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The Potential of SSR Markers to Reveal the Genetic Diversity among Wheat and its Wild Relatives and to Test the Hybridity of F_1s

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The SSR technique was used to reveal the genetic diversity among wheat (*Triticum aestivum*) and its wild relatives and secondly to test the hybridity of wheat (*Triticum aestivum*) F_1s. All the thirteen SSR primers showed 100% polymorphism and except one, all the primers generated unique bands, so these primers can be used for genotype identification. Also four primers showed heterozygous nature of F_1s by giving two bands, one from each parent at a particular locus, so these markers can be used for screening purpose also. Use of these primers resulted in 180 polymorphic bands out of which 47 were unique.

Introduction

Bread wheat (*Triticum aestivum*) is one of the big three globally important crops accounting for 20% of the calories consumed by the people and a staple crop of nearly 35% of the global population. The huge bread wheat genome is comprised of 17 Gb (17,000,000,000) base pairs which is about 5 times the human DNA content and about 40 times of rice genome size. However 80- 90 % of the genome is made up of repetitive sequences. This offers an ample scope for the use of SSRs- the Simple Sequence Repeats as molecular markers studies. Molecular genetics, or the use of molecular techniques for detecting differences in the DNA of individual plants, has got numerous applications in crop improvement.

The differences are called molecular markers because they are often associated with specific genes and act as “signposts” to those genes. Such markers, when very tightly linked to genes of interest, can be used to select indirectly for the desirable allele, and this

represents the simplest form of Marker Assisted Selection (MAS). Molecular markers used to probe the level of genetic diversity among different cultivars, within populations, among related species, etc. have many applications like varietal fingerprinting for identification and protection, understanding relationships among the units under study, efficiently managing genetic resources, facilitating introgression of chromosomal segments from alien species and even tagging of specific genes (Hoisington *et al.,* 2002).

SSRs involve the use of specifically chosen primers to amplify the repetitive sequences through Polymerase chain reaction. The repetitive DNA of all the species is highly polymorphic in nature. These regions contain genetic loci comprising several hundred alleles, differing from each other with respect to length, sequence or both and they are interspersed in tandem arrays ubiquitously. The term microsatellite was coined by (Litt and Lutty 1989). SSRs are increasingly being used as genetic markers of chromosome segments (Dib *et al.,* 1996), for identification of individuals (Anon, 1996), studying evolution and orthologous and paralogous relatedness (Rubinsztein *et al.,* 1995 and Ali *et al.,* 1999) and wildlife conservation (Roca *et al.,* 2001). The present study aimed at the use of SSRs to study the genetic diversity of wheat and its wild relatives at all ploidy levels (diploid, tetraploid and hexaploid) and secondly the codominant marker was also used to to test the hybridity of wheat (*T. aestivum*) F_1s.

Materials and Methods

The study was conducted at G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India during 2004-08. The seeds of wild relatives of wheat were obtained from Directorate of Wheat Research, Karnal, Haryana, India. The experimental material consisted of 41 genotypes which included 10 wild relatives of wheat, 2 *Triticum durum* varieties, 15 *Triticum aestivum* varieties and 14F_1s among them. The parentage of *T. aestivum* genotypes is given in Table 1. DNA characterization was done using 13 SSR primers. Primers were provided by Integrated DNA Technologies, Inc. Details of primers are given in Table 2.

Genomic DNA extraction

CTAB procedure was used for the isolation of DNA. CTAB (Cetyl trimethyl ammonium bromide) is a cationic detergent which solubilizes membranes and forms a complex with DNA. After cell disruption and incubation with hot CTAB isolation buffer, proteins were extracted by chloroform: isoamyl alcohol. CTAB–DNA was precipitated with isopropanol. The DNA pellet resulting after centrifugation was washed, dried and redissolved. RNase A treatment was given to remove RNA contamination.

Protocol followed

Two g of fresh wheat seedling leaves were ground to a fine powder using liquid nitrogen and a mortar and pestle.

The powder was transferred as fast as possible into 15 ml of pre-warmed (60° C) isolation buffer in an oakridge tube.

The oakridge tubes were then incubated in a water bath at 60° C for 30 minutes. It was mixed gently after every 10 minutes.

One volume of chloroform: isoamyl alcohol (24: 1) was then added. The tube was capped and shaken gently and thoroughly for 10 minutes by hand, enough to ensure emulsification of the phase.

Then it was centrifuged for 10 minutes (5000 rpm, room temperature). The (upper) aqueous phase was extracted once again with fresh chloroform: isoamyl alcohol.

The final aqueous phase was transferred to a

fresh tube using micropipette with a wide bore microtip (that of 1000 µl capacity).

0.6 volume of chilled isopropanol was added, the tube was capped and mixing was done gently but thoroughly by inverting the tube several times. At this stage, the DNA–CTAB complex precipitated as a whitish network. The solution was placed at -20° C for 30 minutes to overnight.

Then it was centrifuged (10 min., 5000 rpm, 4° C). It was then washed with 70% ethanol; the pellet was gently agitated for a few minutes, and collected by centrifugation (10 min., 5000 rpm, 4° C). Residual CTAB was removed by this step.

The tubes were inverted and drained on a paper towel for about 1 hour taking care that pellet does not slip down the wall of the tube. It was ensured that it neither contained residual ethanol nor it was too dry. In both cases redissolving might be difficult.

An appropriate volume of 1 X TE buffer was added (say 500 µl) and the pellet was allowed to dissolve at 4° C without agitation.

Purification and quantification of genomic DNA

5 µl RNase (10 mg/ml) was added to 100 µl of dissolved DNA and incubated at 37° C for 1 hour. Equal volume of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added and mixed gently by inverting the tubes. The tubes were spun at 10,000 rpm for 5 minutes and aqueous layer (i.e. upper layer) was collected and equal amount of chloroform + isoamyl alcohol (24: 1) was added. The tubes were spun at 10,000 rpm for 5 min and the top layer of DNA was removed. To this, sodium acetate (1/10 vol, pH=5.2) and chilled absolute ethanol was added. The contents were mixed and kept at –20°C for 30 min. Finally the pellet was washed with 70 per cent ethanol, dried and dissolved in 100 µl TE buffer.

The quantification of genomic DNA was done by taking the absorbance on Genesys UV spectrophotometer. The optical density was measured at 260 and 280 nm. The concentration of the DNA in the sample is related to optical density by the following formula:

$$\text{Conc. of DNA } (\mu g/ml) = \frac{OD_{260} \times 50 \times \text{Dilution factor}}{1000}$$

The ratio of $OD_{260/280}$ was an indication of the amount of RNA or protein contamination in the preparation. A value of 1.8 is optimum for the best DNA preparation. A value of the ratio below 1.8 indicates the presence of protein in the preparation and a value above 1.8 indicates that the sample has RNA contamination.

PCR amplification

The reaction mixture consisted of genomic DNA, d NTPs, Taq polymerase, reaction buffer, primers (forward and reverse) and double distilled water. The concentrations and quantity of components is given in Table 3. The PCR thermocycler was programmed according to the Table 4. In PCR programming 3 annealing temperatures were used: 51°C for Barc 019, Barc 119, Barc 025, Barc 028, Barc 062, Barc 065, Barc 142, Barc 154 and Barc 228. 52°C for Barc 003 and Barc 111 and 54°C for Barc 124 and Barc 159.

Electrophoresis of the amplified PCR products was done in horizontal gel electrophoresis assembly using agarose gel of 2.5 % concentration. Electrophoresis was done at 50 V for 4 hours in 0.5 X TBE buffer. After 75% run of the gel, its image was viewed and its photograph saved in a gel documentation system.

Data analysis

Gels were documented using Gel Doc system (Bio-Rad). Pair-wise similarity and cluster analysis were done on the basis of presence and absence of bands. Computer software (NTSYS) was used to perform the similarity matrix analysis using 'UPGMA' with Jaccard's coefficient of similarity.

Results and Discussion

All the13 SSR primers used in the study were polymorphic. They amplified total 180 bands out of which 47 bands were unique. 12 primers gave unique bands. The size of bands ranged from 100 to 3000 bp. The details of amplification pattern are provided in the Table 5.

All the thirteen SSR primers showed 100% polymorphism and except one, all the primers generated unique bands, so these primers can be used for genotype identification. Also four primers showed heterozygous nature of F1s by giving two bands, one from each parent at a particular locus, so these markers can be used for screening purpose also. Primer Barc 019 amplified maximum number of loci (24) and also gave maximum number of unique bands (10) followed by Barc 062 (7), Barc 142 (6), Barc 028 (5), and Barc 228 (4), whereas Barc 065, Barc 119 and Barc 154, each gave 3 unique bands whereas Barc 145, Barc 159 and Barc 025 gave 2, Barc 124 gave one unique band. So these markers can be used for the identification of genotypes.

The wild species *T. dicoccum* showed highest number of unique bands (18) from 6 primers – Barc 019, Barc 025, Barc 142, Barc 154, Barc 159 and Barc 228 followed by *Ae. Squarrosa* (5) from 4 primers – Barc 019, B. Other wild species *T. sphaerococcum, T. polonicum, T. monococcum* also showed unique bands. *T. durum variety* PDW 289 and *Secale cereale* accession EC 481695 also showed unique bands. It can be inferred that these wild germplasms harbour drought tolerance characteristics and can be used as donor of drought tolerance trait in wheat breeding programmes.

T. aestivum variety WH 730 showed maximum number of unique bands. Varieties like UP 2565 and PBW 373 also showed unique bands which incates the possibility of developing drought tolerance in these varieties. UP 2425 showed 4 unique bands. The study of R. P. Meena *et al.,* (2015) also suggests that UP 2425 performs better under moisture stress conditions based on several stress indices. The hills variety VL 804 also showed unique band confirming its drought tolerant nature. The cross Job 666 X UP 2565 showed unique band as well as the bands present at a particular locus in the parents were also present in the cross, primer Barc 154. This codominant nature of marker was shown in the cross NP 846 x UP 2425 by 2 primers Barc 028 and Barc 159. Barc 025 also revealed its codominant nature in the cross NIAW 34 x UP 2590 (Fig. 1–3).

SSR cluster analysis

The dendrogram that was constructed using NTSYS software divided the genotypes in several clusters. Firstly 2 major groups were formed. Group 1 comprised of genotypes 1,2,11 –*Secale cereale* EC 481697, *Secale cereale* EC 481695 and *T. dicoccum*. All other genotypes formed group 2. Group 2 was further divided in group 2a and group 2b.

2a comprised of 2a sub group 1 and 2a sub group 2. First cluster of 2a sub group 1 included the genotypes 3, 12, 15, 13, 5, 6, 7 – *T. timopheevii*, WH730 x UP 2425, Job 666 x UP 2565, Job 666 x UP 2425, *T. tauschii*, *T. sphaerococcum*, *Ae. Squarrosa*. Out of these *T. timopheevii* was clustered as a separate small cluster, WH 730 x UP 2425, Job 666 x UP 2565 and Job 666 x UP 2425 in another

small cluster. *T. tauschii*, *T. sphaerococcum* and *Ae. squarrosa* in a third small cluster. Second cluster of 2a sub group 1 had genotypes 14 and 16 – Job 666 and UP 2565.

2a sub group 2had 4, 8, 9, 10 – *T. polonicum*, *T. turgidum*, PDW 291 and PDW 289. Out of these *T. polonicum* existed as a separate cluster other than *T. turgidum*, PDW 291 and PDW 289. Group 2b had 2 sub-groups 2b sub group 1 and 2b sub group 2. 2b sub group 1 existed as a single genotype 17 – T. monococcum.

Rest other genotypes were present in 2b sub group 2 i.e. 18, 19, 23, 24, 25, 27, 28, 31, 29, 33, 34, 20, 21, 22, 30, 32, 35, 40, 41, 38, 39, 26, 36, 37 – Halna, PBW 175, PBN 51 x UP 2554, UP 2554, WH 730 x UP 2554, WH730 x UP 2338, UP 2338, NIAW 34 x PBW 373, PBN 51 x UP 2338, NIAW x UP 2590, UP 2590, VL 804, PBN 51 x VL 804, PBN 51, PBW 373, NIAW 34, NIAW 34 x UP 2565, HI 385 x UP 2425, HI 385, NP 846 x UP 2425, NP 846, WH 730, NIAW 34 x UP 2425, UP 2425.

Relationship among wheat genotypes

Based on the estimated genetic similarity matrix using UPGMA method, the primers revealed highest genetic similarity value 0.7895 between VL 804 and its cross PBN 51 x VL 804 indicating the involvement of drought lines, followed by 0.7143 between 2 crosses WH 730 x UP 2425 and Job 666 X UP 2565 indicating the presence of drought tolerant parents WH 730 & Job 666 in the crosses, also the other parents UP 2425 and UP 2565 are the varieties released from the same place i.e. Pantnagar and both are recommended for irrigated late sown conditions. It was followed by the similarity value 0.6857 between HI 385 and its cross HI 385 x UP 2425, followed by 0.6786 between PBW 175 and a cross PBN 51 x UP2554 as PBW 175 is drought tolerant and the parental line PBN 51 of the cross is also drought tolerant. It was followed by the similarity value 0.6765 between UP 2590 and its cross NIAW 34 x UP 2590, followed by 0.6744 between 2 crosses Job 666 X UP 2425 and Job 666 x UP 2565 due to the common parent Job 666 between them.

It was followed by the similarity value 0.6667 between 2 *Triticum durum* varieties PDW 291 and PDW 289, followed by 0.6563 between UP 2338 and a cross NIAW 34 x PBW 373. It was followed by the similarity value 0.6486 between UP 2554 and its cross WH 730 x UP 2554, followed by 0.6250 in 3 pairs i.e. between UP 2338 and its cross PBN 51 x UP 2338, between 2 crosses PBN 51 x UP 2554 and PBN 51 x UP 2338 and between Halna and PBW 175.

The results indicate that SSRs can be very effectively used for molecular characterization of genotypes. SSRs are codominant markers which show bands in both the parents at different loci as well as both the parental bands in the cross. As we know that drought tolerance is a complex Quantitative trait loci therefore a lot of variations can be observed in the banding pattern of crosses. Further for QTL mapping or Gene tagging purposes the populations like Nearly Isogenic Lines, Doubled Haploids, Recombinant Inbred Lines, F_2, Back cross should be used.

Table.1 List of various wheat (*Triticum aestivum,* genome AABBDD, 2n= 42)

Varieties	Parentage	Remarks
Halna (K 7903)	HD 1982 / K 816	Drought tolerant (gene introgressed)
UP 2565	PBW 352 / CPAN 4020	
HI 385 (MUKTA)	HYB 633 // GAZA // PR / PKD 25	Drought tolerant (gene introgressed)
PBW 373	ND / VG 9144 // KAL / BB / 3 / YACO '5' / 4 / VEE # 5 'S'	
NIAW 34	CNO 79 / PRL "S"	Drought tolerant (gene introgressed)
UP 2425	HD 2320/UP 2263	Drought susceptible
NP 846	NP 760 / RN	Drought tolerant (gene introgressed)
UP 2338	UP 368 / VL 421 / UP 262	Drought susceptible
PBW 175	HD 2160 / WG 1025	Drought tolerant (gene introgressed)
PBN 51	BUC 'S' / FLK 'S'	Drought tolerant (gene introgressed)
UP 2554	SM4 – HSN 24E / CPAN 2099	
UP 2590	Not available	
VL 804	CPAN 3018/CPAN 3004//PBW 65	Drought tolerant
WH 730	CPAN 2092/ Improved Lok- 1	Drought tolerant (gene introgressed)
JOB 666	K 65 / HD 2009	Drought tolerant (gene introgressed)

List of related species

Species	Variety/ accessions	Parentage	Genome	Chromosome no.
Aegilops squarrosa	-	-	DD	14
Triticum monococcum	-	-	AA	14
T. tauschii	-	-	DD	14
T. dicoccum	-	-	AABB	28
T. durum	PDW 289	-	AABB	28
	PDW 291	BOOMER 21/ MOJO 2	AABB	28
T. turgidum	-	-	AABB	28
T. polonicum	-	-	AABB	28
T. sphaerococcum	-	-	AABBDD	42
T. timopheevii	-	-	AAGG	28
Secale cereale	EC 481695	-	RR	14
Secale cereale	EC 481697	-	RR	14

Table.2 Characteristics of SSR Primers

Sl. No.	Operon Code	Forward Sequence (5′-3′)	GCcontent (%)	Reverse Sequence(5′-3′)	GC content (%)
1.	Barc 003	TTCCCTGTGTCTT TCTAATTTTTTTT	26.9	GCGAACTCCCG AACATTTTTAT	40.9
2.	Barc 019	GCGACCCGAGTA GCCTGAA	63.1	GGTGGACCATTA GACGCTTACTTG	50.0
3.	Barc 025	GCGGTGCATCAA GGACGACAT	57.1	GCGTAGTTC ATCCACCGTAAT	45.4
4.	Barc 028	CTCCCCGGCTAG TGACCACA	65.0	GCGGCATCTTTCA TTAACGAGCTAGT	46.1
5.	Barc 062	TTGCCTGAGACAT ACATACACCTAA	40.0	GCCAGAACAGAA TGAGTGCT	50.0
6.	Barc 065	CCCATGGCCAAG TATAATAT	40.0	GCGAAAAGTCCAT AGTCCATAGTCTC	46.1
7.	Barc 119	CACCCGATGATGA AAAT	41.1	GATGGCACAAG AAATGAT	38.8
8.	Barc 124	TGCACCCCTTCC AAATCT	50.0	TGCGAGTCGTGT GGTTGT	55.5
9.	Barc 142	CCGGTGAGAGGA CTAAAA	50.0	GGCCTGTCAATT ATGAGC	50.0
10.	Barc 145	GCAGCCTCGA ATCACA	56.2	GGGGTGTTGAAG ATGA	50.0
11.	Barc 154	GTAATTCCGGTT CCACTTGACATT	55.5	GGATGGGCAGCT TCAAGGTATGTT	50.0
12.	Barc 159	CGCAATTTATTAT CGGTTTTAGGAA	32.0	CGCCCGATAGTTT TTCTAATTTCTGA	38.4
13.	Barc 228	CCCTCCTCTCT TTAGCCATCC	57.1	GCACGTACTATTC GCCTTCACTTA	56.8

Table.3 Standard concentration of components for PCR amplification

Components (Conc.)	Final Conc./25 µl	Single tube µl
DNA template (20 ng/µl)	40 ng	2.0 µl
d NTPs (2.5 mM each)	200 µM each	2.0 µl
Taq polymerase (3 U/µl)	0.76 U	0.5 µl
Reaction buffer (10 X)	1 X	2.5 µl
Primer (50 ng/µl) forward	50 ng	1.0 µl
Primer (50 ng/µl) reverse	50 ng	1.0 µl
dd H_2O		16.0 µl
	Total	**25.0 µl**

Table.4 Protocol for PCR amplification

Cycle	Denaturation		Annealing		Polymerization	
	Temp.	Time	Temp.	Time	Temp.	Time
First cycle	94° C	5 min	–	–	–	–
44 cycles	94° C	1 min	51° C, 52° C, 54° C,	1 min	72° C	2 min
Last cycle	–	–	–	–	72° C	7 min

Table.5 Details of amplification pattern

S.No.	Name of Primer	No. of amplified loci	Size of bands (bp)	No. of Unique bands	Genotypes having unique bands		Codominant nature of marker		
					Genotype	Size of unique band (bp)	Bands in cross	Bands in parents	
1.	Barc 003	10	150-3000						
2.	Barc 019	24	110 - 3000	10	*Ae. squarrosa*	3000			
					UP 2425	1200			
					T. dicoccum	1000			
						850			
						520			
						410			
						390			
						340			
					NIAW 34 x UP2425	330			
					UP 2565	200			
3.	Barc 025	8	200 - 2800	2	*T. dicoccum*	550	NIAW 34 x UP2590 250 bp & 200 bp	NIAW34 250 bp	UP 2590 200 bp
						360			
4.	Barc 028	19	230 - 2000	5	*Ae. squarrosa*	2000	NP 846 x UP 2425 270 bp 250 bp	NP 846 270 bp	UP 2425 250 bp
						1500			
					Job 666 x UP 2565	750			
					T. sphaerococcum	300			
					T. polonicum	260			
5.	Barc 062	13	100 - 1200	7	WH 730	1200			
						900			
						700			
						600			

						520			
						375			
						360			
6.	Barc 065	7	100 - 490	3	*T. monococcum*	410			
						290			
					PBW 373	275			
7.	Barc 119	20	180 - 1400	3	UP 2425	580			
						330			
						310			
8.	Barc 124	12	210 - 1100	1	HI 385	1000			
9.	Barc 142	18	160 - 1500	6	*T. dicoccum*	1500			
						720			
						480			
						250			
					PDW 289	200			
					S. cereale EC 481695	800			
10.	Barc 145	17	150 - 1200	2	*Ae. squarrosa*	440			
					PBW 373	320			
11.	Barc 154	10	250 - 600	3	*T. dicoccum*	500	Job 666 x UP 2565 300 bp 280 bp	Job 666 280 bp	UP 2565 300 bp
					Ae. squarrosa	480			
					T. polonicum	320			
12.	Barc 159	8	180 - 700	2	*T. dicoccum*	700	NP 846 x UP 2425 220 bp 210 bp	NP 846 210 bp	UP 2425 220 bp
						400			
13.	Barc 228	14	180 - 1500	4	*T. dicoccum*	1500			
						520			
						270			
					VL 804	230			

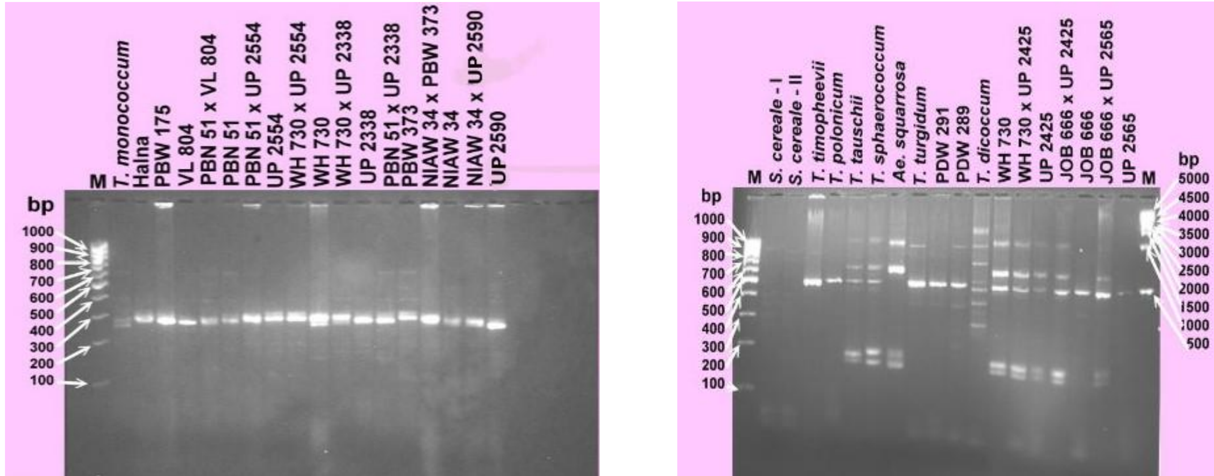

Fig.1&2 SSR profiles generated by primer Barc 142

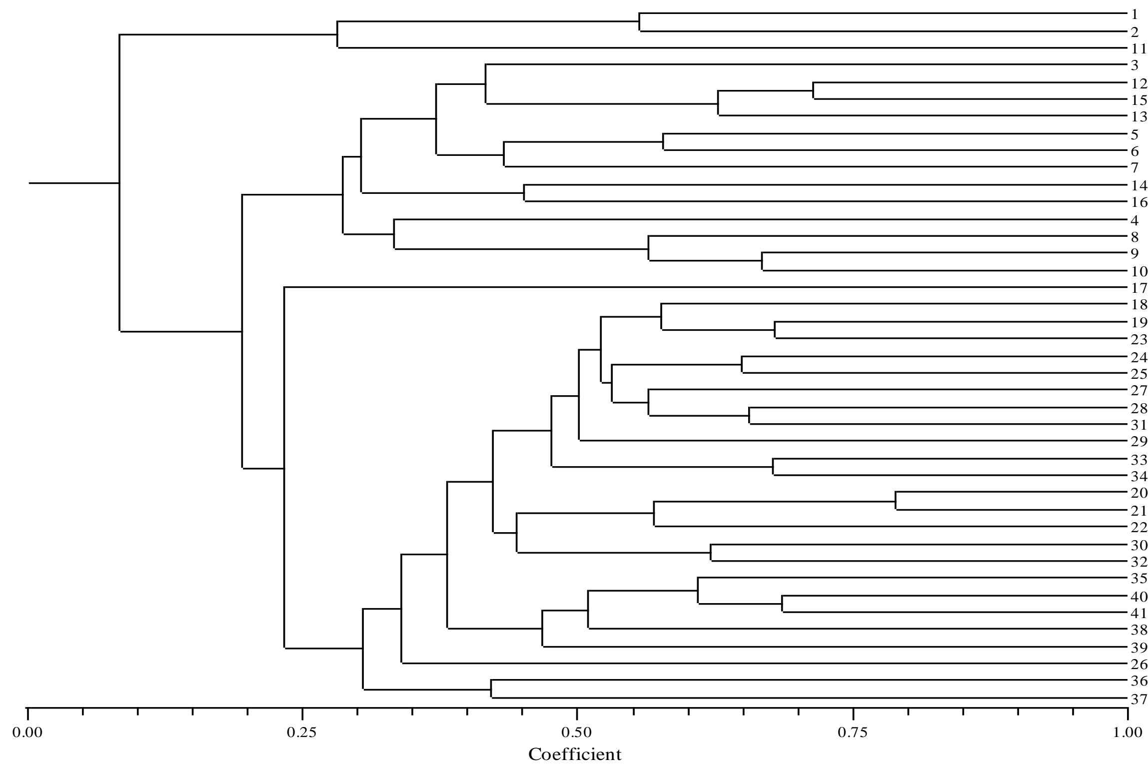

Fig.3 Dendrogram of wheat genotypes constructed using Jaccard's coefficient of similarity

Wheat genotypes as represented in SSR dendrogram

1. *Secale cereale* EC 481697 2.*Secale cereale* EC 481695 3. *Triticum timopheevii* 4. *Triticum polonicum* 5. *Triticum tauschii* 6.*Triticum sphaerococcum* 7.*Aegilops squarrosa* 8.*Triticum turgidum* 9. PDW 291 10. PDW 289 11. *Triticum dicoccum* 12.WH 730 x UP 2425 13. JOB 666 x UP 2425 14.JOB 666 15.JOB 666 x UP 2565 16. UP 2565 17.*Triticum monococcum* 18. HALNA 19. PBW 17 20.VL 804 21. PBN 51 x VL 804 22. PBN 51 23. PBN 51 x UP 2554 24. UP 2554 25. WH 730 x UP 2554 26. WH 730 27. WH 730 x UP 2338 28. UP 2338 29. PBN 51 x UP 2338 30. PBW 373 31. NIAW 34 x PBW 373 32. NIAW 34 33. NIAW 34 x HD 2590 34. HD 2590 35. NIAW 34 x UP 2565 36.NIAW 34 x UP 2425 37. UP 2425 38. NP 846 x UP 2425 39.NP 846 40.HI 385 x UP 2425 41.HI 385

The SSR primers revealed the lowest genetic similarity value 0.0208 between *Secale cereale* EC 481697 and *Triticum monococcum*, followed by 0.0282 between *Triticum dicoccum* and *Triticum monococcum,* followed by 0.0290 between *Triticum dicoccum* and a cross NIAW 34 x UP 2590, followed by 0.0364 between *Secale cereale* EC 481695 and VL 804, followed by 0.0380 between *Aegilops squarrosa* and *Triticum dicoccum*, followed by 0.0385 between *Secale cereale* EC 481695 and UP 2554, followed by 0.0390 between *Secale cereale* EC 481697 and UP 2425, followed by 0.0392 between *Secale cereale* EC 481695 and *Triticum monococcum*, followed by 0.0400 between *Triticum dicoccum* and NIAW 34, followed by 0.0408 between *Secale cereale* EC 481695 and a cross NIAW 34 x PBW 373, followed by 0.0426 between *Secale cereale* EC 481697 and Halna, followed by 0.0435 between *Triticum dicoccum* and UP 2590, followed by 0.0444 between *Secale cereale* EC 481697 and a cross NIAW 34 x UP 2590, followed by 0.0448 between *Triticum dicoccum* and a cross PBN 51 x UP 2338, followed by 0.0455 between 2 pairs i.e. between *Secale cereale* EC 481697 and a cross PBN 51 x UP 2338 and between *Triticum teemopheevii* and *Triticum dicoccum*, followed by 0.0462 between 2 pairs i.e. between *Triticum dicoccum* and UP 2338 and between *Triticum dicoccum* and a cross PBN 51 x UP 2554, followed by 0.0469 between *Triticum dicoccum* and PBN 51, followed by 0.0476 between *Secale cereale* EC 481697 and UP 2338, followed by 0.0484 between *Triticum dicoccum* and NP 846, followed by 0.0488 between *Secale cereale* EC 481697 and PBN 51 and so on.

The low similarity matrix values clearly indicate low genetic similarities or high genetic diversity and may be attributed to genomic differences in the study material.

Relationship among wheat and its wild relatives

SSR amplification patterns were analyzed to study the genetic diversity between wheat and its wild relatives. The F_1s were excluded from this analysis. Based on the estimated genetic similarity the highest genetic similarity value (0.667) was observed between PDW 291 and PDW 289, as both of them are the two varieties of *T. durum* followed by 0.625 between Halna and PBW 175, followed by 0.620 between PBW 373 and NIAW 34, followed by 0.604 between UP 2554 and PBW 373, followed by 0.578 between *T. tauschii* and *T. sphaerococcum*, followed by 0.556 between *Secale cereale* EC 481697 and *Secale cereale* EC 48195, followed by 0.550 between *T. turgidum* and PDW 291 followed by 0.545 between PBW 175 and UP2590, followed by 0.525 between Halna and UP 2554, followed by 0.516 between PBW 175 and UP 2338 followed by 0.514 between 2 pairs i.e. UP2554 and UP 2338; PBW 175 and UP 2554.

The lowest genetic similarity value 0.021 was observed between *Secale cereal* EC 481697 and *T. monococcum*, followed by 0.028 between *T. dicoccum* and *T. monococcum*, followed by 0.038 between 2 pairs i.e. Secale cereal EC 481695 and UP 2554; *Aegilops squarrosa* and *Triticum dicoccum*, followed by 0.039 between 2 pairs i.e. Secale cereal EC 481697 and UP 2425; *Secale cereale* EC 481695 and *T. monococcum.*

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