

Original Research Article

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Study of Genetic Polymorphism in Wheat and its Wild Relatives using ISSR Markers

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ABSTRACT

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Inter Simple Sequence Repeats, the PCR based technique was used to reveal the genetic diversity among wheat and its wild relatives. DNA profiling of 27 wheat genotypes at all the existing ploidy levels (diploid, tetraploid and hexaploid) was done using ISSR primers. All the primers were polymorphic. The primers resulted in the amplification of 73 bands out of which 17 bands were unique. Unique bands resulted in case of all the primers. According to these 4 ISSR primers genetic similarity value was observed ranging from 0 to 1, the highest genetic similarity value (1.00) was noticed between *T. timopheevii* and *T. sphaerococcum* followed by 0.909 between *T. tauschii* - *T. timopheevii* and *T. tauschii* - *T. sphaerococcum*. The lowest genetic similarity value (0.00) was noticed between PBN 51 and NIAW 34 owing to their different places of release. It was followed by 0.032 between PBW 373 and PDW 291 as one is hexaploid and the other one is tetraploid wheat.

Introduction

Wheat is a staple food of a majority of the population. Wheat occupies second position worldwide only after corn with a production of about 735 million metric tonnes. The genus *Triticum* comprises of several species, *Triticum aestivum* being the commonly grown wheat throughout the world. The wild relatives of wheat though poor in yield but possess several desirable characters like resistance to biotic and abiotic stresses, short height, strong culms etc. There is always ample requirement to find out markers linked to these traits. The present study aims at finding out the polymorphism among various

members of wheat group. About 30-90 per cent of the genome of virtually all the species is constituted by regions of repetitive DNA, which are 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.

Inter simple sequence repeat (ISSR)-PCR is a technique, which involves the use of microsatellite sequences as primers in a polymerase chain reaction to generate multilocus markers. They amplify inter-SSR DNA sequences. ISSRs have been

successfully used to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of crop species which include rice, wheat, finger millet, Vigna, sweet potato, plantago, Vanilla etc.

ISSR-PCR overcomes most of the limitations of PCR based techniques like low reproducibility of RAPD, high cost of AFLP and the need to know the flanking sequences to develop species specific primers for SSR polymorphism.

Materials and Methods

DNA isolation

The genomic DNA from 27 genotypes of wheat and its wild relatives (Table 1) was isolated from 15 days old germinated seedlings using CTAB procedure. The quantification of DNA was done by taking the absorbance on Genesys UV-spectrophotometer. The concentration of DNA was analysed by taking OD_{260/280} of each sample i.e. the ratio of the optical density measured at 260 nm and 280 nm.

ISSR analysis

Four ISSR primers (Table 2) UBC- 808, UBC- 841, UBC- 856 and UBC- 873 were used arbitrarily to screen 27 genotypes of wheat. The reaction consisted of genomic DNA, dNTPs, Taq polymerase, reaction buffer, primer, MgCl₂ and double distilled water according to Table 3.

The thermocycling was programmed as given in Table 4. PCR products were fractionated by horizontal gel electrophoresis on Agarose gel 1.5% in 1X TBE buffer at 50 Volts for 4 hours in 1X TBE electrophoresis buffer. The gel was then stained with Ethidium Bromide and visualised under UV illumination.

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' Unweighted Pair Group Method Average with Jaccard's coefficient of similarity.

Results and Discussion

Four primers were used to amplify the DNA of 27 genotypes. They amplified total 73 number of bands with 18.25 as the average number of bands per primer. All of the primers showed 100% polymorphism and a few unique bands (Table 5). Total 17 bands were found to be unique (Table 6). The amplified products ranged in size from 300-2000 bp.

Genetic variation based on ISSR markers

Data scored from amplification products was used to generate similarity coefficients which ranged from 0 to 1. Association among the genotypes revealed by UPGMA with Arithmetic Mean Cluster Analysis. The dendrogram generated is as shown in figure 1.

Genotype identification

All the primers gave polymorphic bands and unique bands

UBC 808

On agarose gel this primer revealed 18 amplified ISSR loci. This primer amplified the products in the range of 300 bp to 1300 bp. All the loci amplified by this primer were 100% polymorphic. One band at the position 1100 bp was unique to PBN 51, another one band at 700 bp was unique to UP 2565 and

one band at 500 bp was unique to *Triticum monococcum*.

UBC 856

On agarose gel this primer revealed 18 amplified ISSR loci. This primer amplified the products in the range of 500 bp to 1600 bp. All the loci amplified by this primer were 100% polymorphic. Two bands at 1600 bp and 1300 bp were unique to PBN 51. Three bands at 1250 bp, 950 bp and 800 bp were unique to UP 2338 and one band at 750 bp was unique to UP 2425.

UBC 841

On agarose gel this primer revealed 19 amplified ISSR loci. This primer amplified the products in the range of 490 bp to 2000 bp. All the loci amplified by this primer were 100% polymorphic. Two bands at 2000 bp and 1700 bp were unique to *Aegilops squarrosa*, one band at 1200 bp was unique to *Secale cereale* EC 481695 and two bands at 650 bp and 640 bp were unique to HI 385.

UBC 873

On agarose gel this primer revealed 18 amplified ISSR loci. This primer amplified the products in the range of 500 bp to 1800 bp. All the loci amplified by this primer were 100% polymorphic. One band at 1800 bp was unique to *Triticum dicoccum*, one band at 1700 bp was unique to PBW 373 and one band at 750 bp was unique to NP 846.

Relationship among wheat genotypes using ISSR markers

Based on the estimated genetic similarity matrix table the highest genetic similarity value (1.00) was noticed between *T. timopheevii* and *T. sphaerococcum* followed by 0.909 between *T. tauschii* and *T.*

timopheevii and *T. tauschii* and *T. sphaerococcum*, 0.828 between *Secale cereale* EC 481695 and *Secale cereale* EC 481697, 0.824 between Halna and *T. polonicum*, 0.733 between PBW 373 and UP 2565, 0.719 between VL 804 and HD 2590, 0.677 between HD 2590 and WH 730, 0.647 among four pairs viz. NP 846 and WH 730, Halna and *T. timopheevii*, Halna and *T. sphaerococcum* and PBW 373 and *T. polonicum* and 0.636 between HI 385 and VL 804 indicating their close genetic similarity with each other.

These markers showed the lowest genetic similarity value (0.00) was noticed between PBN 51 and NIAW 34 owing to their different places of release. It was followed by 0.032 between PBW 373 and PDW 291 as one is hexaploid and the other one is tetraploid wheat and 0.034 between UP 2565 and PDW 291 as again one is hexaploid wheat and the other one is tetraploid. The genetic similarity value 0.036 was found between PBN 51 and PBW 175 as first one is the variety of Parbhani area and the second one is adapted to Punjab area and 0.043 between Halna and NIAW 34 as Halna is a local cultivar of U. P. area and NIAW 34 belongs to Wellington. The genetic similarity value 0.048 was found between *Triticum monococcum* and NIAW 34 as the first one is diploid wheat and the other one is a hexaploid variety of wheat. The genetic similarity value 0.050 was observed among three pairs i.e. PBN 51 and PDW 289 being hexaploid and tetraploid varieties, respectively, PBW 373 and NIAW 34 due to different places of their release, where PBW 373 been released from Punjab and NIAW 34 from Wellington and *T. polonicum* and NIAW 34, *T. polonicum* being a tetraploid wheat and NIAW 34, a hexaploid variety. The genetic similarity value was 0.056 between UP 2565 and NIAW 34 due to their different places of release, UP 2565 released from Pantnagar and NIAW 34 from

Wellington. The genetic similarity value 0.059 was observed among three pairs i.e. *T. timopheevii* and NIAW 34, *T. timopheevii* being a wild relative of wheat and NIAW 34, a hexaploid cultivated variety; UP 2554 and NIAW 34 due to different places of their release, UP 2554 released from Pantnagar and

NIAW 34 from Wellington; 0.063 between *T. tauschii* and NIAW 34, *T. tauschii* being a diploid wheat and NIAW 34, a hexaploid variety; 0.065 between *T. monococcum* and PDW 291, *T. monococcum* being a diploid wheat and PDW 291, a tetraploid variety.

Table.1 List of various wheat genotypes

Species	Cultivars	Parentage	Genome	2n
<i>T. aestivum</i> L.	Halna	HD 1982 / K 816	AABBDD	42
<i>T. aestivum</i> L.	UP 2565	PBW 352 / CPAN 4020	AABBDD	42
<i>T. aestivum</i> L.	HI 385 (MUKTA)	HYB 633 // GAZA // PR / PKD 25	AABBDD	42
<i>T. aestivum</i> L.	PBW 373	ND / VG 9144 // KAL / BB / 3 / YACO '5' / 4 / VEE # 5 'S'	AABBDD	42
<i>T. aestivum</i> L.	NIAW 34	CNO 79 / PRL "S"	AABBDD	42
<i>T. aestivum</i> L.	UP 2425	HD 2320/UP 2263	AABBDD	42
<i>T. aestivum</i> L.	NP 846	NP 760 / RN	AABBDD	42
<i>T. aestivum</i> L.	UP 2338	UP 368 / VL 421 / UP 262	AABBDD	42
<i>T. aestivum</i> L.	PBW 175	HD 2160 / WG 1205	AABBDD	42
<i>T. aestivum</i> L.	PBN 51	BUL 'S' / FLS 'S'	AABBDD	42
<i>T. aestivum</i> L.	UP 2554	SM4 – HSN 24E / CPAN 2099	AABBDD	42
<i>T. aestivum</i> L.	HD 2590	Not available	AABBDD	42
<i>T. aestivum</i> L.	VL 804	CPAN 3081/CPAN 3004/PBW 65	AABBDD	42
<i>T. aestivum</i> L.	WH 730	Not available	AABBDD	42
<i>T. aestivum</i> L.	JOB 666	K 65 / HD 2009	AABBDD	42
<i>Aegilops squarrosa</i>			DD	14
<i>Triticum monococcum</i>			AA	14
<i>T. tauschii</i>			DD	14
<i>T. dicoccum</i>			AABB	28
<i>T. durum</i>	PDW 289		AABB	28
	PDW 291		AABB	28
<i>T. turgidum</i>			AABB	28
<i>T. polonicum</i>			AABB	28
<i>T. sphaerococcum</i>			AABBDD	42
<i>T. timopheevii</i>			AAGG	28
<i>Secale cereale</i>	EC 481695		RR	14
<i>Secale cereale</i>	EC 481697		RR	

Table.2 Characteristics of ISSR primers

Sl. No.	Operon Code	Sequence	GC content (%)
1.	UBC-808	AGAGAGAGAGAGAGAGC	52.9
2.	UBC-841	GAGAGAGAGAGAGAGACC	55.5
3.	UBC-856	ACACACACACACACACCA	50.0
4.	UBC-873	GACAGACAGACAGACA	50.0

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)	0.5 U	0.16 µl
Reaction buffer (10 X)	1 X	2.5 µl
Primer (50 ng/µl)	50 ng	1.0 µl
dd H ₂ O		14.44 µl
MgCl ₂ (25 mM)	3.0 mM	3.0 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	1.5 min	–	–	–	–
44 cycles	94° C	40 sec	44° C	45 sec	72° C	21.5 min
Last cycle	–	–	–	–	72° C	5 min

Table.5 Summary of primers amplification

S.No.	Primer code	Total number of ISSR loci (TB)	Number of polymorphic bands	Range of base pairs amplified	Number of unique bands	PB/TB x 100
1.	UBC- 808	18	18	300- 1300	3	100
2.	UBC- 856	18	18	500- 1600	6	100
3.	UBC- 841	19	19	490- 2000	5	100
4.	UBC- 873	18	18	500- 1800	3	100

Table.6 Unique bands amplification

S. No.	Primer	Number of unique bands	Genotype	Size of unique band
1.	UBC-808	3	PBN 51	1100 bp
			UP2565	700 bp
			<i>T. monococcum</i>	500 bp
2.	UBC-856	6	PBN 51	1600 bp and 1300 bp
			UP2338	1250 bp, 950 bp and 800 bp
			UP 2425	750 bp
3.	UBC-841	5	<i>Ae. squarrosa</i>	2000 bp and 1700 bp
			<i>Secale cereale</i> EC 481695	1200 bp
			HI 385	650 bp and 640 bp
4.	UBC-873	3	<i>T. dicoccum</i>	1800 bp
			PBW 373	1700 bp
			NP 846	750 bp

Fig.1 Dendrogram of 27 wheat genotypes with similarity coefficient on horizontal axis and genotypes (1 to 27) on vertical axis

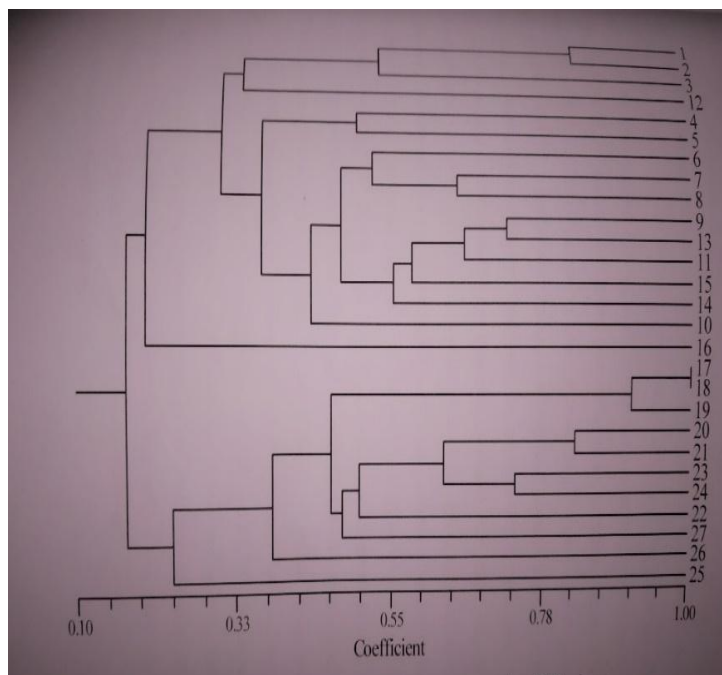
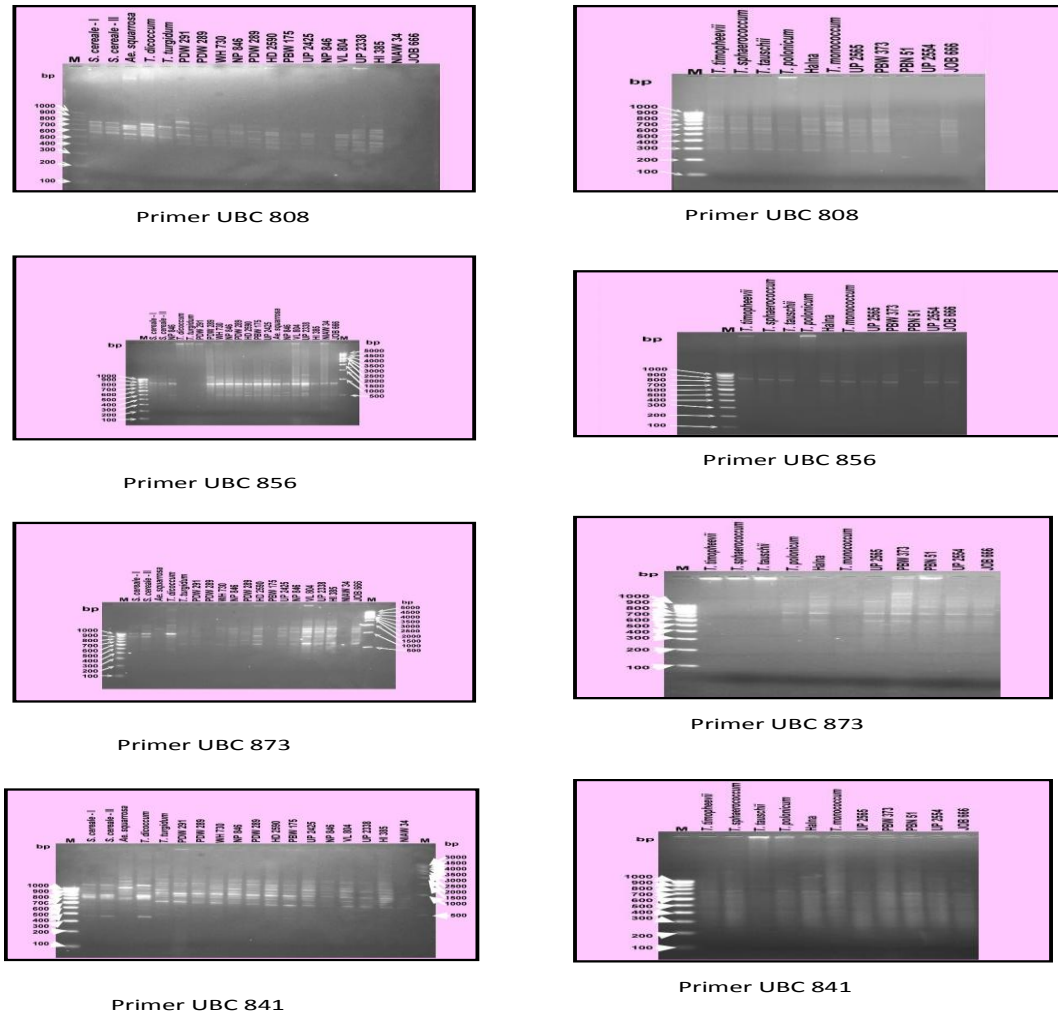


Fig.2 ISSR amplification pattern generated on 27 wheat genotypes



The genetic similarity value 0.069 was observed among three pairs i.e. PBN 51 and *T. turgidum*, PBN 51 and PDW 291, Job 666 and NIAW 34, where Job 666 was released from Jobneir, Rajasthan and NIAW 34 from Wellington. So we conclude that the above pairs of genotypes have got negligible similarity between them.

As shown in the ISSR dendrogram (Fig. 2), *T. timopheevii* and *T. sphaerococcum* had the maximum similarity value (1.00). Both of them had 0.909 similarities with *T. tauschii*. *S. cereale* EC 481695 and *S. cereale* EC 481697 had 0.828 similarity value. Halna and *T. polonicum* had 0.824 similarity value on

dendrogram. VL 804 and HD 2590 had 0.719 similarity coefficient on dendrogram. WH 730 and NP 846 had 0.647 similarity coefficient between them. *T. turgidum* and PDW 291 had formed one group and had 0.500 similarity coefficient between them.

Thus the study shows that the ISSR primers were well suited for genetic diversity studies. However the study for linkage of markers with genes would require quite a big number of primers to be used and the genotypes differing in one or a very few characters. This type of study has also been done by Sreedhar *et al.*, (2007), Verma *et al.*, (2009), Abdel *et al.*, (2014), Linda *et al.*, (2014,) Moradkhani

et al., (2015), Seyedeh *et al.*, (2015), Todorovska *et al.*, (2015), Ashraf *et al.*, (2016), Villanueva *et al.*, (2017).

List of genotypes of wheat and its wild relatives as represented in ISSR dendrogram

1. *Secale cereale* EC 481697, 2. *Secale cereale* EC 481695, 3. *Triticum dicoccum*, 4. *Triticum turgidum*, 5. PDW 291, 6. PDW 289, 7. WH 730, 8. NP846, 9. HD 2590, 10. PBW 175, 11. UP 2425, 12. *Aegilops squarrosa*, 13. VL 804, 14. UP 2338, 15. HI 385, 16. NIAW 34, 17. *Triticum timopheevii*, 18. *Triticum sphaerococcum*, 19. *Triticum tauschii*, 20. *Triticum polonicum*, 21. Halna, 22. *Triticum monococcum*, 23. UP 2565, 24. PBW 373, 25. PBN 51, 26. UP 2554, 27. Job 666.

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