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Website : www.jeb.co.in
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Journal of Environmental Biology

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

DNA fingerprinting and genetic diversity studies in wheat genotypes using SSR markers

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Publication Info

Paper received:
30 October 2015

Revised received:
04 January 2016

Accepted:
09 January 2016

Abstract

Fifty four wheat genotypes comprising of 41 Indian origin and 13 exotic genotypes were characterized using 39 polymorphic SSR markers for DNA fingerprinting and extent of genetic diversity. A total of 112 alleles ranging from 1 (*Barc1*, *Barc26* and *Barc147*) to 7 alleles (*Barc77*) were generated with an average of 2.87 per SSR marker. The PIC value of SSR markers ranged from 0.03 to 0.49 with 0.29 mean PIC value indicating lower level of genetic diversity among genotypes. Similarity values ranged from 22.8% (between MP-4161 and K-424) to 78.7 % (between GP-350 and GP-361) with an average of 51.23 %. UPGMA based cluster analysis, broadly grouped 54 genotypes into four clusters were represented as A, B, C and D. Cluster D included maximum number of genotypes (43) which was further divided into seven sub-clusters. Out of thirteen exotic genotypes used in the present study, six were included in sub-cluster D4. However, two exotic genotypes each were sub-clustered with Indian genotypes into three sub-clusters, D-2, D-6 and D-7 revealing their genetic similarity with genotypes of Indian origin. Genotypes from same origin were usually clustered together, e.g. K-65 and K-68 belonged to Kanpur region were clustered into 'Cluster B'. Similarly, MP-4115, MP-4131, MP-4136 and MP-4010 released for Madhya Pradesh were clustered into 'D-3' sub-cluster. Present study also showed the potentiality of SSR markers for study of genetic diversity and DNA fingerprinting in wheat.

Key words

Cultivar identification; DNA Fingerprinting; PIC values; SSR markers; Wheat

Introduction

Wheat is the staple food of about 35% world population and most preferred cereals in the world. Bread wheat (*Triticum aestivum* L.) is a difficult material for genome-wide studies due to its hexaploid nature, comprising of three closely related genomes (A, B and D), which makes it largest genome (16×10^9 bp) in plant kingdom with 80% repetitive DNA (Gupta *et al*., 1991; Bennett and Leitch, 1995). Due to all these constraints, it is also difficult to establish genetic diversity successfully among wheat genotypes. Due to genomic complications, only few studies have been carried out successfully to establish genetic diversity among wheat genotypes using molecular markers. Chances of mixing of seeds of varieties is very high, in many poor countries, seeds of inferior varieties are getting mixed with improved varieties and selling them by putting the tag of improved cultivar is also in practice in the market. Visual observations or existing morphological characterization methods are not efficient enough for precise and rapid estimation of genetic diversity and to distinguish similar cultivars. Since, varieties with similar appearance are increasing very rapidly every year, this is highly required to fingerprint wheat varieties using molecular markers to distinguish similar varieties and establish diversity among them to make them more useful for breeding purpose. The advancement in molecular markers in last two to three decades, more reliable estimation of genetic diversity has

become possible, which was otherwise not possible by indirect diversity measures based on agronomic traits or geographic origin. Molecular markers have been successfully used in genetic mapping, population genetics and marker assisted selection (Rehem *et al.,* 2010). Simple sequence repeat (SSR) is a known molecular marker for plant cultivar identification (Crespel *et al.,* 2009; Lu *et al.,* 2009) and considered as powerful molecular markers among hexaploid wheat due to their high level of polymorphism (Röder *et al.,* 1995; Bryan *et al.,* 1997; Yifru *et al.,* 2006).

Due to high-throughput and high accuracy of SSR markers, they have been successfully used for genotype identification of Sorghum (Li *et al.,* 2005); rice (Rahman *et al.,* 2009); pigeonpea (Khalekar *et al*., 2014); Indian bitter gourd (Behera *et al.,* 2008); rose (Crespel *et al.,* 2009); pear (Kimura *et al.,* 2002); potato (Coombs *et al.,* 2004); rape (Louarn *et al.,* 2007); soybean (Tantasawat *et al.,* 2011) etc. In case of wheat, SSR markers have been used to characterize genetic diversity among wild relatives of wheat (Hammer *et al.,* 2011) and among improved wheat germplasm (Börner *et al.,* 2000; Huang *et al.,* 2002; Fujita *et al.,* 2009; Zhu *et al.,* 2011; Singh *et al*., 2012; Kumar *et al*., 2013a and 2013b). Hence, keeping in view the above facts, the present study was undertaken to discriminate genotypes based on their genetic similarity using SSR markers.

Materials and Methods

Plant materials : A set of fifty four (54) bread wheat genotypes comprised of 41 Indian germplasm lines and released varieties; and 13 exotic genotypes were used for fingerprinting (Table 1). The seed materials of these genotypes were procured from Indian Institute of Wheat and Barley Research (IIWBR), Karnal and National Gene Bank, National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Seeds were sown in small plastic pots in green house where normal agronomical practices were followed for raising healthy plants during the winter season 2010-2011. After one month, leaves were collected and packed immediately in plastic bags and stored in deep freeze at -80 ºC for DNA extraction.

DNA extraction : Genomic DNA was extracted from one month old leaves (~ 100 mg each) using the modified CTAB method (Saghai-Maroof *et al.,* 1984). CTAB extracting buffer was prepared with a final concentration of CTAB 2.0%, NaCl 5M, Tris buffer 100mM, Na_2EDTA 20mM and β-Mercaptoethanol 0.2%. For the preparation of 100ml of extraction buffer, 20ml of 10% CTAB, 20ml of 1M Tris, 28ml of 5M NaCl, 4ml of 0.5M Na_2EDTA was added with 28ml of distilled water. 10µl β-Mercaptoethanol was added in 5ml of extraction buffer (just before use) and adjusted the pH to 8.0 and made up the volume to 100 ml. DNA was diluted to a final concentration of 20 ng μl^{-1} with TE buffer (10 mM l^{-1} Tris-HC1, pH 8.0; 1 mM l^{-1} EDTA, pH 8.0) and stored at (-20^{0}C) for further use. The quality and concentration of extracted DNA was estimated by Nano-drop (ND1000, Thermo Scientific, USA) followed by gel electrophoresis.

SSR marker based genotyping : The work was initiated with 250 *Barc* series SSR markers which were synthesized commercially. These primers were tested initially on eight genotypes for polymorphism and only 39 polymorphic and reproducible primers were selected for fingerprinting of 54 wheat genotypes (Table 2).These thirty nine polymorphic SSR markers distributed over 1B, 1D, 2B, 2D, 3A, 3B, 3D, 4A, 4D, 5A, 5B, 5D, 6B, 7A, 7B and 7D chromosomes. DNA amplification was conducted in a 10 µl volume containing 20 ng of genomic DNA, 0.2 U *Taq* DNA polymerase, 1.5 mM Mg^{2+}, 0.25 mM dNTPs and 0.2 µM primer (Table 2). The PCR cycle consisted of an initial denaturation at 94ºC for 4.5 min, followed by 36 cycles of 94ºC for 55 sec, annealing at

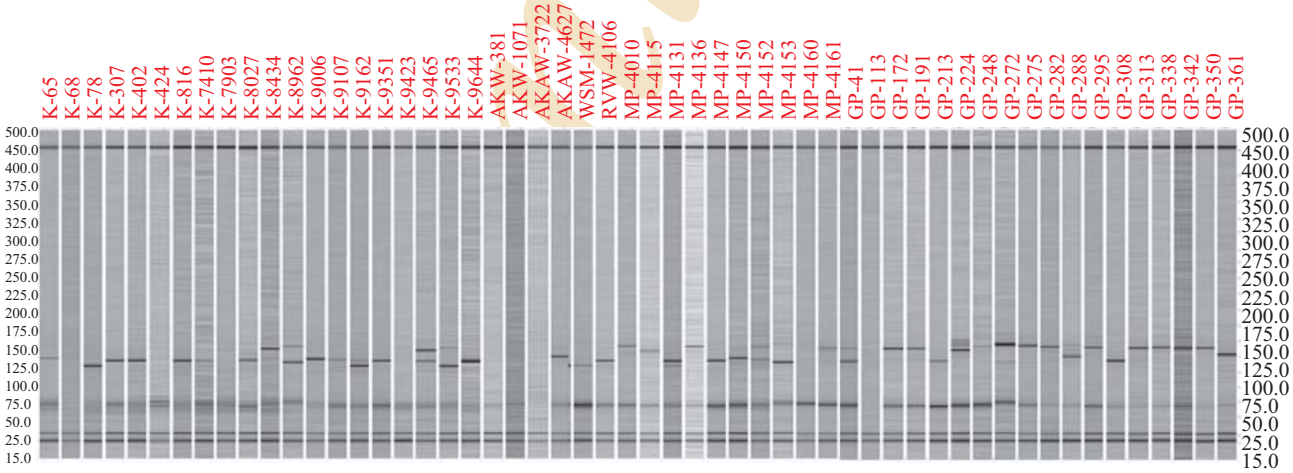

Fig. 1 : DNA profiling of 54 wheat genotypes with SSR marker *Barc 61* on automated capillary based electrophoresis (QIAXCEL, Germany)

Table 1 : Wheat genotypes along with its pedigree used in the present study

Genotype	Pedigree	Indigenous or exotic	Species
K-65	NP773/PB591	Indigenous	*T. aestivum*
K-68	NP773/C13	Indigenous	*T. aestivum*
K-78	HD1982/K816//K65	Indigenous	*T. aestivum*
K-307 (Shatabdi)	K9321/UP2003	Indigenous	*T. aestivum*
K-402 (Mahi)	HP1731/UP2425	Indigenous	*T. aestivum*
K-424 (Golden Halna)	K8020/K9162	Indigenous	*T. aestivum*
K-816	CN051B//SN64/KLRE/3/8156	Indigenous	*T. aestivum*
K-7410 (Purvi)	K816/51B/Kalyan Sona	Indigenous	*T. aestivum*
K-7903 (Halna)	HD1982/K816	Indigenous	*T. aestivum*
K-8027 (Maghar)	HD1969/*2K852	Indigenous	*T. aestivum*
K-8434 (Prasad)	HD2160/K68	Indigenous	*T. aestivum*
K-8962 (Indra)	K7401/HD2160	Indigenous	*T. aestivum*
K-9006 (Ujiyar)	CPAN1687/HD2204	Indigenous	*T. aestivum*
K-9107 (Deva)	K8102/K68	Indigenous	*T. aestivum*
K-9162 (Gangotri)	K7827/HD2204	Indigenous	*T. aestivum*
K-9351 (Mandakini)	K72/K8027/K72	Indigenous	*T. aestivum*
K-9423 (Unnat Halna)	HP1633/Kalyan Sona/UP262	Indigenous	*T. aestivum*
K-9465 (Gomati)	B1153/CB85	Indigenous	*T. aestivum*
K-9533 (Naina)	HI1077/HUW234	Indigenous	*T. aestivum*
K-9644 (Atal)	HD2402/K8305	Indigenous	*T. aestivum*
AKW-381	S-308/NI-5439	Indigenous	*T. aestivum*
AKW-1071 (Purna)	VEE"/FLN-ACC//ANA	Indigenous	*T. aestivum*
AKAW-3722 (Vimal)	WH147/SUNSTAR*/CO-80-1	Indigenous	*T. aestivum*
AKAW-4627	Selection from Vimal	Indigenous	*T. aestivum*
WSM-1472	Selection from HD-2815	Indigenous	*T. aestivum*
RVW-4106	CHIBIA//PRL11/CM 65531	Indigenous	*T. aestivum*
MP-4010	ANGOSTURA-88 (CM50123-3M-Y-2M-1Y-2M-Y-2M-2Y-0M-OMR/S)	Indigenous	*T. aestivum*
MP-4115	SYNTH 2/ VL-818	Indigenous	*T. aestivum*
MP-4131	DF2K4-800	Indigenous	*T. aestivum*
MP-4136	DF2K3-635	Indigenous	*T. aestivum*
MP-4147	WORRAKATTA/PASTOR	Indigenous	*T. aestivum*
MP-4150	MILAN/S87230//BABAX	Indigenous	*T. aestivum*
MP-4152	HD 2768/WH-913	Indigenous	*T. aestivum*
MP-4153	DWR 241/HW1081-5	Indigenous	*T. aestivum*
MP-4160	TEPOKA/DL 547-2-51	Indigenous	*T. aestivum*
MP-4161	TEPOKA/DL 547-2-56	Indigenous	*T. aestivum*
GP-41	IC-309868	Indigenous	*T. aestivum*
GP-113	EC-556506	Exotic	*T. aestivum*
GP-172	(EC-556506) Bodallin//Gamenya/Inia66	Exotic	*T. aestivum*
GP-191	(EC-533532)PI531244/TAM 200	Exotic	*T. aestivum*
GP-213	IC-296729	Indigenous	*T. aestivum*
GP-224	(IC-539580) KYZ-0286	Indigenous	*T. aestivum*
GP-248	CRDH/SIH	Exotic	*T. aestivum*
GP-272	PASTOR/CP68-88.5.6	Exotic	*T. aestivum*
GP-275	CBRD/KAUZ	Exotic	*T. aestivum*
GP-282	WR-1204	Exotic	*T. aestivum*
GP-288	MP-3054	Indigenous	*T. aestivum*
GP-295	PBW 343/CAR422/ANA	Indigenous	*T. aestivum*
GP-308	PJN/BOW/OPATA/3/MILLAN/4/ATTILA	Exotic	*T. aestivum*
GP-313	OTUS/TOBA-97	Exotic	*T. aestivum*
GP-338	(EC-721852) FRET-2	Exotic	*T. aestivum*
GP-342	OPATA/RAYON//KAUZ	Exotic	*T. aestivum*
GP-350	CHOIX/STAR//3/HE1*CNO79/2*C4EN	Exotic	*T. aestivum*
GP-361	ALLILA*2/9/KT/BAJE/FN/4/3/BLA/4/TAAM/5	Exotic	*T. aestivum*

50-60ºC (varied from primer to primer) for 55 sec, 72ºC for 55 sec, and a final extension step of 72ºC for 5 min before cooling at 4°C. All PCR (polymerase chain reaction) amplifications were carried out in G-Storm (Gene Technology Ltd UK) thermocycler. PCR products were analyzed by automated capillary based electrophoresis (QIAXCEL, Germany) system which separates and sizes the amplified products very precisely (variation up to 5 bp) (Fig. 1).

Statistical analysis : SSR bands were scored as present (1) or absent (0) for each genotype and scored binary data matrix was analyzed for establishing genetic relationship among the genotypes using the software NTSYSpc 2.1 (Rohlf, 1993). Simple matching similarity coefficients were calculated for all pair wise comparisons among the genotypes. Based on the simple matching similarity values, UPGMA cluster analysis was performed to generate a dendrogram. Simple matching similarity values were also used for the principal component analysis to generate a two dimensional diagram showing genetic association among the cultivars. Polymorphism information content (PIC) of SSR loci was calculated by the following formula :

$$PIC = 1- \Sigma Pij^2$$

where, P_{ij} is the frequency of j^{th} allele of i^{th} locus, summed across all the alleles for the locus over all genotypes as suggested by Anderson *et al.* (1993).

Results and Discussion

In the present study a set of 39 polymorphic SSR markers identified from the initial screening of 250 SSRs were used for DNA fingerprinting and assessing genetic diversity of 54 Indian bread wheat genotypes. These markers amplified a total of 112 alleles and number of alleles per locus ranged from 1 (*Barc1*, *Barc26*, *Barc44* and *Barc147*) to 7 (*Barc77*) with an average of 2.87 allele per locus (Table 2).

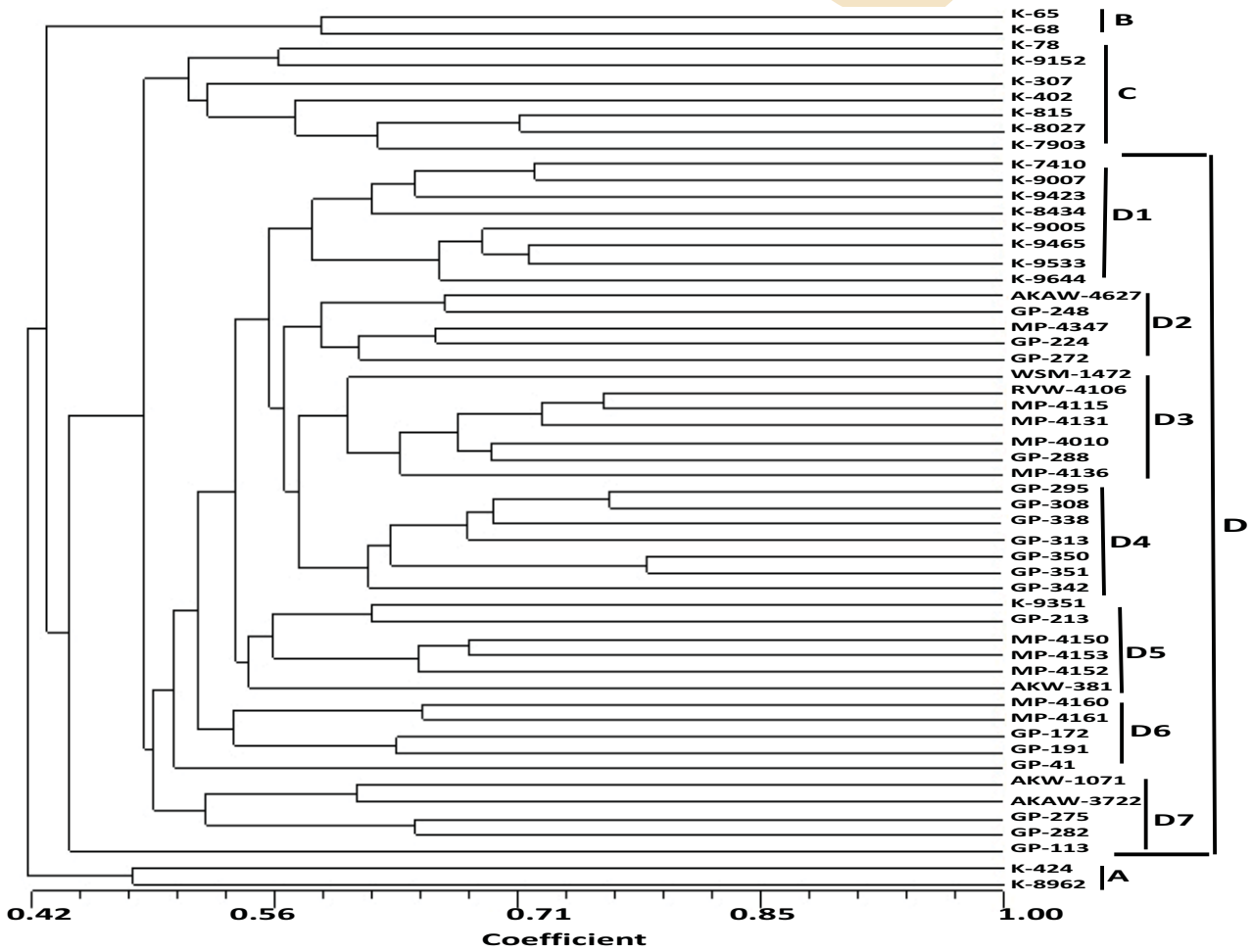

Fig. 2 : Genetic relationship among 54 bread wheat genotypes (cultivars and germplasm lines) based on 39 SSR markers

Table 2 : Details of 39 SSR primers alongwith number of amplicons produced and their PIC value

Primer	Repeats	Forward 5‘ 3‘	Reverse 5‘ 3‘	Chrom-osome	Amplicons size	Amplicon number	PIC Value
Barc-1	TTA 8	GCGATGCTTTTGC CTTGTTTCAG	GCGGCCCCTTTGA CTCTTCATAG	7BD/5AL	150	1	0.37
Barc-4	TTA15	GCGTGTTTGTGTC TGCGTTCTA	CACCACACATGC CACCTTCTTT	5BS	170, 180, 200	3	0.45
Barc-24	TCA10+ TAA9	CGCCTCTTATGGA CCAGCCTAT	GCGGTGAGCCATCG GGTTACAAAG	6BL	110, 90, 80,	3	0.03
Barc-26	ATT8	GCGCTGGGTAAAA AGTGAAATTC	TGCAAGTGGAGGG GGAGGCGAGAG	7DL	100,	1	0.35
Barc-32	TAA10	GCG TGA ATC CGG A AACCC AAT CTG TG	TGG AGA ACC TTC GCA TTG TGT CAT TA	5BL	500,400, 320,300,200	5	0.07
Barc-37	(TTA)8	CAG CGC TCC CCG ACT CAG ATC CTT	GCG CCA TGT TTC TTT TAT TAC TCA CTT T	6A	220, 200, 100	3	0.31
Barc-42	TTA12	GCGATCT CCT ACT G TT GAT AGT TC	GCG TTC TTT TAT TAC TCA TTT TGC AT	3DL	125,100,	2	0.21
Barc-44	TTA 13	CCC TAC AAA ATA C GAACA TGA AGT CAG	GGG TCC TAC TCA GAT AGT GAC AGT CAA C	5DL	125,120	2	0.18
Barc-45	TTA 10	CCC AGA TGC AAT GAA ACC ACA AT	GCG TAG AAC TGA AG C GTA AAA TTA	3AS	130, 140,	2	0.49
Barc-49	TAGA 8	GTC CCA CCA AAT TAA CAG CTC CTA	AGG CGC AGT GCT CG A AGA ATA TTA T	7AL	348, 220, 215, 88	4	0.33
Barc-56	TAGA12	CGC GGA ATT TAC GGG AAG TCA AGA A	GCG AGT GGT TCA AA T TTA TGT CTG T	1BS	150,115, 110	3	0.34
Barc-61	TAGA14	TGC ATA CAT TGA TTC ATA ACT CTC T	TCT TCG AGC GTT AT G ATT GAT	1BS	155, 148, 138, 136, 444	5	0.28
Barc-62	TAGA8	TTG CCT GAG ACA TAC ATA CAC CTA A	GCC AGA ACA GAA TGA GTG CT	1DL	155,150, 140,	3	0.34
Barc-67	GATA 9+3	GCG GCA TTT ACA TTT CAG ATA GA	TGT GCC TGA TTG TAG TAA CGT ATG TA	3AS	450, 350, 250, 240	4	0.36
Barc-70	TATCTA3+ TCTA7	GCG AAA AAC GA T GCG ACT CAA AG	GCG CCA TAT AAT TCA GAC CCA CAA AA	4AL	236, 331, 221, 200	4	0.39
Barc-75	TAG 2 TAGA5	AGG GTT ACA GTT T GC TCT TTT AC	CCC GAC GAC CTA TCT ATA CTT CTC TA	3BS	115,110, 155	3	0.39
Barc-76	TATC 7	ATT CGT TGC TGC CAC TTG CTG	GCG CGA CAC GGA G TA AGG ACA CC	7DL	210,205,	2	0.25
Barc-77	(ATCT)6+18	GCGTATTCTCCCTC GTTTCCAAGTCTG	GTGGGAATTTCTTGG GAGTCTGTA	3B	110, 210, 220, 230, 310, 325, 350	7	0.23
Barc-88	TGA 9	AGG CCT AGA GAC TCA AAG CTG	GCG CTC TTC ATC AAC ACA TTC CTC ATC G	5B	115,100	2	0.07
Barc-97	TC9+6 TAA 9	GCG CCA ACT ACG G AG CTC GAG GAA T	GCA GGA TCA AAC GT A GCC ATG GTG	7D	310, 290, 255,	3	0.36
Barc-98	TAA 10	CCG TCC TAT TCG C AA ACC AGA TT	GCG GAT ATG TTC TCT AAC TCA AGC AAT G	4D	160, 155, 150	3	0.43
Barc- 99	TAG3 TAA10	CGC ATT CTT TCG CAT TCT CTG TCA TA	CGC ATA CTG TGT CGT GTT CCT GGT TTA GA	1D	135, 125,	2	0.39
Barc-109	ATT(35)	GGC AAA AGA GAA GGC TCG GAA GAA CC	CGC ATC GAC GTA ACA TCA CCA CAA TCA	5BS	360, 350, 370	3	0.44
BARC- 134	CT21	CCG TGC TGC AAA TGA ACA C	AGT TGC CGG TTC CC A TTG TCA	6BL	215, 200	2	0.49
BARC-138	CT 8	CTC GAT TCG CCG TCA G	GTG GGG GAA GAA GAA ACC	4AS	255, 180, 175, 110	4	0.30
BARC-142	CT 13	CCG GTG AGA GGA CTA AAA	GGC CTG TCA ATT ATG AGC	5BL	200, 150, 125,	3	0.31
BARC-143	-	TTG TGC CAA ATC AAG AAC AT	GGT TGG GCT AGG ATG AAA AT	5DL	300, 175	2	0.26

Cont....

BARC-144	CA15	GCG TTG TAG GTG GAC GAC ATA GAT AGA	GCG CCA CGG GCA TTT CTC ATA C	5DL	250, 240	2	0.08
Barc-147	CA14	GCGCCATTTATTCA TGTTCCTCAT	CCGCTTCACATGCA ATCCGTTGAT	3B	90	1	0.06
BARC-159	TAA9	CGC AAT TTA TTA TCG GTT TTA GGA A	CGC CCG ATA GTT TT CTA ATT TCT GA	2DL	250, 200	2	0.38
BARC-164	ATT18	TGC AAA CTA ATC ACC AGC GTA A	CGC TTT CTA AAA CTG TTC GGG ATT TCT AA	3B	180, 160, 140	3	0.46
BARC-168	ATT20	GCG ATG CAT ATG A GA TAA GGA ACA AAT G	GCG GCT CTA AGG CGG TTT CAA AT	2DL	200, 190, 185, 100,	4	0.27
BARC-172	ATT19	GCG AAA TGT GAT GGG GTT TAT CTA	GCG ATT TGA TTT AAC TTT AGC AGT GAG	7DL	195, 190, 180, 175	4	0.09
BARC-181	CT17	CGC TGG AGG GGG ATA GTC ATC AC	CGC AAA TCA AGA ACA ACA CGG GAG AAA GAA	1 BL	250, 150, 90	3	0.16
BARC-184	CT21	TTC GGC GAT ATC T TT TCC CCT TGA	CCG AGT TGA CTG TGT GGG GTT GCT G	4AL	350,275, 270,265,200	5	0.21
BARC-197	TAA15	CGC ATG GTC AGT TTT CTT TTA ATC CT	GCG CTC TCC TTC ATT TAT GGT TTG TTG	3AL /5AL	175, 80	2	0.21
BARC- 198	ATT19	CGC TGA AA GAA GTG CCG CAT TAT GA	CGC TGC CTT TGG ATT GCT TGT CA	6BS	175,150,3 140,	0.27	
BARC-200	ATT24	GCG ATA TGA TTT G GA GCT GAT TG	GCG ATG ACG TTA GA T GCG GAA TTG T	2BS	166, 115. 112,110	4	0.18
Barc-352	-	CCC TTT CTC GCT CGC CTA TCC C	CCC TTT CTC GCT CGC CTA TCC C	7D	250,260, 325	3	0.48

The heighest number of alleles (7 alleles) were obtained with *Barc77* followed by *Barc24* (6 alleles) and *Barc32* and *Barc184* (5 alleles) (Table 2). These 39 markers produced lesser number of average alleles as compared to earlier studies (Spanic *et al.,* 2012; Dresigackers *et al.,* 2004; Dvojkovi *et al.*, 2010). Interestingly, the mean PIC value of SSR markers recorded in the present study was also much lower (0.032-0.499 with the mean PIC value of 0.29) as compared to PIC values reported in earlier studies (Arora *et al.* 2014; Mir *et al.* 2012). A low mean PIC value and less number of average alleles for SSR markers (Table 2) may be attributed to comparatively lower level of genetic diversity in the wheat genotypes than those included in the earlier studies. Genotypes showed higher level of similarity due to presence of common parents in their ancestry *i.e.,* out of 54 wheat genotypes analyzed in the present study, 20 genotypes shared 10 common parents in their pedigree (Table 1). Furthermore, 7 SSR markers generated alleles specific to some of the wheat genotypes (Table 3). These unique SSR alleles may be potentially used as markers for identifying corresponding genotypes.

The genetic similarity coefficient value for the present set of wheat genotypes ranged from 22.8 % (between MP-4161 and K-424) to 78.7 % (between two exotic genotypes i.e., GP-350 and GP-361) with an average similarity of 51.23 %. In UPGMA dendrogram all the 54 genotypes were grouped into four clusters (A, B, C and D) at 48% genetic similarity (Fig 2). Cluster 'D' consisted of maximum number of genotypes (43) which were distributed into seven sub-clusters *i.e.*, D1 (8 genotypes), D2 (5 genotypes), D3 (7), D4 (7), D5 (6), D6 (5) and D7 (5) (Fig.1). Cluster 'A', 'B', 'C' and sub-cluster 'D1' were comprised of only K-series genotypes released from CSA, Kanpur (Uttar Pradesh), India and shared a common gene pool. K-424 and K-8962 were clustered into Cluster A and showed 48% genetic similarity while, K-65 and K-68 were clustered into Cluster B and showed 59.2 % genetic similarity to each other. D3 consisted of seven genotypes; all were collected from Madhya Pradesh (Central part of India). D5 also consisted genotypes collected from and grown in Central part of India, except K-9351 (Mandakini) - a variety released from Kanpur (Uttar Pradesh) for North East Plain Zones (NEPZ) that showed 62.3% genetic similarity with GP-213 (Fig. 2). Surprisingly, AKAW-4627 selected from Vimal (Peninsular Zone) showed closeness to GP-248, MP-4147, GP-224 and GP-272, (Sub-cluster 'D2'). Four elite parental lines i.e., MP-4115, MP-4131, MP-4136 and MP-4010 released for Madhya Pradesh were clustered into 'D3' sub-cluster. This implied that these genotypes shared common ancestry that could be further characterized using additional number of polymprphic markers (Table 1).

Out of 13 exotic genotypes, 6 exotic genotypes GP-308, GP-313, GP-338, GP-342, GP-350 and GP-361 were clustered in to sub-cluster D4. However, some exotic genotypes showed higher level of similarity with Indian genotypes. For example, exotic genotype GP 248 showed 66.7% similarity with AKAW-4627- a variety released from Central part of India. Similarly, exotic genotype GP-272

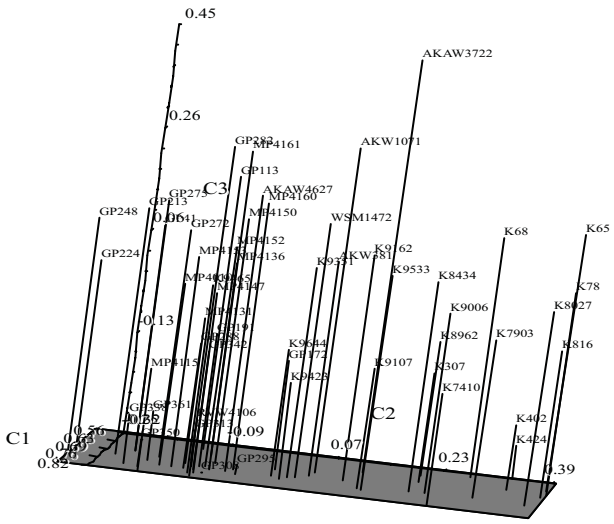

Fig. 3 : Principal components analysis (3D plot) of 54 wheat accessions based on 39 SSR markers

Table 3 : List of SSR markers which produced genotype specific amplicons in 7 wheat genotypes

Primer	Genotype name	Unique Amplicon size (bp)
Barc-24	K-307	110, 120, 130, 135, 150, 165
Barc-32	K-816	200
Barc-77	K-68	110
Barc-77	K-9644	210
Barc-168	GP-191	185
Barc-172	K-65	175
Barc-181	GP-41	150

showed 62.5% and 60.4% similarity with indigenous genotypes GP-224 and MP-4147, respectively, (Fig 2) were sub-clustered into 'D-2'. These genotypes need to be distinguished further with the help of additional SSR markers. The present study show the efficiency of marker system in grouping diverged genotypes into different groups according to their origin and the parents present in their ancestry. Similar results were obtained by principlal component analysis that also differentiated 54 genotypes into four major groups with clear cut differentiation (Fig 3) Overall, except few exceptions, the results of PCA and dendrogram analysis, using UPGMA, showed good correspondence with each other.

Genetic diversity is the base of any genetic improvement breeding program. Therefore, it is necessary to investigate genetic diversity in wheat germplasm in order to broaden the genetic base in future wheat breeding. Number of studies has been done in past to establish genetic diversity in common wheat using different molecular markers such as RAPD (Joshi and Naguyen 1993), AFLP (Barret and Kidwell 1998) and SSR (Röder *et al.,* 1995, Röder *et al.,* 1998). Highly polymorphic SSRs may be useful for genetic diversity studies and cultivar identification required for variety protection in wheat in similar way as it has been proposed for soybean (Khalekar *et al*., 2014; Rongwen *et al*., 1995). SSRs show a much higher level of polymorphism and are more informative in hexaploid wheat than any other marker system (Röder *et al.,* 1995; Bryan *et al.,* 1997; Plaschke *et al.,* 1995). Present study revealed considerable amount of genetic diversity among 54 wheat genotypes using SSR markers. The diverse bread wheat genotypes of Cluster B and C can be utilize in breeding program with Cluster A, and different sub-clusters of D with the aim to create genetic variability in Indian bread wheat. This in terms will be crucial in utilizing the genetic potential of these genotypes for improvement of traits needed for adaptation to various stress conditions either biotic or abiotic, to gain maximum value and practical impact on breeding program. Further, SSR markers used in the present study were found fit for establishing diversity, fingerprinting and clearly differentiating wheat genotypes. Since, number of new similar or related cultivars are increasing very fast, this rapid, unambiguous economic method of genotyping will be a useful tool for cultivar registration, certification and identification of our precious indigenous germplasm lines and to address IPR issues in better way.

Acknowledgment

The authors are grateful to Indian Council of Agricultural Research, Government of India for funding this research.

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