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Genotypic characterization of elite Indian wheat genotypes using molecular markers and their pedigree analysis

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Abstract

Forty eight elite wheat genotypes including popular cultivars of India were analysed using 56 SSR and 12 STS markers. These markers were robust markers chosen out of 400 SSRs and 100 STS markers, showing good polymorphism and representing each chromosome of wheat genome. Estimated values of coefficient of parentage (COP) for the pair-wise combinations of 48 genotypes ranged from 0.0 to 0.58 with an average of 0.047. The average number of alleles per locus was 2.42 and average polymorphism information content of 0.469. Cluster analysis clearly demonstrated the bread wheat AVTs and popular genotypes in one group while durums, dicoccums and triticale in a separate group. Our results provide information for the discriminative SSR/STS markers for varietal characterization and genetic variation available in at molecular level in Indian wheat and enhance genetic variability in bread wheat breeding program.

Keywords: Genetic variation, SSR markers, Coefficient of Parentage, STS.
Abbreviations: AICWBIP- All India coordinated wheat & barley improvement programme; COP- Coefficient of parentage; IWIS- International wheat information system; PCA- Principal correlation analysis; PIC- Polymorphism information content; SSR- Simple sequence repeats; UPGMA- Unweighted pair group method with arithmetic mean.

Introduction

Common wheat (*Triticum aestivum* L.) is a self-pollinating staple crop that has been bred for higher yield, specific end-use like bread, chapatti, biscuits, pasta etc. quality traits and adaptive characteristics. This resulted in the development of distinct cultivars tailored to specific production environments and for specialized end uses. Over the last 50 years, Indian agriculture has witnessed spectacular advances in both production and productivity after the introduction of dwarf wheats during the mid-sixties. This could be effectively achieved with the introduction of promising genetic resources to widen the genetic base required in wheat improvement for diversified national requirements, trials of straight introductions and acclimatization of exotic genetic stocks (Nagarajan, 2004). India has produced over 85.43 million tonnes of wheat in 2010-11 (source: Directorate of Economics and Statistics, Ministry of Agriculture, India). In the aegis of Indian Council of Agricultural Research (ICAR), Directorate provides required scientific support in evaluating the new materials for their superiority for yield and other economic traits in six wheat agro-climatic conditions of India. Since 1965, more than 350 varieties have been evolved and released through All India Coordinated Wheat Improvement Programme (AICWIP) for wheat cultivation (Kundu et al., 2006). Progress in wheat breeding also requires a broad genetic base with an availability of promising and diverse germplasm collection (Gautam et al., 1997). Therefore, an analysis of genetic diversity among registered/advance lines/stocks can be a useful tool to get information about the genetic variability of the varieties/stocks and possibly change the direction of breeding programs and strategies (Kleshtkina et al., 2004). Consequently, such knowledge can also contribute to a purposeful and focused utilization of germplasm. Recent advances in molecular biology have created new opportunities for evaluating and characterizing wheat genotypes beyond the traditional phenotypic limits. Molecular markers are particularly suited for wheat germplasm characterization since their detection is independent from the field conditions under which plants are grown and phenotypic traits are analyzed for traditional genotypic characterization (Grewal et al., 2007; Salem et al., 2008 and Sud et al., 2005). These factors make the advanced test entries and popular cultivars extremely interesting for genetic variability studies using molecular markers. Microsatellite repeats based molecular markers appear most promising as they are available in large numbers, detect high levels of polymorphism and are amenable to detection and recording (Landjeva et al., 2006). Therefore, DNA profiling based on microsatellites is more popular and has been extensively reported for construction of molecular database of wheat genotypes. In the present work, we analyzed a collection of 48 genotypes belonging to advanced varietal trials and popular wheat varieties that are distinguished for six agro-climatic regions of India. This gene pool was analysed by 68 (SSRs and STS) DNA markers randomly chosen for wheat genome. Coefficient of Parentage (COP) calculates a matrix of measures of pedigree-based co-ancestry. It is used by the plant breeders to determine the genetic diversity across various varieties so as to incorporate the useful characters of the two varieties to develop a new crop variety with particular useful characters. It depends upon

Table 1. Indian wheat genotypes and their pedigree and classification.

Sr. No	Genotype	Pedigree	Wheat
		Northern Hill Zone	
1.	HS 514	INQILAB91*2/TUKURU	BREAD WHEAT
2.	TL 2969 (C)	JNIT141/TL1210//JNIT141	TRITICALE
3.	HS 240 (C)	AV/KAL/BB/WOP"S"/PVN"S"	BREAD WHEAT
4.	HS 295 (C)	COT/AZ//IAS55/ALD"S"/3/ALD"S"/NAFN"S"/4/PIN"S"/PEZSE127	BREAD WHEAT
5.	VL 804 (C)	CPAN3018/CPAN3004//PBW65	BREAD WHEAT
6.	VL 892 (C)	WH542/PBW246	BREAD WHEAT
		North Western Plain Zone	
7.	HD 3043	PJN/BOW//OPATA*2/3/CROC_1/AE.SQUARROSA(224)//OPATA	BREAD WHEAT
8.	HI 8703	HD4502/HI8498//HI8498	DURUM
9.	PBW 639	HW2019/PBW49	BREAD WHEAT
10.	PBW 644	PBW175/HD2643	BREAD WHEAT
11.	PDW 322	PDW278/HD4502//PBW34/PDW276	DURUM
12.	WHD 946	SOMAT3/YEBAS8//RASCON3//2*TARO2	DURUM
13.	C 306 (C)	REGENT1974/3*CHZ//*2C591/3/P19/C281	BREAD WHEAT
14.	DBW 17 (C)	CMH79A.95/3*CNO79//RAJ3777	BREAD WHEAT
15.	HD 2967 (C)	ALD/CUC//URES/HD216OM/HD2278	BREAD WHEAT
16.	PBW 175 (C)	HD2160/WG1025	BREAD WHEAT
17.	PBW 343 (C)	ND/VG1944//KAL//BB/3/YACO'S'/4/VEE#5'S'	BREAD WHEAT
18.	PBW 373 (C)	ND/VG1944//KAL/BB/3/YACO'S'/4/VEE#5'S'	BREAD WHEAT
19.	PBW 550 (C)	WH594/RAJ3856//W485	BREAD WHEAT
20.	PDW 233 (C)	YAV'S'/TEN'S'	DURUM
21.	WH 1080 (C)	21STSAWSN151	BREAD WHEAT
22.	WHD 943 (C)	GLARE/PLATA-16//AJAIA-3/SILVER16	DURUM
		North Eastern Plain Zone	
23.	DBW 14 (C)	RAJ3765/PBW343	BREAD WHEAT
24.	HD 2733 (C)	ATTILA/3/TUI/CARC//CHEN/CHTO/4/ATTILA	BREAD WHEAT
25.	HUW 234 (C)	HUW12*2/CPAN1666	BREAD WHEAT
26.	K 8027 (C)	HD1696/2*K852	BREAD WHEAT
27.	NW 2036 (C)	BOW/CROW//BUC/PVN	BREAD WHEAT
		Central Zone	
28.	HI 1572	SKAUZ*2/FCT	BREAD WHEAT
29.	HI 8704	HG822/HI8498	DURUM
30.	MP 3304	GW322/J485	BREAD WHEAT
31.	HI 1500 (C)	HW2002*2//STREMPALLI/PNC5	BREAD WHEAT
32.	HI 1544 (C)	HINDI62/BOBWHITE/CPAN2099	BREAD WHEAT
33.	LOK 1 (C)	S308/S331	BREAD WHEAT
34.	MPO 1215 (C)	GW1113/GW1114//HI8381	DURUM
		Peninsular Zone	
35.	AKAW 4210-6	DF-99-186SELECTION FROM 3RD SSN99-2000	BREAD WHEAT
36.	HD 3040	WR1027/HD2851	BREAD WHEAT
37.	HI 1571	RAJ3777/WLT277//HW2045	BREAD WHEAT
38.	LOK 62	SHARBATISONARA/C306/LOK1//HS295	BREAD WHEAT
39.	MP 3299	DOVE/BUC/DL788-2	BREAD WHEAT
40.	UAS 320	UAS257//GW322/DWR195	DURUM
41.	UAS 428	GREEN-14/YAV-10/AUK/UAS402	DURUM
42.	MACS 1967 (C)	GULAB/CPAN6078	DURUM
43.	NI 5439 (C)	NI8883/MP1055	BREAD WHEAT
44.	NIAW 34 (C)	CNO79/PRL"S"	BREAD WHEAT
		Southern Hills Zone	
45.	HW 2044 (C)	PBW226*5//SUNSTAR*6/C80-1	BREAD WHEAT
		Special Trial	
46.	KHARCHIA 65 (C)	SELECTION FROM KHARCHIA LOCAL	BREAD WHEAT
47.	KRL 210 (C)	PBW65/2*PASTOR	BREAD WHEAT
48.	MACS 2971 (C)	KRT5*2/NP200	DICOCCUM

*C: Prominent cultivar used as check

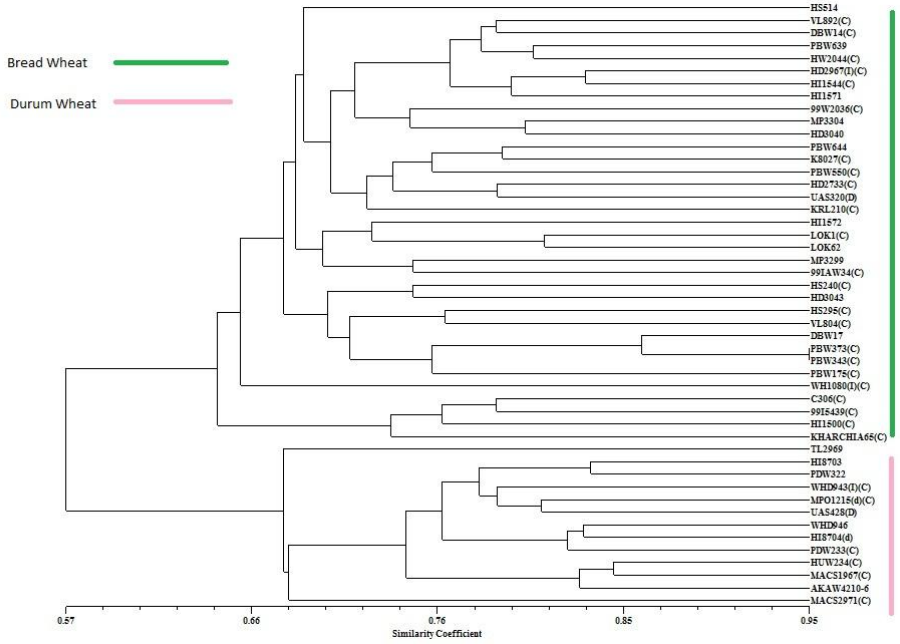

Fig 1. UPGMA based genetic variability analysis of Indian wheat genotypes using STS/SSR markers

the pedigree information of the varieties based on particular levels.

Results and Discussion

SSR markers based DNA fingerprinting analysis: The 48 wheat genotypes were evaluated using a set of 56 SSR and 12 STS markers selected on the basis of their known genetic locations to give a uniform coverage of the chromosomes in the three wheat genomes (A, B and D). Out of the 56 SSRs surveyed, 44 markers gave polymorphic profiles while 12 were found monomorphic (Table 2). Total 136 alleles were detected and the number of alleles per locus ranged from 1 to 5 with an average of 2.42 alleles per locus. The PIC values ranged from a minimum of 0.2491 (WMC194) to a maximum of 0.9520 (GWM389), with an average of 0.4596 (Table 2). The high frequency allele percentage ranged from 41.67 % (GWM484) to 97.92 % (WMC160, WMC233 & WMC455) with an average of 72.02 % for 44 polymorphic SSR markers. More alleles were observed in B genome as compared to A and D genome. This confirmed that there was a higher polymorphism in the B genome. Similar observation of higher polymorphism was also reported by Wang et al. (2007). The less value of average PIC and greater value of average high frequency alleles were expected with the use of agarose gel based separation of amplified products in this study. However, PIC values recorded for SSR markers in this study are comparable as PIC values reported from previous studies for respective markers in wheat (Prasad *et al*, 2000; Medini *et al*, 2005; Roder *et al*, 2002). A genotype was assigned a null allele for an SSR locus whenever an amplification product(s) was not detected in repeated PCR reaction for the particular genotype X marker combination. In this study, null alleles were prominently observed for markers located on D genome for durum, dicoccum and triticale genotypes with few exceptions of homeoloci amplifications. Similarly null alleles were observed for Ethiopian tetraploid wheat germplasm for microsatellite markers screening (Alamerew et al., 2004). Multiple alleles (more than one allele per locus) were detected at GWM2, GWM111 and GWM519 loci that were identified when more than one bands had the same intensity in a given accession. These additional loci in wheat are homoeologous or non-homoeologous amplified products that were smaller in size and showed lower level of polymorphism as reported for European and other exotic wheat in previous studies (Prasad et al., 2000).

STS markers based trait analysis

Of the 48 genotypes, screened with different gene specific markers (STS), the frequency of leaf rust resistant allele *Lr10* was 31.25% and for *Lr 34* it was 56.25%. Former is a seedling resistance gene while later one is well characterized adult plant resistance gene. The *1B/1R* translocation, documented to be associated with better yield but with inferior bread making properties was recorded in 29.16% genotypes. Majority of wheat genotype were photoperiod insensitive at *Ppd-D1* locus. The *Wx-B1b* allele i.e. null allele of Wx-B1 protein was present in 22 genotypes. Only four bread wheat genotypes possessed the *Ppo-A1b* allele associated with low polyphenol oxidase activity. Several Indian wheat genotypes possessed the *Wx-B1b* i.e. null allele of Wx-B1 protein associated with improved starch quality for udon noodles (Zhao et al., 1998). Amongst physiological traits, reduced height gene *RhtB1b* was observed in 56.25% while for vernalization genes *VrnA1a* and Vrna1b an allele frequency of about 35.42% and 64.58% was observed respectively. In the present study, SSR/STSs markers clearly revealed the well-documented differentiation among the genotypes except for sister-line varieties PBW343 and PBW373 at all the loci studied except at six SSR loci (GWM225, GWM255, GWM256, GWM261, GWM389 and GWM519).

Genetic similarity analysis in Indian wheat

Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based cluster analysis of genetic similarity values for SSR/STS alleles from all the wheat accessions was used to construct a dendrogram (Fig. 1). The cluster analysis

Table 2. Allelic variation of the polymorphic SSR loci in the Indian wheat genotypes on the basis of allele richness and PIC.

Sr. No	Molecular marker	Chromosomal location	Annealing Temp. (^{0}C)	No. Of Alleles (A)	Allele size range	High frequency allele	% of high frequency allele	PIC
1.	GWM219	6B	60	3	140-220	220	68.75	0.4596
2.	GWM312	2A	60	2	150-230	150	64.58	0.4717
3.	GWM484	2D	55	2	150-200	200	41.67	0.7638
4.	GWM374	2B	60	3	180-220	200	58.33	0.5863
5.	GWM519		60	5	130-550	130	75.0	0.8935
6.	GWM 44	7D	60	4	100-200	200	93.75	0.7159
7.	GWM 190	5D	60	2	200-230	210	54.17	0.6853
8.	GWM 337	1D	55	2	180-220	220	35.42	0.7769
9.	WMC 617	4A, 4B, 4D	60	4	180-310	250	66.67	0.8331
10.	GWM 265	2A	55	2	150-250	150	47.92	0.6297
11.	WMC 200	4B	60	2	150-200	200	62.5	0.7636
12.	WMC 160	5B	50	4	169-300	169	97.92	0.5047
13.	GWM 273	1B	55	4	180-200	190	66.67	0.6681
14.	WMC 255	-	60	2	250-300	250	60.42	0.6182
15.	WMC 232	4A, 7B	60	2	120-160	120	60.0	0.4861
16.	WMC 225	-	60	4	160-180	180	63.5	0.5466
17.	WMC 227	-	60	4	140-160	160	56.5	0.7992
18.	WMC 233	5D	60	2	200-250	250	97.92	0.4687
19.	WMC 245	2B, 2D	60	2	120-150	150	95.83	0.4989
20.	WMC 242	-	60	2	150-220	150	91.67	0.5466
21.	WMC 153	1D,3A	60	2	150-200	200	83.33	0.6161
22.	WMC 261	2A	60	2	100-150	150	58.33	0.5909
23.	GWM 2	3D	60	5	110-270	110	75.0	0.5399
24.	GWM 149	4B	60	2	150-180	180	54.17	0.5171
25.	GWM 160	5B	60	2	180-230	180	72.92	0.3949
26.	GWM 428	7D	60	4	120-180	160	56.25	0.5260
27.	GWM 165	4B	60	2	180-280	180	72.92	0.3949
28.	GWM 437	7D	50	3	100-160	120	66.67	0.6299
29.	GWM 256	-	60	2	100-160	130	77.08	0.3702
30.	GWM 458	1D	60	2	100-150	110	72.92	0.3949
31.	GWM 325	6D	60	2	100-280	250	54.17	0.4965
32.	GWM 194	4D	50	2	120-160	160	85.42	0.2491
33.	GWM 111	7D	55	5	120-300	150	72.92	0.6884
34.	GWM 493	3B	60	3	150-250	200	62.5	0.6085
35.	GWM 408	5B	55	3	160-210	190	74.78	0.4177
36.	GWM 46	7B	60	2	150-200	200	66.67	0.4445
37.	GWM 186	5A	60	2	150-190	190	58.33	0.4861
38.	WMC 54	3B	60	2	150-200	150	50.0	0.5399
39.	WMC274	3B	60	3	150-200	200	60.42	0.5646
40.	WMC 340	-	60	3	140-200	180	75.0	0.4589
41.	WMC455	2A	55	4	140-400	150	97.92	0.8209
42.	GWM 5	3A	50	3	100-200	172	87.5	0.5570
43.	WMC 265	2B	60	3	180-253	180	77.08	0.9243
44.	GWM 389	3B	60	4	100-180	140	93.75	0.9520

Note: Null alleles observed for Durum, Dicoccum and Triticale genotypes for most of D-genome based SSR markers.

revealed two major clusters (Group I and II) with a similarity coefficient varying between 0.15 and 0.90 indicating significant genetic variation among the wheat accessions studied. First major cluster grouped 72% of total genotypes and mainly grouped bread wheat advanced breeding lines, prominent cultivars and genotypes bred for abiotic stress tolerance. The few popular varieties included in cluster I were PBW343, DBW17, C306, Lok1 and PBW550. Only single durum line (UAS320) was observed to be grouped with bread wheat genotypes in cluster I. Similar grouping of prominent cultivars of Indian was reported in previous molecular markers based genetic diversity studies in wheat. The second cluster predominantly grouped durum genotypes along with dicoccum and triticale genotypes with exception of three bread wheat genotypes (HS240, HUW234 and AKAW4210-6). These bread lines grouped with dicoccum wheat lines MACS2971 and MACS1967 in the distant group of UPGMA tree. Triticale genotype, TL2969 was placed uniquely in cluster II. Therefore, genetic distance-based results in UPGMA tree efficiently differentiated three major germplasm groups of bread wheat, durum and dicoccum. In earlier studies, grouping of wheat varieties were reported using molecular markers within a specific group for evaluating genetic diversity or similarity (Ben-Amer et al., 2001, Grewal et al., 2007). The UPGMA clustering was further validated by principle correlation analysis (PCA) that gave clear distinctions between the ploidy levels of wheat and all genotypes clustered into two distinct groups. First group was composed of bread wheat varieties and advanced lines bred for high yield performance and abiotic stress tolerance for six different agro-climatic wheat growing zones. The

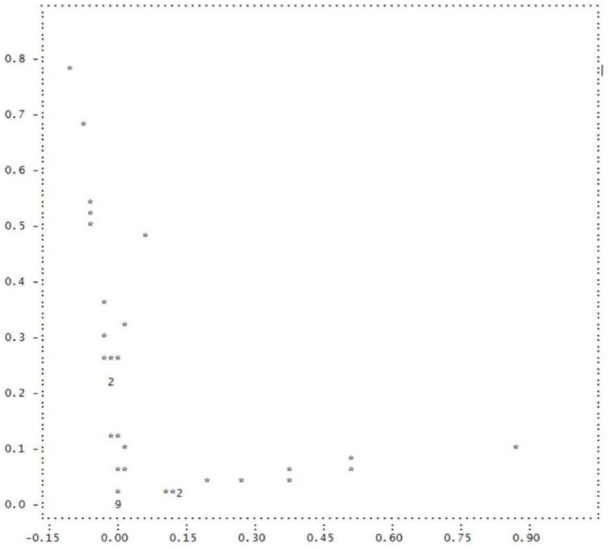

Fig 2. Plot of Axis 1 vs Axis 2 for spectral composition of COP matrix contains 14. % of trace, Axis values scaled by ROOT EIGENVALUE

second important grouping of PCA mainly consisted of varieties and advanced breeding lines of durum wheat and a dicoccum check cultivar, MACS29H. As expected, TL2969, a triticale line was uniquely placed in PCA.

Coefficient of Parentage

A coefficient of parentage analysis of all the 48 genotypes, selected for marker based variability analysis, was performed. The Figure 2 shows a spectral composition of COP matrix. Values estimated for COP for 1176 pair-wise combinations of 48 genotypes ranged from 0.0 to 0.58 with an average of 0.047. About 56 percent of COP values (656) showed no genetic similarity among the pair of cultivars. Approximately 3% combinations showed COP values ranging from 0.01 to 0.1 indicating thereby limited similarity by descent in any pair of cultivars. Only 25 out of 1176 combinations (less than 2%) showed COP values of 0.2 to 0.58. These were LOK62-LOK1, HD2733-DBW14, NIAW34-HS240, UAS428-MPO 1215, HI 1572-HD2733, PBW343-HS240, PBW373-HS 240, HI1572-HS240, WHD943-UAS428, MPO1215-PDW233, HI1572-VL892, PBW175-PBW644, WHD943-PDW233, HI 1572-PBW343, HI1572-PBW373, LOK1-K8027, DBW14-PBW373, UAS428-PDW233, HD2733-PBW343, HD2733-PBW373, HI1577-HD3043, HI1544-PDW233, Lok62-C306, DBW14-PBW343 and PBW343-PBW373. The similarity values based on pedigree and SSRs are not of the similar magnitude. The one through COP analysis may be found low since similarities obtained from parentage analysis could be due to distinct names often given to parents which actually trace back to common progenitors and incomplete pedigree records (Sud *et al*. 2005). A weak correlation among molecular and pedigree data have been reported in earlier studies also in wheat and other crops (Barbosa-Neto et al., 1996; Barrett et al., 1998). UPGMA clustering, number of alleles available and range of PIC values in different sets of wheat genotypes was further evaluated to define genetic variability being explored in present wheat improvement program under AICW&BIP. Of the 48 selected wheat lines, thirty one genotypes, prominent cultivars were taken as check varieties and seventeen were the advanced entries tested at multilocational trial for their yield and disease responses. Almost similar genetic variability was observed in check and advanced varietal entries for number of alleles, PIC values and genetic similarity in UPGMA clustering. Genepool of Northern and Central-southern India gave comparable values in terms of genetic variations. Similar values were also observed for bread and durum wheat group except for the higher number of alleles observed in advanced varietal entries as compare to durum check lines as given in table 4. This reflected use of more diverse parental lines in durum wheat breeding program whereas the parental combination explored in bread wheat breeding program are contributing almost same genetic variation. Therefore, it is suggested to ensure more use of diverse parents in bread wheat program to afford futuristic challenges of high yield in Indian bread wheat varieties as suggested by Sud et al., (2005). This finding clearly demonstrated the reliability, usefulness, and efficiency of SSRs/STS in analyzing genomic diversity. The genetic similarity values varied in the similar range as that of PIC values calculated by SSRs markers thus suggesting high variability and wide range of genetic base in Indian wheat. This cluster analysis concluded that it should be possible to establish a collection of highly polymorphic SSRs for genetic diversity studies, cultivar identification, and plant variety protection in Indian wheat improvement programs. This study further established the possible use of cluster analysis to assess genetic diversity of elite genotypes and to select genetically diverse genotypes for breeding purposes.

Materials and methods

Plant material

In total, 48 bread and durum wheat genotypes from advanced varietal trials 2010-11 of all Indian coordinated wheat improvement programme representing six different agro-climatic wheat sowing zones of India (Table 1) were used to study the DNA fingerprinting profile. The seeds were procured from Germplasm Resource Unit, Directorate of Wheat Research, Karnal.

DNA extraction and molecular marker genotyping

Equal number of fresh, young leaves (fourteen days old seedlings) of at least five plants from each genotype was bulked for DNA extraction. Total genomic DNA was isolated using the modified CTAB method (Saghai Maroof et al., 1984). The DNA samples were analyzed both qualitatively and quantitatively using 0.8% agarose gel electrophoresis.
Total 56 SSR and 12 STS molecular markers randomly located on twenty one chromosomes of wheat (Supplementary data 1) were used to develop amplification profiles of selected genotypes (Table 2 & 3). PCR reaction was conducted in a reaction volume of 20 µl containing 1X PCR buffer. 200 m M dNTPs, 0.25 µM of primer, 2Mm $MgCl_2$, 1U *Taq* polymerase and 50 ng template DNA. PCR amplification was performed using BIORAD S 1000 thermocycler. PCR products were resolved by electrophoresis on 2 % agarose gels (HiMedia) at 4v/cm in 0.5 X TBE buffer. Fragment sizes were approximately calculated by interpolation from the migration distance of marker fragments of 100-bpDNA ladder (Invitrogen, USA) and corroborated with the reported amplified fragment size of respective molecular marker. The occurrence of 'null' alleles was verified by re-amplification under similar PCR conditions. Gels were stained with ethidium bromide (0.5ug/ml) and DNA banding patterns were visualized under UV light (Syngene Synoptics Ltd. USA).

Table 3. STS marker based allelic variation in Indian wheat genotypes.

Sr No	STS Marker	Chr location	Annealing Temp. (^{0}C)	No. Of Alleles	Allele size range	No of lines scored
1.	*Vp1B3*	3B	62	3	569,652, 845	36
2.	*DuPw004*	4A	60	2	250, 350	26
3.	*Ppd**	2D	60	2	288,414	34
4.	*Vrn-A1a*	5A	50	1	965	14
5.	*Vrn-A1b*	5A	58	1	1068	31
6.	*DREB*	3B	60	2	600, 700	25
7.	*PPO18*	3A	62	2	685, 876	31
8.	*Wx-B1*	4A	62	2	425, null	22
9.	*Lr10*	1A	60	1	280	15
10.	1B/1R	1B / 1R	55	1	1.5Kb	14
11.	*Lr34*	7D	55	1	150, 220	31
12.	*RhtB1b*	4B	58	1	273	27

*Null alleles observed for Durum, Dicoccum and Triticale genotypes for D-genome based STS markers.

Table 4. Evaluation of genetic variability on the basis of allele richness, PIC and GS values in different sets of Indian wheat.

Sr No.	Wheat Type	Wheat Set	Number of Genotypes	Allele Numbers	Presence/absence of STS allele
1.	All Type Wheat	Selected Set	48	157	All present
2.		Varieties used as checks	31	157	All present
3.		Advanced varietal lines	17	155	*Vp1B3b* absent
4.	Bread Wheat	Selected Set	36	154	*Ppdc* Absent
5.		Varieties used as checks	25	153	*Ppdc* , *Wx-B1d* Absent
6.		Advanced varietal lines	11	151	*Ppdb, Vp1B3c, Wx-B1d,* Absent
7.	Durum Wheat	Selected Set	10	135	*Lr10, Ppdb, Vp1B3c, Vrn-A1a, Wx-B1a*
8.		Varieties used as checks	4	101	*1B1R, Lr10, DuPw004, Ppda, Wx-B1b,* Absent
9.		Advanced varietal lines	6	132	*Lr10,Ppdb,Vp1B3b, Vp1B3c, Vrn-A1a Wx-B1a,* Absent
10.	Wheat set for North India	Selected Set	27	154	All present
11.	Wheat set for Central & South India	Selected Set	21	154	*Vp1B3b* Absent

Analysis of molecular data

Molecular weights for microsatellite products, in base pairs, were estimated and the summary statistics including the number of alleles per locus, major allele frequency and polymorphism information content (PIC) values were determined (Anderson et al, 1993). Allele molecular weight data were also used to export the data in binary format (allele presence = "1" and allele absence = "0") for analysis with NTSYS-PC version 2.1. The 0/1 matrix was used to calculate genetic similarity as DICE coefficient using SIMQUAL subprogram and the resultant similarity matrix was employed to construct dendrograms using Sequential Agglomerative Hierarchical Nesting (SAHN) based Unweighted Pair Group Method of Arithmetic Means (UPGMA) as implemented in NTSYS-PC (version 2.1) to infer genetic relationships and phylogeny. For estimating the similarity matrix, null alleles were treated as missing data to reduce the biased genetic or similarity measures.

Coefficient of Parentage

Pedigrees of 48 cultivars were obtained from cultivar descriptions in communications with the germplasm resource unit, DWR, Karnal. The pedigree trees of each of these 48 cultivars (at the expansion level of 5) were generated using the external pedigree input tool of the International Crop Information System (ICIS) Software (Mclaren and White 1999). The COP values were then estimated using the WCOP function of the International Wheat Information System (IWIS) software, version 4.0.

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