Society for Advancement of Wheat Research
ICAR-Indian Institute of Wheat & Barley Research
Karnal - 132 001, India

Journal of Wheat Research
7(1):18-26
Homepage: http://epubs.icar.org.in/ejournal/index.php/JWR

Research Article

Assessment of genetic diversity in elite wheat genotypes using simple sequence repeat and quality protein markers

Sonia Sheoran, Pradeep Sharma*,Virender Singh, Sushma Pawar, Davender Sharma, Neha Jain, Rajendra Kumar, Vidisha Thakur, Girish Chander Pandey, Rekha Malik, Ratan Tiwari, Vinod Tiwari, Raj Kumar Gupta and Indu Sharma

ICAR-Indian Institute of Wheat and Barley Research, Karnal-132001, Karnal, India

Article history

Received: 30 April, 2015
Revised : 10 June, 2015
Accepted: 10 June, 2015

Citation

Sheoran S, P Sharma,V Singh, S Pawar, D Sharma, N Jain, R Kumar, V Thakur, GC Pandey, R Malik, R Tiwari, V Tiwari, R K Gupta and I Sharma. 2015. Assessment of genetic diversity in elite wheat genotypes using simple sequence repeat and quality protein markers. *Journal of Wheat Research* 7(1):18-26

****Corresponding author***

Email: *neprads@gmail.com*
Tel.: *01842209192*

Abstract

In present investigation, genetic diversity (GD) analysis of forty two elite Indian wheat genotypes representing different agro-climatic zones of India was determined using set of sixteen STS and 36 SSR markers. In total, 86 alleles were detected and polymorphism information content (PIC) ranged from 0.09 *(Xwmc227)* to 0.99 (*Xgwm165*) with a mean of 0.56, indicating sufficient discrimination by markers. Among the seven homologous chromosome groups, GD was highest in group 4 followed by lowest in group 3 and 6. Average number of allele were highest in B- genome (2.6) followed by the A- and D- genomes (2.3 and 2.1, respectively). Cluster analysis based on SSRs and quality protein markers identified three distinct clusters.Principal component analysis (PCA) obtained from molecular data shows that the first two components justified 38.04% of total variance. Further, these elite lines were also characterized for HMW using SDS-PAGE. For the *Glu-1* loci, 10 alleles were detected, 3 at the Glu-A1 locus, 5 at the *Glu-B1* and 2 at the *Glu-D1*. The frequency range in 10 HMW-GS combination was 3.2% to 74.2% in the advanced lines. At the *Glu-D1* locus, the subunits 1Dx5+1Dy10 was present in 74.2% of the lines followed by the subunit 1Dx2+1Dy12 with a frequency of 25.8%. The subunit 1Dx5+1Dy10 was predominantly observed in these advanced lines having a stronger effect on bread making quality. This study provides evidence about genetic divergence and distinctness including quality traits in elite Indian wheat genotypes evaluated under the umbrella of All India coordinated wheat and barley improvement programmeand serve as new source of genetic variation for wheat improvement.

Keywords: genetic variability, HMW, SSRs, SDS-PAGE, *Triticum aestivum*

1. Introduction

India ranks second in wheat production across the world and produced over 95.76 million tonnes of wheat during the crop year 2014-15 (www.agricoop.nic.in; 2nd Advance Estimate). This spectacular increase has been possible due to the technological advancement including varietal improvement. So far, in India, more than 380 varieties of wheat and *triticale* have been released for different wheat sowing conditions, thereby providing a wider choice for each agro-climatic zone. In recent years, a number of molecular markers have been used to study the trends of changes in genetic diversity in wheat germplasm released during the last century (Mir *et al.*, 2012). The genes encoding HMW-GS reside at the *Glu-1* locus (*Glu-A1, Glu-B1* and *Glu-D1*), which is located on the long arm of the group 1 homologous chromosomes. The extensive allelic variations of HMW glutenin subunits, which could be considered as a useful genetic marker, have been reported in germplasm evaluation and breeding work (Pfluger *et al.*, 2001; Degaonkar *et al.*, 2005). Crop genotypes include inbred or pure lines hybrids, landraces, wild relatives, germplasm accessions, cultivars or varieties. These genotypes have wide and diverse origin and genetic background known as genetic diversity.

Morphological traits can be used to characterize genetic diversity, and are often influenced by environmental factors. Genetic diversity study is a major breakthrough in understanding intra-specific crop performance leading to crop improvement. Genetic diversity studies therefore, is a stepwise process through which existing variations in the nature of individual or group of individual crop genotypes are identified using specific statistical method or combination of methods.

The loss of genetic diversity not only limits further improvement of yield and quality, but also increases the vulnerability of wheat to biotic and abiotic stresses. Therefore, wheat breeding programs all over the world aims to improve grain yield with better quantity and quality, disease resistance and agricultural performance. Genetic diversity is basis for selection of crossing parents, creating heterotic groups and has a significant effect on the amelioration of crops. In this study, we used a set of 42 elite breeding material advanced material. This material comprised of 13 checks of different zones and 29 elite lines of wheat, which were multi-tested at different locations of India under AICWB&IP were evaluated. Therefore, the main objective of the present study was (i) to assess the genetic diversity among Indian wheat registered/advanced varietal lines from six agro-climatic zones of India using SSR and STS markers (ii) to detect the variations of HMW glutenins subunits in selected *Triticum* genotypes for better end use quality. The molecular characteristics, genetic diversity and phylogenetic relationships concluded in this study will help in the selection of parents to develop highly-docile varieties in breeding programs.

2. Materials and methods

2.1. Plant material: A set of 42 elite Indian bread and durum wheat genotypes (Table 1) evaluated for various traits during the year 2011-12 and 2012-13 under All India Coordinated Wheat and Barley Improvement Programme representing six agro-climatic wheat sowing zones of India were used to study the genetic diversity.

2.2. DNA extraction and PCR analysis: Genomic DNA was extracted from fresh young leaves of plants (15 days old) of each genotype using a CTAB method (Saghai-Maroof*et al.*, 1984). The DNA samples were analyzed both quantitatively and qualitatively using 0.8% agarose gel electrophoresis. PCR analysis of molecular markers (panel of 16 STS and 36 SSRs markers spanning whole genomic region of wheat) was carried and the amplified products were resolved on 2% agarose gel (Hi Media).

For HMW, single grains were crushed and suspended with 25 µl/mg of 62.5 mM Tris–HCl buffer, pH 6.8, containing 2% (v/v) SDS, 1.5% (v/v) dithiothreitol, 10% (v/v) glycerol and 0.002% (v/v) bromophenol blue to extract total proteins. The tubes were vortexed for 3 min, placed in a boiling water bath for 5 min and then immediately centrifuged for 3 min at 15,000 rpm, the supernatant being retained for SDS-PAGE. SDS-PAGE was routinely conducted with 10%, 12% and 15% (w/v) running gels and 4% (w/v) stacking gels, but a 17.5% (w/v) separating gel was used to determine subunit 1Ax2* in the presence of subunit 1Dx2. The gels were run at room temperature and at a constant voltage (130 V) for about 1 h, followed by 240 V for 3–4 h. The separating gel was then stained overnight with 0.1% (w/v) Coomassie Brilliant Blue G250, in 25% (v/v) propan-2-ol and 10% (v/v) acetic acid, and de-stained with tap water. HMW glutenin subunits were designated according the nomenclature of Payne and Lawrence (1983) and the international nomenclature of McIntosh *et al.* (1998). Sedimentation volume of the whole meal is calculated by SDS-Sedimentation method (FMBRA Bulletin, 1977). Hardness index is determined using SKCS (Perten).

2.3. Statistical analysis: The genetic diversity (GD) in the genotypes was assessed by using various statistical–parameters like average number of alleles/locus, major allele frequency and polymorphism information content (PIC) values for each SSR locus. All the above parameters were analyzed using Powermarker 3.25 (Liu and Muse, 2005). Allele molecular weight data were used to export the data in binary format (allele presence="1" and allele absence="0"). DARwin version 5.0 was used for calculating pair- wise genetic distances and for constructing the dissimilarity matrix (Perrier *et al.*, 2003). The dissimilarity matrix thus obtained was subjected to cluster analysis using the un-weighted neighbour-joining (UNJ) method (Gascuel, 1997), followed by bootstrap analysis with 1,000 permutations to obtain a cluster for all the selected genotypes. Principal component analysis (PCA) was done by using SAS version 9.3.

3. Results

3.1. Polymorphism in elite Indian wheat genotypes using SSRs: In this study, a set of 36 most discriminating markers out of 300 SSR markers surveyed, were used to characterize and estimate the genetic diversity of the 42 genotypes (Table 2). Total 86 alleles were detected and the number of alleles per locus ranged from 1 to 5 with an average of 2.65 alleles per locus. The PIC values ranged from of 0.09 (*Xwmc227*) to 0.9929 (*Xgwm165*), with an average of 0.56 (Table 2). The high frequency allele ranged from 0.3214 (*Xwmc617*) to 1.0 (*Xgwm325, Xgwm458, Xwmc227, Xwmc274*). North Western Plains Zone showed the highest genetic diversity whereas in the Southern Hills Zone less diversity was found which could be due to less number of genotypes selected in this study (Fig. 1).

Table 1. List of elite breeding wheat genotypes and their pedigree used in this study

G.No.	Genotypes	Pedigree	Zone	Genotypes
1	HS526	CMH82A1294/2*KAUZ//MUNIA/CHATO/3/MILAN	NHZ	Bread wheat
2	HPW349	OASIS/SKAUZ//4*BCN/3/PASTOR/4/KAUZ	NHZ	Bread wheat
3	HS 507	KAUZ/MYNA/VUL//BUC/FLK/4/MILAN	NHZ	Bread wheat (C)
4	WH 1105	MILAN/S87230//BABAX	NWPZ	Bread wheat
5	DPW 621-50	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	NWPZ	Bread wheat (C)
6	HD3059	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	NWPZ	Bread wheat
7	HD3065	PBW65/2*PASTOR	NWPZ	Bread wheat
8	PBW658	CS/*Th.sc*//3*PBN/3/MIRLO/BUC/4/MILAN/5/TULHI	NWPZ	Bread wheat
9	WH1100	PBW65/2*PASTOR	NWPZ	Bread wheat
10	DBW71	PRINIA/UP2425	NWPZ	Bread wheat
11	DBW90	HUW468/WH730	NWPZ	Bread wheat
12	WH1098	TILHI/PASTOR	NWPZ	Bread wheat
13	PBW660	WG6761/WG6798	NWPZ	Bread wheat
14	PBW644	PBW175/HD2643	NWPZ	Bread wheat (C)
15	HI1579	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	NWPZ	Bread wheat
16	WH1097	ATTILA/BABAX//PASTOR	NWPZ	Bread wheat
17	DBW74	RWP2008-26/WBLLL*2/BRAMBLING	NWPZ	Bread wheat
18	NIAW1594	BEE/PJN/2*KAUZ/3/PASTOR	PZ	Bread wheat
19	HD3043	PJN/BOW//OPATA*2/3CROC_1/*Ae.sqarrosa*(224) //OPATA	NWPZ	Bread wheat (C)
20	RAJ4229	HW2048/RAJ4000	NEPZ	Bread wheat
21	K0906	UP2338/PBW373	NEPZ	Bread wheat
22	DBW39	ATTILA/HUI	NEPZ	Bread wheat (C)
23	K0911	K9533/DBW14	NEPZ	Bread wheat
24	HI1563	MACS2496*2/MC10	CZ	Bread wheat (C)
25	HD3070	TAM200/TUI/3/URES/JUN//KAUZ	NWPZ	Bread wheat
26	HD2888	C306/*T.sphaerococcum*//HW2004	NWPZ	Bread wheat (C)
27	RAJ4238	HW2021/RAJ3765	NWPZ	Bread wheat
28	MP3336	HD2402/GW173	CZ	Bread wheat
29	HD2932	KAUZ/STAR//HD2643	NWPZ	Bread wheat (C)
30	HW5216	HW3094//HW4028	SHZ	Bread wheat
31	COW(W)1	HD2646//HW2002A/CPAN3057	All zones	Bread wheat (C)
32	KRL210	PBW65/2*PASTOR	NWPZ	Bread wheat (C)
33	KRL283	CPAN3004/KHARCHIA65//PBW343	NWPZ	Bread wheat
34	KHARCHIA65	SELECTION FROM KHARCHIA LOCAL	All zones	Bread wheat
35	DDK1009	NP200*4//NP200/ALTAR84	PZ	Dicoccum (C)
36	MACS2971	KRT5*2/NP200	PZ	Dicoccum (C)
37	HW1098	NILGIRI LOCAL (MUTAGEN TREATED-25Kr)	SHZ	Dicoccum
38	WHD948	ALTAR84/STINT//SILVER	NWPZ	Durum
39	PDW314	AJAIA12/F3LOCAL(SEL.ETHION.135.85) //PLATA13/3/SOMAT3/4/SOOTY9/RASCON37	NWPZ	Durum (C)
40	MACS3828	ALTAR84/STINT//SILVER44/3/SOMAT3.1/4	PZ	Durum
41	HI8713	HD4672/PBW233	CZ	Durum
42	HI8498	RAJ6070/RAJ911	CZ	Durum

Note: (C) denotes cultivar used as check

Table 2. Allelic variation of the polymorphic SSR loci on the basis of allele and PIC

Marker	Chromosome Location	Major Allele Frequency	No. of alleles	Gene Diversity	Heterozygosity	PIC
Xgwm111	7D	0.512	5	0.665	0.743	0.7117
Xgwm149	4B	0.527	2	0.498	0.000	0.6315
Xgwm160	5B	0.731	2	0.392	0.146	0.432
Xgwm165	4B	0.500	2	0.500	1.000	0.992
Xgwm186	5A	0.579	2	0.487	0.052	0.580
Xgwm190	5D	0.512	2	0.499	0.000	0.5686
Xgwm219	6B	0.511	3	0.593	0.023	0.5933
Xgwm256	-	0.642	3	0.494	0.309	0.4897
Xgwm265	2A	0.885	2	0.202	0.057	0.446
Xgwm273	1B	0.611	4	0.534	0.750	0.664
Xgwm312	2A	0.761	2	0.362	0.000	0.363
Xgwm325	6D	1.000	1	0.000	0.000	0.587
Xgwm337	1D	0.576	2	0.488	0.000	0.804
Xgwm374	2B	0.726	3	0.423	0.119	0.424
Xgwm408	5B	0.804	2	0.314	0.000	0.337
Xgwm428	7D	0.985	2	0.029	0.029	0.364
Xgwm437	7D	0.529	2	0.498	0.000	0.671
Xgwm44	7D	0.525	2	0.498	0.850	0.546
Xgwm458	1D	1.000	1	0.000	0.000	0.224
Xgwm46	7B	0.500	2	0.500	0.000	0.634
Xgwm484	2D	0.617	2	0.472	0.000	0.655
Xgwm493	3B	0.581	3	0.497	0.027	0.606
Xgwm519	-	0.381	4	0.691	0.833	0.692
Xwmc153	1D,3A	0.547	2	0.495	0.714	0.495
Xwmc227		1.000	1	0.000	0.000	0.092
Xwmc232	4A, 7B	0.4250	3	0.642	0.950	0.646
Xwmc233	5D	0.500	2	0.500	1.000	0.611
Xwmc242	-	0.535	2	0.497	0.738	0.510
Xwmc245	2B, 2D	0.559	2	0.492	0.881	0.493
Xwmc255	-	0.437	3	0.626	0.718	0.838
Xwmc265	2B	0.397	3	0.653	0.923	0.580
Xwmc274	3B	1.000	1	0.000	0.000	0.223
Xwmc455	2A	0.595	3	0.563	0.809	0.806
Xwmc54	3B	0.678	2	0.436	0.024	0.564
Xwmc160	5B	0.500	2	0.500	0.850	0.436
Xwmc617	4A,4B,4D	0.321	5	0.742	0.905	0.743

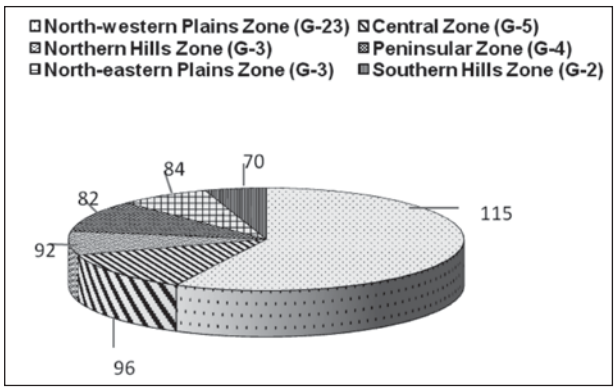

Fig 1. Genetic variability among six agro-climatic zones of India on the basis of number of alleles.*G denotes genotypes.

The highest mean number of alleles was detected in the B genome (2.6) followed by the A genome (2.25) and the lowest number were detected in the D genome (2.1). The highest mean PIC was also recorded in the B genome (0.57), followed by the D genome (0.56) and the A genome (0.54) (Fig 2). The highest PIC value of 0.8 was observed for the fourth homologous chromosome group markers, and the lowest value of 0.5 was observed for the third and sixth homologous group markers. Among the seven homologous chromosome groups in wheat, the ranking for the allele diversity was 4 (0.6)>7 (0.5) >5 (0.45) = 2 (0.45) >1 (0.38) > 6 (0.3) = 3 (0.3) (Fig.2).

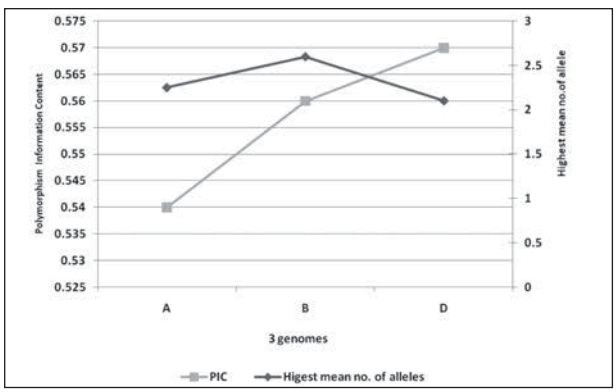

Fig 2. Comparison of genetic diversity (PIC and Highest mean number of allele) among 3 genomes of wheat from 36 SSRs.

3.2. Cluster analysis: According to Neighbour joining cluster analysis (DARWin 5), the 42 genotypes formed three separate major clusters (Fig.3). Cluster I consisted of 74% of total genotypes having bread wheat advanced lines bred mainly for abiotic stress and high yield performance for six different agro-climatic zones of India. Cluster II accounted 7% of the total genotypes of *T. dicoccum* lines namely DDK 1009, MACS 2971 and HW 1098, while Cluster III predominantly grouped with 12% cultivars consisted of durum wheat advanced lines namely WHD 948, HI 4498,

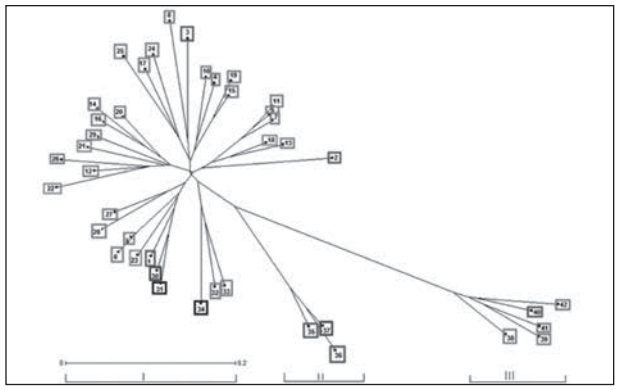

Fig 3. Unrooted neighbour joining tree showing genetic relationship among 42 genotypes(as shown in Table 1) using 36 SSR markers. The genotypes of different agro-climatic zones represented (blue for CZ, purple for SHZ, green for NWPZ, red for NHZ, orange for NEPZ, pink for PZ, black for all zones).

PDW 314, HI 8713 and MACS 3828. Three clusters of unrooted neighbour joining tree efficiently differentiated the germplasm into three major groups of bread wheat, durum wheat and *dicoccum*. Genetic relationship and grouping of genotypes on the basis of SSR analysis was further investigated using principal component analysis (PCA) (Fig 2). Unlike cluster analysis, PCA classified the 42 genotypes into two groups. First group included both *T. aestivum* and *T.dicoccum* while second group included the durum lines only (Fig. 4). The first component of PCA (PC1) explained approximately 38.04% of the variation and the second (PC2) explained approximately 9.05% of variation of the data and overall the first two major axis of differentiation (PC1 and PC2) explained about 47.55% of the total variations on an average.

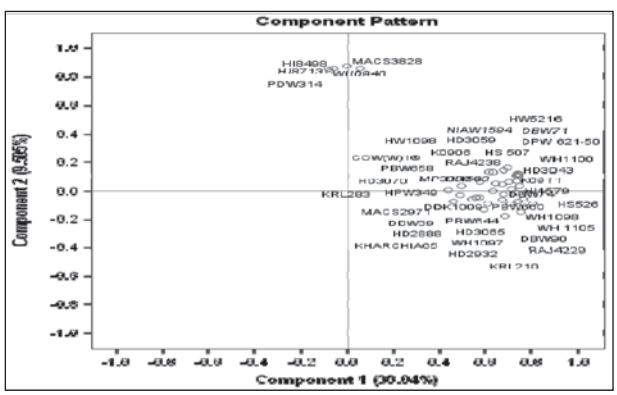

Fig 4. PCA of 42 wheat genotype lines using first two principal components from 36 SSR markers.

3.3. STS marker analysis: Advance Varietal trails (AVTs) advanced lines were also characterized for quality related genetic traits using gene-specific markers for HMW-GS, *polyphenol oxidase* (PPO) and waxy genes (Table 3). For *Glu-1* locus, *Ax2** allele accounted 33.0% while *Dx5* was found in 30.0% of the advanced lines.

Table 3. Gene based/STS markers allelic variations

Sr. No.	Gene based markers	Chr location	Allele size range (bp)	PIC
1	*1B/1R*	1B/1R	1.5 kb	0.963719
2	*Ax2**	1A	1.3kb	0.888889
3	*Bx7/17*	1B	447, 669	0.904195
4	*DREB*	3B	600, 700	0.888889
5	*DuPw004*	4A	250, 350	0.418084
6	*Dx10/Dy12*		576/612	0.793367
7	*Dx5*	1D	450	0.904195
8	*Lr10*	1A	280	0.854875
9	*Lr34*	7D	150, 220	0.673469
10	*PinA*	5D	450	0.997732
11	*PinB*	5D	450	0.382653
12	*PPO18*	3A	685/876	0.877268
14	*Vp1B3*	3B	569, 652, 845	0.386621
15	*VrnA1a*	5A	965	0.693311
16	*Wx-B1*	4A	425, null	0.30839

For the *Ppo-A1* locus tested with the marker *PPO18,* the frequencies of alleles *Ppo-A1a* and *Ppo-A1b* were 35.17% and 7.1% respectively, which are associated with higher and lower PPO activities. Sixteen lines did not yield any amplification. For waxi loci, tested by the marker *Wx-B1*, 38% of lines possessed the *Wx-B1b* allele. In vernalization gene, the frequency of allele *Vrn A1a* and *Vrn A1b* was observed 23.0% and 50.0%, respectively in the advanced lines.Leaf rust caused by the biotrophic species *Puccinia triticina* (*Pt*) is one of the most common diseases of wheat, causing yield loss and decreasing grain quality. The frequency of leaf rust resistant allele *Lr10* was 38% which is seedling resistance gene and for *Lr 34* it was 57 % which is adult plant resistance gene.

3.4. Allelic diversity of high-molecular weight glutenin subunits in elite Indian wheat genotypes: In total, thirty one genotypes were selected for the quality marker based analysis. Sedimentation value of these selected genotypes was observed from 38 ml to 60 ml (Table 4). Grain hardness

Table 4. Allele frequency of three HMW glutenin subunits and other quality parameters in selected Indian wheat genotypes

AVTs	Sedimentati-on value (ml)	Grain hardness index	GLU-D1	GLU-A1	GLU-B1	Total quality score	Zones
HS526	56	72	5+10	2**	7+10	10	NHZ
HPW349	53	76	5+10	2**	17+18	10	NHZ
HS507	41	67	5+10	1	7+8	10	NHZ
WH1105	53	68	5+10	2**	7	8	NWPZ
DPW621-50	52	75	5+10	2**	17+18	10	NWPZ*
HD3059	57	69	5+10	2**	17+18	10	NWPZ
HD3065	48	70	5+10	1	17+18	10	NWPZ
PBW658	58	75	5+10	1	17+18	10	NWPZ
WH1100	55	76	5+10	1	17+18	10	NWPZ
DBW71	42	71	5+10	2**	17+18	10	NWPZ
DBW90	52	76	5+10	1	17+18	10	NWPZ
WH1098	51	68	5+10	2**	7+8	10	NWPZ
PBW660	44	76	5+10	1	7+9	9	NWPZ
PBW644	47	81	2+12	2**	7+8	8	NWPZ
HI1579	60	79	5+10	2**	7	8	CZ
WH1097	55	80	5+10	2**	7+8	10	NWPZ
DBW74	48	69	5+10	2**	7+8	10	NWPZ
NIAW1594	41	79	5+10	1	7	8	PZ
HD3043	45	76	5+10	2**	7	8	NWPZ
RAJ4229	38	69	2+12	2**	17+18	8	NWPZ
K0906	51	62	5+10	1	7+9	9	NEPZ
DBW39	43	68	5+10	2**	7+9	9	NEPZ
K0911	41	76	2+12	2**	7+8	8	NEPZ
HI1563	41	80	2+12	2**	7+8	8	CZ*
HD3070	50	86	5+10	2**	7	8	NWPZ
HD2888	41	80	2+12	N	20	4	NWPZ*
RAJ4238	45	73	2+12	2**	17+18	8	NWPZ
MP3336	39	77	2+12	2**	7+8	8	CZ
HD2932	45	72	2+12	2**	17+18	8	NWPZ*
HW5216	48	75	5+10	2**	7	8	SHZ
COW1	47	88	5+10	1	7+9	9	SHZ*

*Note: *check for respective zone*

Table 5. Frequency of HMW-GS subunits in hexaploid wheat

locus	Allele	Subunits type	Number	Frequency (%)	H(Nei's index)
GLU-A1	a	1	9	29	
	b	2*	21	67.7	0.5
	c	Null	1	3.2	
GLU-B1	a	7	6	19.3	
	b	7+8	8	25.8	
	c	7+9	5	16.1	0.7
	e	20	1	3.2	
	i	17+18	11	35.5	
GLU-D1	a	2+12	8	25.8	0.4
	d	5+10	23	74.2	

in selected set of lines ranged from 62 to 80 index (Table 4). At the *Glu-A1* locus, the *Glu-A1b* (subunit 1Ax2*) had the highest frequency of 67.7%, followed by the *Glu-A1a* allele (subunit 1Ax1) with a frequency of 29.0% (Table 5). These two alleles (subunit 1Ax2* and 1Ax1) together accounted for 96.7% of the variation in the genotypes which impart better quality to wheat flour, while *Glu-A1c* allele (subunit null) showed the lowest frequency (3.2%) found in only one accession. Greater allelic variations was observed at the *Glu-B1* locus, with the *Glu-B1i* allele (subunits 1Bx17+1Bx18) being dominant with a frequency of 35.5%. The frequency of other five alleles varied from 3.2% to 25.8%. The *Glu-B1e* allele (subunit 1Bx20) had the lowest frequency of 3.2%, being present in only one genotype (HD 2888). The genetic diversity calculated by Nie's index at this locus was the highest (0.7). At the *Glu-D1* locus, the *Glu-D1d* allele (subunits 1Dx5+1Dy10) was present in 74.2% of the lines followed by *Glu-D1a* allele (subunit 1Dx2+1Dy12) with a frequency of 25.8%. The subunit 1Dx5+1Dy10 was predominantly observed in these advanced lines. On the basis of quality score presented in the table 4, it was observed that twelve genotypes besides check of different zones exhibited the highest quality score of ten and these could be used for breeding high quality wheat varieties.

4. Discussion

Wheat in India occupies 28-29 million ha and nearly 150 cultivars are in the seed-chain of breeder seed production. Changing climate is supposed to affect both yield and quality of wheat directly and also indirectly through changing dynamics of biotic stresses. Besides, reliable elite genotypes and dependable grain parameters, the varietal registration under Central Varietal Release Committee (CVRC) requires information on diversity, distinctness and uniformity status of the genotypes, before it is notified as cultivar. Therefore, characterization of genetic variation among breeding lines is crucial for effective conservation and exploitation of genetic resources for crop improvement programs. Therefore, an analysis of genetic diversity among registered, advance lines/genetic stocks can be useful tool to get information about the genetic variability of the varieties/stocks and possibly change the direction of breeding programs and strategies. In present study, total 86 alleles were detected in 42 genotypes. The PIC values ranged from of 0.09 (*Xwmc227*) to of 0.9929 (*Xgwm165*), with an average of 0.56. Our results are in congruence with the earlier results of Akfirat*et al.* (2013), but lower than reported by Kelestanie*et al.* (2013) and higher than those of Islam *et al.* (2012). Twenty three SSR primers recorded PIC values more than 0.5 suggesting the discriminating nature of these markers. Markers with PIC more than 0.5 are efficient in discriminating genotypes and extremely useful in detecting the polymorphism rate at a particular locus (DeWoodyet al., 1995). In this study, the highest mean number of alleles was detected in the B genome (2.6) followed by A genome (2.25) and D genome (2.1). While Chao *et al.* (2007) reported a ranking of B>D>A among US wheat genotypes. The B genome chromosomes appear to have high genetic richness compared to A and D genomes, in various classes of repetitive DNA, particularly microsatellites (Cuadrado and Schwarzacher, 1998). Among the seven homoeologus groups, the allelic diversity of group 3 and 6 was lowest while group 4 had the highest. Other studies reported highest PIC values for homologous chromosome group 2 (Huang *et al.*, 2002a, b; Roder*et al.*, 2002).

Based on molecular markers, cluster analysis clearly differentiated the genotypes into three major groups-bread wheat, durum wheat and *dicoccum*. The clustering pattern of genotypes obtained in the present study is in agreement with their pedigree information. Unlike cluster analysis, PCA classified the 42 genotypes into two groups and contributed 47.55% of the total variation.

Advanced lines were also characterized for quality related genetic traits using gene-specific markers for HMW-GS, *polyphenol oxidase* (PPO) and waxy genes. The analysis

of storage protein variation in wheat has proved to be a useful tool not only for diversity studies but also to optimize the variation in germplasm collections and, to breed cultivars with improved bread making quality. The importance of wheat seed storage proteins in determining dough properties and bread-making quality has long been recognized because they are the major components of the gluten fraction which confers visco-elasticiy to dough (Shewry*et al.*, 2003). Sedimentation value which measure gluten strength ranged from 38ml to 60 ml. For making good quality bread, chapatti and biscuit, the required sedimentation values lie in the group >60ml, 30-60 ml and <30 ml respectively. Sedimentation value is generally low in the Indian materials and only six lines could be grouped in 55-60 ml range. Grain hardness is an important determinant of the end-product quality in wheat. Grain hardness in selected set of lines ranged from 62 to 80 index which is good for making bread and chapatti. The best studied gluten proteins are the high molecular weight (HMW) subunits of glutenin which are encoded by the *Glu-A1, Glu-B1* and *Glu-D1* loci located on the long arms of the homoeologous group 1 chromosomes of wheat (Payne, 1987). In addition, HMW glutenin subunits useful markers to evaluate the diversity of wheat genetic resources (Caballero *et al.*, 2004). In this study, the subunit 1Ax2* and 1Ax1 together accounted for 96.7% of the variation in the genotypes. Generally "null" allele at *Glu-A1* locus is predominantly observed in the hexaploid wheat cultivars (Payne and Lawrence, 1983) and the germplasm accessions (Cross and Guo, 1993). Here, it is interesting to note that except one genotype, none of the selected Indian wheat genotypes possessed the "null" allele at the *Glu-A1* locus. It assumes that the wheat breeders of India, in way of developing superior quality cultivars, have replaced the "null" allele with 2* which imparts better quality to wheat flour. `

Greater allelic variations were observed at the *Glu-B1* locus, with the *Glu-B1i* allele (subunits 1Bx17+1Bx18) being dominant (35.5%) in this study. The genetic diversity calculated by Nie's index at this locus was the highest (0.7). Earlier, Li *et al.* (2009) and Moragues*et al.* (2006) observed higher diversity (H) at the *Glu-B1* locus in wheat. In bread wheat, the *Glu-D1* locus is usually characterized by subunits 1Dx2+1Dy12 and 1Dx5+1Dy10 with the latter having a stronger effect on bread-making quality. At the *Glu-D1* locus, the *Glu-D1d* allele (subunits 1Dx5+1Dy10) was found predominantly distributed in the North West Plains genotypes of India. The inferior subunit 1Dx2+1Dy12 showed a comparative low frequency in the advanced lines. The allelic richness at *Glu-D1* is higher than *Glu-B1* but the genetic diversity is low due to the fact that the two subunit pairs 2+12 and 5+10 have greater proportion in these advanced lines. Combination of the ten alleles resulted in different combinations of HMW glutenin subunits. Since there is a significant association between certain HMW glutenin subunits and bread making quality, variation in these loci were essential for the plant breeders to develop cultivars with improved bread making quality (Payne *et al.*, 1981).

In summary,this study not only provides useful information about the genetic divergence and distinctness of elite breeding wheat material but also highlights the importance of quality traits. Besides breeders, the information generated will also be quite valuable to the researchers engaged in wheat genomics studies thereby involving diverse material in their programmes to understand molecular mechanisms in a better way.

Acknowledgement

The authors thankfully acknowledge the financial support from Indian Council of Agricultural Research (ICAR), Government of India, New Delhi Grant-in-Aid No. DWR/RP/10-1. Authors greatly appreciates assistance from Dr. Apoorva Arora and Mr. Manoj Saini.

References

1. Akfirat FS and AA Uncuoglu. 2013. Genetic Diversity of Winter Wheat (*Triticumaestivum* L.) revealed by SSR Markers. *Biochemical Genetics* **51:** 223-229.
2. Caballero L, LM Martin and JB Alvarez. 2004. Variation and genetic diversity for gliadins in Spanish spelt wheat accessions. *Genetic Resources and Crop Evolution* **51**: 679-686.
3. Chao S, W Zhang, J Dubcovskyand M Sorrel. 2007. Evaluation of genetic diversity and genome-wide linkage disequilibrium among US wheat (*Triticumaestivum* L.) germplasm representing different market classes. *Crop Science* **47**:1018-1030.
4. Cross RJ and B Guo. 1993. Glutenin variation in adverse pre-1935 world wheat germplasm collection. In: Damania AB (ed) Biodiversity and wheat improvement. ICARDA, Syria.
5. Cuadrado A and T Schwarzacher. 1998. The chromosomal organization of simple sequence repeats in wheat and rye genomes. *Chromosoma* **107**: 587-594.
6. DegaonkarAM, SA Tamhankar and S Rao. 2005. An assessment of cultivated emmer germplasm for gluten proteins: polymorphism of gluten proteins in cultivated emmer germplasm. *Euphytica* **145**: 49-55.
7. Dewoody JA, RL Honeycutt and LC Skow. 1995. Microsatellite markers in white tailed deer. *Journal of Heredity* **86**: 317-319.
8. Gascuel O. 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution* **14**: 685-695.

9. Huang S, A Sirikhachornkit, JDFaris,X Su, BS Gill,R Haselkorn and P Gornicki. 2002b. Phylogenetic analysis of the acetyl-CoA carboxylase and 3-phosphoglycerate kinase loci in wheat and other grasses.*Plant Molecular Biology* **48**: 805-820.

10. Huang S, A Sirikhachornkit, X Su, J Faris, BS Gill, R Haselkorn and P Gornicki. 2002a. Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Triticum/*Aegilops complex and the evolutionary history of polyploid wheat. *Proceedings of the National Academy of Sciences* **99**: 8133-8138.

11. Islam S, MS Haque, RM Emon, MM Islam and SN Begum. 2012. Molecular characterization of wheat (*Triticum aestivum* L.) genotypes through SSR markers. *Bangladesh Journal Agricultural Research* **37**: 389-398.

12. Kelestanie ARA, AAsadi, SRMirfakhraei and AR Abasi. 2013. Genetic Diversity in Twenty Bread Wheat Cultivars Using Microsatellite Markers. *International Journalof Agronomy Plant Production* **4**: 1920-1927.

13. Li Y, H Chengyan, S Xinxia, F Qingqi, L Genying and C Xiusheng. 2009. Genetic variation of wheat glutenin subunits between landraces and varieties and their contributions to wheat quality improvement in China.*Euphytica* **169**: 159-168.

14. Liu K and SV Muse. 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* **21**: 2128-2129.

15. Mcintosh RA, GE Hart, KM Devos, MD Gale and WJ Rogers. 1998. Catalogue of gene symbols for wheat. In: Proceedings 9th International Wheat Genetics Symposium Saskatoon, pp., 236.

16. Mir RR, J Kumar, HS Balyanand PK Gupta. 2012. A study of genetic diversity among Indian bread wheat (*Triticum aestivum* L.) cultivars released during last 100 years. *Genetic Resources and Crop Evolution* **59**: 717-726.

17. Moragues MJ, M Zarco-hernández, A Moralejo and C Royo. 2006. Genetic diversity of glutenin protein subunits composition in durum wheat landraces (*Triticumturgidum* L.Var*durum*) from the Mediterranean basin. *Genetic Resources and Crop Evolution* **53**: 993-1002.

18. Payne PI, LM Holt and CN Law. 1981. Structural and genetical studies on the high molecular subunits of wheat glutenin.Part 1.Allelic variation in subunits among its varieties of wheat (*T. aestivum* L.). *Theoretical and Applied Genetics* **60**: 229-236.

19. Payne PI. 1987. Genetics of wheat storage proteins and the effects of allelic variation on bread-making quality. *Annual Review of Plant Physiology* **38**: 141-153.

20. Payne PI and GJ Lawrence.1983. Catalogue of alleles for the complex gene loci., Glu-A1., Glu-B1., Glu-D1., which code for high molecular weight subunits of glutenin in hexaploid wheat. *Cereal Research Communication* **11**: 29-35.

21. Perrier X, AFlori and F Bonnot. 2003. Data analysis methods. In: Hamon P., Seguin M., Perrier X., Glaszmann J.C., editors. Genetic diversity of cultivated tropical plants Montpellier (France): Enfield., Science Publishers, pp., 43-76.

22. Pfluger LA, LM Martin and JB Alvarez. 2001. Variation in the HMW and LMW glutenin subunits from Spanish accessions of emmer wheat (*Triticum turgidum* ssp *dicoccum* Schrank). *Theoretical and Applied Genetics* **102**: 767-772.

23. Roder MS, K Wendehake, V Korzun, G Bredemeijer, D Laborie, L Bertrand, P Isaac, S Rendell, J Jackson, RJ Cooke, B Vosmann and MW Ganal. 2002. Construction and analysis of a microsatellite-based database of European wheat cultivars. *Theoretical and Applied Genetics* **106**: 67-73.

24. Saghai-maroof MA, KM Soliman, RA Jorgensen and RW Allerd. 1984. Ribosomal spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of the National Academy of Sciences* **81**: 8014-8019.

25. Shewry Y, PR Halford, NG Tatham, AS Popineau, Y Lafiandra and D Belton. 2003. The highmolecular weight subunits of wheat glutenin and their role in determining wheat processing properties.*Advances in Food and Nutrition Research* **45**: 219-301.

26. Third Advance estimates of crop production (www.agricoo.nic.in). Department of agriculture and cooperation, Ministry of Agriculture, Govt. of India.