA-Amber	+	30.5
12	GA-2002	+	20.5	25	KT-2010	+	26.5
13	Moomal-2002	+	24	26	Daman-98	+	27

For *Pinb-D1d* + and - indicates the presence and absence respectively

Table 3. Comparison of marker *Glu-B3* data with Gluten Consistency

No	Variety	*Glu-B3*	Gluten consistency†	Sr. No	Variety	*Glu-B3*	Gluten consistency
1	Pavon-76	-	MS	25	Iqbal-2000	+	W
2	Fsd-83	+	MS	26	Marvi-2000	-	MS
3	Chakwal-86	+	MS	27	Saleem-2000	-	MS
4	Khyber-87	-	MS	28	Marwat-2001	-	MS
5	Shalimar-88	+	W	29	Wafaq-2001	+	MS
6	Pasban-90	+	MS	30	Bhakkar-02	+	MS
7	Soghat-90	-	W –MS	31	GA-2002	+	MS
8	Inqalab-91	+	MS	32	Moomal-2002	+	MS
9	Bakhtawar-92	-	MS	33	Manthar-2003	+	MS
10	Sariab-92	+	MS	34	SH-2003	+	MS-W
11	Kaghan-93	+	MS	35	Imdad-05	+	S-MS
12	Parwaz-94	+	MS-W	36	Rashkoh-05	+	MS
13	Kohsar-95	+	MS	37	Fareed-06	-	MS-S
14	Shahkar-95	-	MS	38	Sehar-2006	+	MS
15	Suleman-96	+	MS	39	Shafaq-2006	+	MS-S
16	B.Pur-97	-	MS	40	SKD1-2006	+	MS
17	Chakwal-97	+	S-MS	41	Fsd-2008	-	S
18	Kohistan-97	+	MS	42	Lasani-2008	+	S-MS
19	MH-97	+	MS-W	43	Mairaj-2008	-	MS-S
20	Margalla-99	+	MS	44	Chakwal-50	+	MS-S
21	Zarlashta-99	-	W	45	Mehran-89	-	MS-W
22	Auqab-2000	+	MS-W	46	Anmol-91	+	W
23	Chenab-2000	-	W	47	Jauhar-78	+	MS
24	Haider-2000	+	MS	48	Sindh-81	+	S-MS

† W = weak, MS = medium strong, S = strong, MS-W = medium strong to weak and W-MS weak to medium strong

For *Glu-B3.* + and - indicates the presence and absence of allele respectively

Table 4. Comparison of marker *Pinb-D1b* data with Particle Size Index (PSI) value

Sr. No	Variety	*Pinb-D1b*	PSI	Sr. No	Variety	*Pinb-D1b*	PSI
1	MH-97	+	38	21	Bhakkar-02	-	46.6
2	Margalla-99	+	37	22	Lasani-2008	-	41
3	Zarlashta-99	+	39.1	23	Imdad-05	-	34
4	Haider-2000	+	34.5	24	Rashkoh-05	-	34
5	Wafaq-2001	+	33	25	Fareed-06	-	33
6	Manthar-2003	+	30	26	Sehar-2006	-	39
7	SH-2003	+	30	27	Shafaq-2006	-	33
8	Mairaj-2008	+	33	28	SKD1-2006	-	31
9	Chakwal-50	+	38	29	GA-2002	-	29
10	Shalimar-88	-	46	30	Saleem-2000	-	30
11	Pasban-90	-	51	31	Marwat-2001	-	38
12	Soghat-90	-	48	32	Kohistan-97	-	33
13	Inqalab-91	-	50	33	Shahkar-95	+	47
14	Bakhtawar-92	-	48	34	B.Pur-97	+	50.5
15	Sariab-92	-	65	35	Parwaz-94	+	49
16	Kohsar-95	-	49	36	Moomal-2002	+	43
17	Chakwal-97	-	50	37	Kaghan-93	+	48
18	Auqab-2000	-	45.7	38	Fsd-2008	+	43
19	Iqbal-2000	-	44	39	Chenab-2000	+	44.3
20	Marvi-2000	-	43.2				

For *Pinb-D1d* + and - indicates the presence and absence of allele respectively

DISCUSSION

The present study evaluated 117 Pakistani adapted spring wheat varieties for presence/absence of quality related genes using DNA markers. Genetic variation was observed for the different quality related genes in the studied genotypes. Our results can provide a basis for incorporating desirable alleles for different quality traits in future wheat varieties for improving different quality traits. Molecular markers particularly gene specific markers are valuable tools for the detection of genes controlling economically important characters and are very helpful in the selection of genotypes with desirable end-use quality in early segregating generations.

Wheat-rye translocation 1BL.1RS is used widely in wheat breeding program (Ko *et al.,* 2002; Zarco-Hernandez *et al.,* 2005). Under optimum growing conditions, the presence of 1BL.1RS translocation increases grain yield upto 1.6%, whereas under drought conditions it increases yield upto 11.3% (Villareal *et al.,* 1998). This translocation is associated with higher kernel weight and greater biomass but it negatively affects the dough quality. Several reports have illustrated that some genotypes with this translocation might have good dough quality (Bullrich *et al.,* 1998; Liu *et al.,* 2005; Xia *et al.,* 2005). Furthermore, several race-specific rust resistance genes are located on 1RS chromosome of rye that enhances rust resistance (Rajaram *et al.,* 1983; Chai *et al.,* 2006). Zhang *et al.* (2008) screened 70 Chinese wheat cultivars and lines and found that 28 genotypes had 1B.1R translocation.

LMW-GS encoded by *Glu-B3* allele plays a key role in gluten strength (Flaete and Uhlen, 2003; Goba *et al.,* 2008). Zhang *et al.* (2008) reported that 40% Chinese genotypes possessed 1B.1R translocation and 60% genotypes had *Glu-B3* allele. This suggested that genotypes with positive allele of 1B.1R are negative for *Glu-B3* allele. In our study, positive allele of *Glu-B3* was present in 65% Pakistani wheat varieties, whereas 35% varieties did not have this allele. Our results are 61% consistent with that of Zhang *et al.* (2008) with regards to the presence of 1B.1R translocation and absence of *Glu-B3* allele or vice versa. However, the remaining 39% genotypes either had positive alleles at both loci or negative alleles. The possible reason for the presence or absence of both alleles may be the heterozygosity of varieties for 1B.1R translocation or the existence of other translocation. *Glu-B3* allele greatly contributes in highest mixing tolerance and mixing time to dough (Liu *et al.,* 2005) and also confers high SDS sedimentation value (Liu *et al.,* 2005; Ragupathy *et al.,* 2008). Genetic variation exists in wheat (Arshad *et al.,* 2012; Khakwani *et al.,* 2011). Our results also revealed positive correlation between *Glu-B3* and SDS sedimentation value. This indicated that marker assisted selection for *Glu-B3* can be useful to select genotypes with desirable SDS sedimentation value. As SDS sedimentation affects bread making quality, *Glu-B3* can be used as an indirect selection criterion for improving bread making quality in early breeding generations. *Glu-B3* showed a weak positive correlation with gluten consistency.

Glu-A3d is a LMW-GS allele, which exerts a positive effect on gluten strength, SDS sedimentation, gluten consistency

and bread making quality (Flaete and Uhlen, 2003; Liu *et al.,* 2005; Zhang *et al.,* 2008). We found *Glu-A3d* allele in 18% varieties including 'Chenab-70', 'Yecora-70', 'Sandal-73', 'Pavon-76', 'Lu26-76', 'Barani-83', 'Fsd-85', 'Pasban-90', 'Rohtas-90', 'Parwaz-94', 'Shahkar-95', 'B.Pur-97', 'Derawar-97', 'B.Pur-2000', 'Chenab-2000', 'Iqbal-2000', 'Wafaq-2001', 'Bhakkar-02', 'AS-2002', 'Pirsabak-04' and 'Pirsabak-08'. Zhang *et al*. (2008) reported the presence of *Glu-A3d* in 46% of the 70 Chinese wheats using PCR and SDS-PAGE, indicating a higher frequency of this allele in Chinese wheats than in Pakistani varieties. As *Glu-A3d* is very important for quality, the frequency of this allele needs to be increased in future wheat varieties of Pakistan.

Glu-D1d is a HMW-GS allele conferring better bread and noodle quality (Yanaka *et al.,* 2007; Guo *et al.,* 2010). Positive allele of *Glu-D1d* was present in 98.5% Pakistani wheat varieties. Sultana *et al*. (2007) screened some Pakistani wheat varieties for HMW-GS allele, *Glu-D1d* (5+10), using SDS-PAGE. Twenty three varieties including 'Yecora-70', 'Lyallpur-73', 'Sandal-73', 'Punjab-76', 'LU 26', 'Sindh-81', 'Pak-81', 'Kohinoor-83', 'Faisalabad-85', 'Khyber-87', 'Shalimar-88', 'Pasban-90', 'Rohtas-90', 'Inqalab-91', 'Parwaz-94', 'Kohsar-95', 'Sulman-96', 'Nowshera-96', 'Chakwal-97', 'Chenab-2000', 'AS-2002', 'Watan' and 'Zardana' showed similar results in our study based on PCR marker *Glu-D1d*. Sultana *et al*. (2007) found that 13 varieties including 'Barani-70', 'Blue silver', 'Chakwal-86', 'Sarsabz', 'Pothowar', 'Pavon', 'Punjab-81', 'Auqab-2000', 'Mexipak-65', 'Bhakkar-2002', 'Punjnad-1', 'Bahawalpur-97' and 'Manthar' did not have the *Glu-D1d* (5+10) allele. However, these varieties showed the DNA fragment associated with the presence of *Glu-D1d* allele in our study.

The *Wx* proteins encoded by *Wx* alleles in wheat have a significant impact on wheat quality (He *et al.,* 2004; Yamamori, 2009). Low amylase is very important for good noodle quality (Urbano *et al.,* 2002; Xu *et al.,* 2005). Yamamori *et al*. (1992) and Miura and Tanii (1994) described positive correlation between lower amylose content and *Wx* alleles in different Japanese cultivars. GBSS I genes are in low number in Waxy wheat, while one or two waxy proteins are absent in partial Waxy wheat (Saito *et al.,* 2009). *Wx-B1* null allele indicates reduced amylase (Briney *et al.,* 1998). Most of the Pakistani wheat varieties, we studied, showed *Wx-B1a* allele, indicating slightly high amylose level. Similarly, *Wx-B1a* allele that indicates the presence of desirable *Wx-B1b* allele, was absent from 41% Pakistani wheat varieties studied.

Varieties with *Pinb-D1b* allele have higher flour yield and low flour ash content (Martin *et al.,* 2001; Chen *et al.,* 2007). Allelic variation in *Puroindoline* determines various grain texture (Bagge *et al.,* 2007; Chen *et al.,* 2007). Feiz *et al*. (2009) described segregation pattern and function of different new alleles of *Puroindoline* in bread wheat. Based on our results, Pakistani wheat may be divided into two categories; hard to medium hard and soft grain. Of 117 Pakistani wheat varieties studied, 42% showed hard grain based on *Puroindoline* alleles. We also compared the marker data of 39 varieties studied with available PSI (Particle Size Index) values. PSI value less than 40 indicates hard to very hard; above 40 indicates medium hard to fairly soft, whereas above 64 indicates soft grain texture. Our results showed that 23% varieties had positive allele of *Pinb-D1b* and had PSI values less than 40, indicating that these have hard grain texture. Similarly, 33% of varieties that had the null allele of *Pinb-D1b* had PSI value greater than 40, suggesting medium hard to soft grain texture. Seventeen varieties did not show an association between *Pinb-D1b* marker data and PSI values. The difference among these varieties may be due to the presence or absence of other alleles for grain hardness.

Polyphenol oxidase (PPO) activity causes undesirable browning and darkening of wheat products during processing or after storage (Raman *et al.,* 2007; Singh *et al.,* 2009). Consumers prefer bright and creamy color noodles (Demeke *et al.,* 2001). High PPO activity produces black pigments in wheat flour (Raman *et al.,* 2007; Martin *et al.,* 2010). We found a high PPO activity in most (70%) of the Pakistani wheat varieties tested. The absence of amplification in five Pakistani varieties might be due to the presence of alternate alleles for PPO in these varieties. Wang *et al*. (2009) also found that *PPO-2Aa* and *PPO-2Db* alleles were associated with high PPO activity, whereas *PPO-2Ab* and *PPO-2Da* were associated with low PPO activity.

Wang *et al.* (2008) developed STS01 marker from the sequence AY515506 to discriminate two alleles of gene *PPO-2D.* They developed another marker "*PPO05*" from the same sequence (AY515506), which produced 685 and 876-bp bands in Chinese wheat genotypes with low PPO activity and a 685-bp band in genotypes with high PPO activity (Wang *et al.,,* 2009). They observed a high frequency of wheat genotypes possessing high PPO activity in Chinese wheat germplasm. Our results were consistent with the findings of Wang *et al.* (2009).

We failed to amplify DNA fragments associated with quality related genes with 7 DNA markers tested. Zhang *et al*. (2008) used gene specific markers *PPO-A1a/b, Ppo-D1a* for detection of PPO activity and *Glu-B1-2a* for improved gluten quality in 70 Chinese wheat genotypes and amplified 685/876, 713 and 527-bp fragments, respectively. These markers did not show polymorphism in the Pakistani wheat varieties we tested. The primer pairs *Wx-B1-W-BDFL, Wx-B1-WBRC1, Wx-B1-N-BFC, Wx-B1-N-BRC2* used by Saito *et al*. (2009) to detect waxy genes, also did not amplify any fragment in Pakistani varieties. Marker GBSS-4A, which detects the presence/absence of a waxy gene (Briney *et al.,* 1998), amplified non-specific PCR fragments in our study. Similarly, marker LMW1/2 for the detection of LMW-GS (Li *et al.,* 2008) did not amplify the DNA fragments

associated with the presence/absence of LMW-GS gene. The reason for no amplification in these markers might be either due to the presence of alternate alleles or presence of mutation in the sequences of these genes in Pakistani wheat.

In the present study, we did find genetic variation at different loci controlling various quality traits using Pakistani wheat. This variation was evident from the difference in the frequencies of genes controlling quality traits. The markers we used were gene-specific and should ideally explain variation with respect of quality traits. However, we did not find a perfect correlation between marker data and the previously taken quality data. One of the possible reasons for this could be due to the use of seeds of wheat varieties from different sources. Moreover, the quality traits we studied are complex traits and are controlled by multiple genes. As we did not screen for all the genes controlling a particular quality traits due to the non-availability of DNA markers, we could not find consistency between marker and quality data. Nevertheless, results of this study have implication in wheat breeding programs aiming at improving quality traits. Early generation marker assisted selection is likely to save time and resources of the breeders in future wheat quality improvement. Moreover, information generated in this study on the presence and/or absence of quality related genes may be useful for wheat breeders wishing to assemble desirable gene combinations in future wheat varieties.

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