Research in Biotechnology 2017, 8: 29-36
doi: 10.25081/rib.2017.v8.3596
http://updatepublishing.com/journal/index.php/rib

ISSN: 2229-791X

REGULAR ARTICLE

MOLECULAR CHARACTERIZATION OF WHEAT GENOTYPES (*TRITICUM AESTIVUM* L.)

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ABSTRACT

Molecular diversity in thirty wheat genotypes was done. For this the genomic DNA isolation was carried out and which were then subjected to PCR amplification using twenty SSR primers. Out of these twenty SSR primers, eighteen yielded amplifications and showed polymorphism. Total 93 loci were generated by amplification with 18 polymorphic primers, all of which 93 loci were polymorphic i.e. 100%. Among the SSR primers, BARC-170, WMC-44, produced maximum number of 2 loci. The size of amplification products ranged from 102 bp to 805 bp. All SSR primers showed 100 % polymorphism and all primers had more than 0.50 PIC value except one primer. Maximum PIC value 0.17 was observed in WMC-468. The maximum number of bands were observed in NIAW-2721 (28 bands), whereas minimum number of bands were present in NIAW-301 and NIAW-2539 (19 bands). The dice similarity coefficient values ranged from 0.50 to 0.95. Maximum similarity value of 0.95 was noticed between NIAW-2891 and NIAW-2837, while minimum similarity value of 0.50 was observed among NIAW-2595, NIAW-2874, NIAW-2995 and NIAW-2725. The consensus tree software revealed two major clusters.

Keywords: Molecular characterization, Wheat, SSR markers

INTRODUCTION

Wheat is the foremost and strategic cereal crop of the world and is the most important and major staple food of more than thirty six percent of world`s population. Globally, it is the most crucial oldest and edible grain cereals. In 2015-16 world production of wheat was estimated to be 727.2 million tons [3]. In India wheat is grown in an area of 30.96 million hectors during 2014-15 [3].

Wheat is highly nutritious and provides almost all the nutrients needed for human. wheat endosperm proteins, is the gluten protein and the vital role of gluten proteins in bread making quality and backing quality in wheat [7].

Molecular markers are relatively new technology in breeding and is widely used by breeders to select variations in genotypes. The main marker technologies RFLP, RAPD, AFLP, SSR, ISSR etc. are becoming important for cultivar identification and diversity analysis [9, 14].

Traditional method for estimating genetic diversity and relationship among groups of plants were using morphological characters, agronomic information and biochemical variations. Agronomical data of wheat is essential in applied wheat breeding but, individual genotypes of wheat are well adopted to certain regions. Besides, collection of such data is laborious. Due to these region, DNA/molecular markers are attractive alternative [12]. The molecular marker reveals genetic diversity at molecular level [5]. DNA marker are unlimited in numbers, selectivity neutral and can be organized in linkage maps [14].

Along with agronomical values the genetic diversity should be considered. New sources of variation give new and improved cultivar. The parents with high diversity will give highly variable progenies. Mahjourimajd et al. [15] showed the genetic basis responsible for grain characteristics and how it influenced with cultural practices like fertilizer application.

The knowledge of diversity also helps to develop strategies to incorporate useful diversity in breeding programs. Characterization of crop by markers reveals similarities (i.e. shared alleles) and diversity (i.e. typical alleles) among cultivars of a crop. This also helps in identifying gene pool or origin of cultivar. For development of elite cultivar, the genetic base has to be enlarged. The species whose gene pool is identified through markers are used for developing elite cultivars by exchange in germplasm. The use of markers characterizes and develops a DNA profile. These DNA profile of crops are used in management of genetic

Received 02 November 2017; Accepted 31 December 2017

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resources of a crop in gene bank. DNA profile help in selection of distinct parents for obtaining higher genetic variation. The present investigation was conducted to Characterize thirty three wheat genotypes (*Triticum aestivum* L.) by molecular methods.

MATERIALS AND METHODS

The study was carried out at State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri (Maharashtra) during year 2016-17.

The plant material for the study comprised of thirty three wheat genotypes, which were collected from Agriculture Research Station Niphad, District Nashik, Maharashtra. The seeds thus obtained were sown in plots inside poly house for genomic DNA isolation. Details of these wheat genotypes along with their pedigree are given in table 1.

Isolation of genomic DNA from leaves

Genomic DNA was isolated from 33 wheat genotypes following CTAB (Cetyl Tri methyl Ammonium Bromide) extraction method with some modifications as described by [4]. 2 µl of all DNA extracts were electrophoresed in 0.8 % (w/v) on agarose gel containing 0.5 µg/ml ethidium bromide. After electrophoresis the band intensity of genomic DNA was visualized on gel documentation unit (Flour Chem. TM Alpha InfoTech, USA) and compared to that of standard Lambda phage DNA. These gels also provided a visual measure of purity of DNA.

Optimization of DNA concentration for PCR

To carry out PCR, required template DNA concentration of 20ng/µl is needed. Optimization was done to base on the intensity of electrophoresed DNA. It was done to bring all DNA concentrations to a relatively equal level (20ng/µl) by appropriate dilutions. Accordingly, dilutions were made with calculated amount with TE Buffer.

Primers used and optimization of annealing temperature of gene specific primers for PCR amplification

Eighteen SSR primers were used for PCR amplification. The details of primers and their sequences are given in table 2. Gradient PCR amplification for different gene specific primers was carried out to determine the annealing temperature of each primer. The PCR programme was set in thermal cycle. (Eppendorf tube, Master Cycler Gradient, Germany).

Table 1: List of wheat genotypes used for analysis

S. No.	Name of genotypes	Pedigree
1.	NIAW-2778	NIAW 1188/NIAW 1161
2.	NIAW-2976	NIAW-1121/NIAW 2844
3.	NIAW-2822	PBW 343/LOK 54
4.	NIAW-2823	NIAW 1161/NIAW 1188
5.	NIAW-2930	POTCH93/4/MILAN/KAUZ//PRINIA/3/BAV92/5/MILAN/KAUZ//PRINIA/3/BAV92
6.	NIAW-2757	NIAW 917/MP 3097
7.	NIAW-2844	NIAW 34/lOK 54
8.	NIAW-2874	PHS 623/NIAW 1121
9.	NIAW-2892	WR 1392/NIAW 1161
10.	NIAW-2959	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/S/FRET2/KUKUNA//....
11.	NIAW-2721	NIAW 301/HD 2781
12.	NIAW-2725	NIAW 301/FLW 6
13.	NIAW-2792	NIAW 1188/NIAW 1161
14.	NIAW-2809	PBW 343/WH147
15.	NIAW-2837	LOK 45/RAJ 4083
16.	NIAW-2891	LOK 54/VW 0514
17.	NI-5439	RFPM 80/NP7103
18.	NIAW-1415	GW 9506/PRL//PRL
19.	NIAW-1885	ALTAR84/AEGILOPS QUARROSSA (TAUS)//OPATA/3/.
20.	NIAW-2565	MILAN/MUNIA/3/PASTOR//MUNIA/ALTAR8414/MILAN/DUCULA
21.	NIAW-1994	NIAW 34/PBW 435
22.	NIAW-34	CNO79/PRL'S'
23.	MACS-6222	HD 2189*2//MACS 2496
24.	HD-2189	HD1963/HD1931
25.	HD-2932	KAUZ/STAR/HD2643
26.	NIAW-2495	NIAW 1161/NIAW 1188
27.	NIAW-2539	PRU/2*PASTOR/ISUNSTATE
28.	NIAW-2547	BAV92//IRENA/KAUZ/3/HUITES/4/DOLL
29.	NIAW-2613	PFAU/SERI.1B//AMAD/3/INQALAB91*2/KUKUNA/4/WBLL1*2/KURUKU
30.	NIAW-2030	Lok 45 X NIAW 34
31.	NIAW-2595	PRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/BOW/URES//2*WEAVER/3/CROC-1/AE. SQUARROSA (213)//PGO
32.	NIAW-917	GW 244/Bob White
33.	NIAW-301	SERI 82/3/MRS/JUP/HORK 'S'

Table 2: Sequenced and fixed optimum annealing temperature for SSR primers

S. No.	Primer	Sequenced of primers (5`-3`)	Ch. No.	Ta
1. .	Barc-8	F-GCG GGA ATC ATG CAT AGG AAA ACA GAA R-GCG GGG GGC GAA ACA TAC ACA TAA AAA CA	1BS	50 °C
2.	Barc-62	F-TTG CCT GAG ACA TAC ATA CAC CTAA R-GCC AGA ACA GAA TGA GTG CT	1DL	58 °C
3.	Barc-71	F-GCG CTT GTT CCT CAC CTG CTC ATA R-GCG TAT ATT CTC TCG TCT TCT TGT TGG TT	3DL	63 °C
4.	Barc-137	F-GGC CCA TTT CCC ACT TTC CA R-CCA GCC CCT CTA CAC ATT TT		57 °C
5.	Barc-170	F-CGC TTG ACT TTG AAT GGC TGA ACA R-CGC CCA CTT TTT ACC TAA TCC TTT TGA A		62 °C
6.	Gmw-60	F-TGT CCT ACA CGG ACC ACG T R-GCA TTG ACA GAT GCA CAC G	7AS	59 °C
7.	Gmw-268	F-AGG GGA TAT GTT GTC ACT CCA R-TTA TGT GAT TGC GTA CGT ACC C		58 °C
8.	Gmw-610	F-CTGCCTTCTCCATGGTTTGT R-AATGGCCAAAGGTTATGAAGG	4AS	58 °C
9.	Wmc-24	F-GTGAGCAATTTTGATTATACTG R-TACCCTGATGCTGTAATATGTG	1A,	51 °C
10.	Wmc-44	F-GGT CTT CTG GGC TTT GAT CCT G R-TGT TGC TAG GGA CCC GTA GTG G	1BL	50 °C
11.	Wmc-89	F-ATG TCC ACG TGC TAG GGA GGT A R-TTG CCT CCC AAG ACG AAA TAA C	4A, 4B	61 °C
12.	Wmc-313	F-GCA GTC TAA TTA TCT GCT GGCG R-GGG TCC TTG TCT ACT CAT GTCT		57 °C
13.	Wmc-419	F-GTT TCG GATAAA ACC GGA GTG C R-ACT ACT TGT GGG TTA TCA CCA GCC		62 °C
14.	Wmc-468	F-AGC TGG GTT AAT AAC AGA GGA T R-CAC ATA ACT GTC CAC TCC TTT C		54 °C
15.	Wmc-497	F-CCC GTG GTT TTC TTT CCT TCT R-AAC GAC AGG GAT GAA AAG CAA		61 °C
16.	Wmc-525	F-GTT TGA CGT GTT TGC TGC TTA C R-CTA CGG ATA ATG ATT GCT GGC T		58 °C
17.	Wmc-707	F-GCT AGC TGA CAC TTT TCC TTT G R-TCA GTT TCC CAC TCA CTT CTT T		56 °C
18.	Psp-3151	F-GGC TGG TGT AGT AGG TTG CGA R-CGC AAC ACC ATA TCA TGT GTC AA		61 °C

Data analysis

The clearly resolved PCR amplified bands of wheat genotypes with different SSR and ISSR primers were scored manually as binary matrix for their presence (1) and absence (0) in the data sheet. The polymorphism information content (PIC) value was calculated as

$$PIC = 1 - \sum_{i=1}^{n} P_i^2$$

Where, n is the number of band positions analysed in the set of accessions and Pi is the frequency of it pattern.

Cluster analysis

The binary data were analysed under the SIMQUAL module of NTSYSpc 2.0i software [10], by using Dice similarity coefficient [8]. SAHN module based on UPGMA unpaired group mean algorithm based clustering method [13] was used to generate a tree (dendrogram).

RESULTS AND DISCUSSION

Aim of this investigation was to study the diversity in the available wheat genotypes. For this purpose, molecular marker was used effectively in the assessment of genetic diversity in wheat.

The genomic DNA was isolated from 33 genotypes and was subjected to PCR amplification using 20 primers. Annealing temperature of each of the primer was optimized by gradient PCR. It was observed that out of these 20 SSR primer pairs, 18 amplified and 18 showed polymorphism.

From the SSR analysis it was observed that a total of 93 bands were generated by amplification with 18 polymorphic primers. 93 of them were polymorphic, from which 16 were unique. Average polymorphism was 100%. Each SSR primer pair thus produced on an average 5.16 polymorphic bands.

Maximum scorable bands were observed using the primer BARC-170 (11 loci), followed by WMC-44 (9 bands). The least number of bands were reported in the primer WMC-468 (2 each), followed by BARC-62, GMW-610 and WMC-313(3 bands). Eighteen primers showed 100 percent polymorphism. All the genotypes evaluated for the molecular diversity with 18 SSR primer showed variation in their banding pattern.

Table 3: Analysis of wheat genotypes with SSR primers

S. No.	SSR analysis	Observations
1.	Total number of primers pairs used	20
2.	Number of primers amplifying DNA	18
3.	Number of primers not amplifying DNA	02
4.	Total number of polymorphic primers	18
5.	Total numbers of bands amplified	93
6.	Total number of polymorphic bands	93
7.	Total number of monomorphic bands	0
8.	Total number of unique bands	16
9.	Percent polymorphism	100
10.	Average number of bands/primers	5.16
11.	Average polymorphic bands produced per primer	5.16
12.	Amplified products size	102-805bp

Table 4: Unique SSR fragments amplified in wheat genotypes

S. No.	Wheat genotypes	Primer revealing unique SSR (Size of base pairs of amplified fragment)
1.	HD-2932	Barc-8 (240bp)
2.	NIAW-34	Barc-71(158bp)
3.	NIAW-2721,NIAW-2613, NIAW2547, NI5439, HD-2189	Barc170 (405bp, 325bp, 310bp, 272bp, 200bp)
4.	NIAW-2874, NI-5439, NIAW-2613	GMW-60 (158bp, 175bp, 145bp)
5.	NIAW-2030, NIAW-2976	Wmc-44 (183 bp,154bp)
6.	NIAW-2792, NIAW-301	Wmc-419 (160bp, 141bp)
7.	NIAW-917	Wmc-497 (245bp)
8.	NIAW-301	Wmc-707 (178bp)

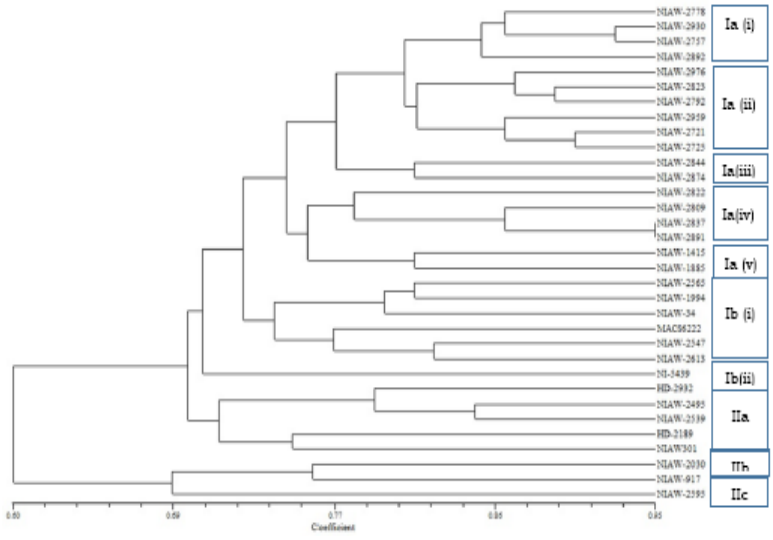


Fig. 1: Consensus tree showing clustering of thirty three wheat genotypes using SSR markers

Thirty-three varieties were used for amplification with 18 SSR primers. From eighteen, eighteen primers showed polymorphic. These 18 primers yielded maximum 11 bands and minimum 2 bands with PIC value ranged from 0.17 to 0.806. A total 16 unique bands were observed in 16 genotypes which could be useful in DNA fingerprinting, molecular characterization and varietal identification purpose.

In the present investigation out of 20 primers used 18 primers were able to amplify the genomic DNA of wheat and 2 primers were not able to amplify the genomic DNA. The total of 95 bands was resolved by 18 SSR primers out of which 95 bands were polymorphic. The PIC values of primers ranged from 0.17 to 0.806. Further, it was observed that there was no correlation between per cent polymorphism and PIC values as SSR primers Wmc-468 showed minimum PIC values but was 100 per cent polymorphic. There are previous reports assessment of molecular characterization between 12 wheat genotypes by using 4 SSR markers [17].

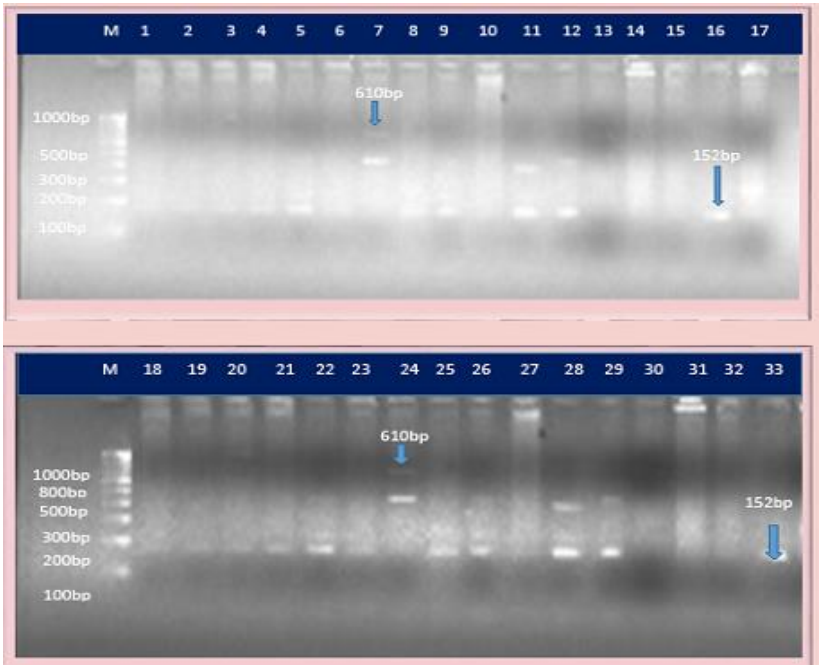
Wheat genotypes were grouped into two major clusters (I and II). First major cluster I consisted of four sub clusters (Ia, Ib, Ic and Id). First sub cluster (Ia) divided into six sub subcluster Ia (i), Ia (ii), Ia (iii), Ia (iv), Ia (v) and Ia (vi). First sub subcluster Ia (i) comprised four genotypes *viz.*, NIAW-2778, NIAW-2930, NIAW-2757, and NIAW-2892. Second sub subcluster Ia (ii) comprised six genotypes *viz.*, NIAW-2976, NIAW-2823, NIAW-2792, NIAW-2959, NIAW-2721 and NIAW-2725. Third sub subcluster Ia (iii) consists two genotypes *viz.*, NIAW-2844 and NIAW-2874. Fourth sub subcluster Ia (iv) consists only one genotypes *viz.*, NIAW-2822. Fifth sub subcluster Ia (v) consists three genotypes *viz.*, NIAW-2809, NIAW-2837 and NIAW-28. Six sub subcluster Ia (vi) consists two genotypes *viz.*, NIAW-1415 and NIAW-1885.

Second sub cluster (Ib) divided into two sub subcluster (Ib (i) and Ib(ii). First sub subcluster Ib (i) comprised three genotypes *viz.*, NIAW-2565, NIAW-1994 and NIAW-34. Second 72 sub subcluster Ib (ii) comprised three genotypes *viz.*, *MACS*-6222, NIAW-2547and NIAW-2613. Third sub cluster Ic formed independent sub cluster *viz.*, NI-5439. Forth sub cluster Id further divided into two sub subclusters (Id (i) and Id (ii). The first sub subcluster of Id (i) comprised of three genotypes *viz.*, HD-2932, NIAW-2445 and NIAW-2539. The second sub subcluster of Id (ii) comprised of two genotypes *viz.*, and HD-2189 and NIAW-301. All the wheat genotypes obtained from ARS Niphad were tightly grouped from others except, HD-2189, HD-2932 and MACS-6222.

Second major cluster II consisted of two sub clusters (IIa and IIb). First sub cluster (II a) comprised two genotypes *viz.*, NIAW-2030 and NIAW-917. Second sub cluster (IIb) consists of independent sub cluster and consists only one genotypes *viz.*, NIAW-2595 and among these three wheat genotypes from ARS Niphad.

Recent development in the field of DNA technology has resulted in the development of several molecular markers, which are linked to many traits that are used in characterizing true species and genera. Taxonomically, wheat has one of the largest gene pool among the cereal crops and is notable for its diversity. Modern day wheat (*Triticum aestivum* L.) is a hexaploid T composed of AABBDD genome. The genome donors are *T. urartu* (A), the Sitopsis section of *Aegilops* (B), and *Aegilops tauschii* (D) [2].

Plate 1: PCR amplification obtained using primer BARC-170 Lane M= Marker 100 bp



Lane No.	Genotypes	Lane No.	Genotypes	Lane No.	Genotypes
1.	NIAW-2778	12.	NIAW-2725	23.	MACS-6222
2.	NIAW-2976	13.	NIAW-2792	24.	HD-2189
3.	NIAW-2822	14.	NIAW-2809	25.	HD-2992
4.	NIAW-2823	15.	NIAW-2837	26.	NIAW-2495
5.	NIAW-2930	16.	NIAW-2891	27.	NIAW-2539
6.	NIAW-2757	17.	NI-5439	28.	NIAW-2547
7.	NIAW-2844	18.	NIAW-1415	29.	NIAW-2613
8.	NIAW-2874	19.	NIAW-1885	30.	NIAW-2030
9.	NIAW-2892	20.	NIAW-2565	30.	NIAW-2595
10.	NIAW-2959	21.	NIAW-1994	32.	NIAW-917
11.	NIAW-2721	22.	NIAW-34	33.	NIAW-301

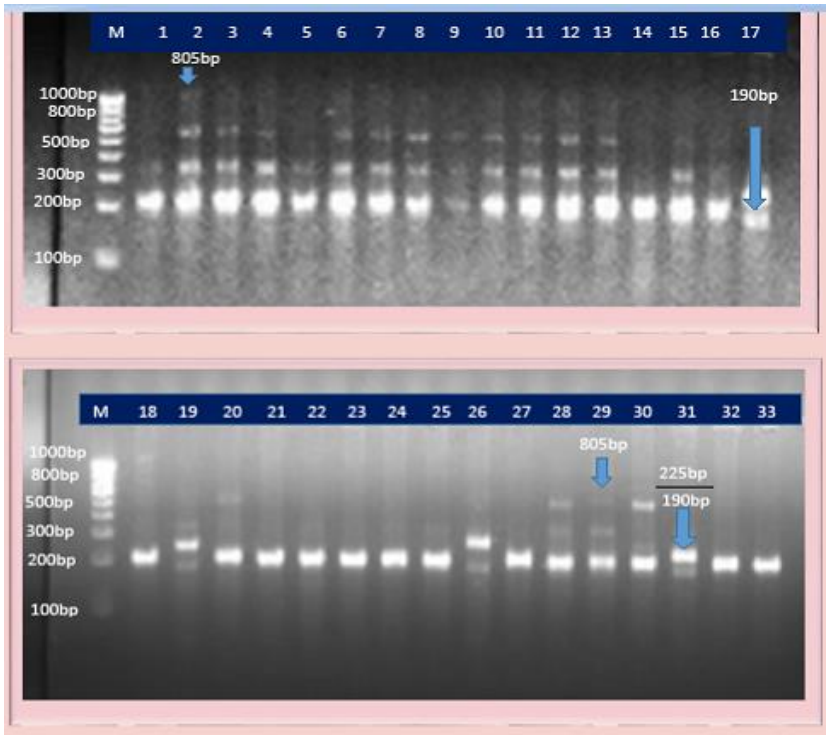
Christiansen *et al.* [2] Assessed genetic diversity in wheat using 47 microsatellite primer pairs were assessed in 75 Nordic spring wheat cultivars bred during the 20th century. Huang *et al.,* [6], assessed genetic diversity using 24 wheat microsatellite markers for 998 accessions of hexaploid bread wheat.

Zala *et al.,* [16] assessed molecular diversity in wheat using18 RAPD and eight SSR markers amplified 5554 fragments with 66.83 % polymorphism and 343 fragments with 90.32% polymorphism respectively. A Jaccard's similarity coefficient of all genotypes derived from RAPD data ranged from 0.65 to 0.90 and that of SSR ranged from 0.45 to 0.94. The PIC value for RAPD and SSR markers ranged from 0.91 to 0.96 and 0.57 to 0.76 respectively, with marker indexes of 11.35 and 5.35, demonstrating its utility in genetic diversity analysis.

Singh *et al.*, [11] screened thirty advance lines of hexaploid wheat (*Triticum aestvium* L.) cultivars by applying microsatellite markers. Seven primers out of thirty five SSR primers showed polymorphism in banding pattern. A total of 15 alleles were detected. The number of alleles per locus ranged from 1-3 with an average of 2.14 alleles per locus. The overall size of PCR products amplified ranged from 100-300 bp. indicating wide genetic diversity and it may be used in wheat hybridization programme for improving grain yield.

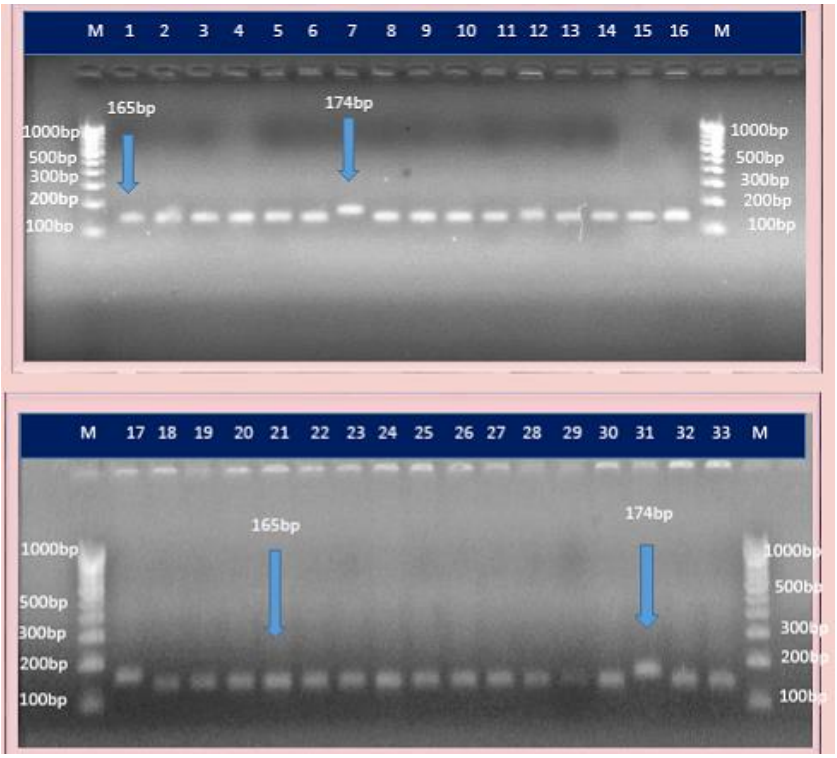
Results from the present study support the potential utility of molecular markers in characterization of the wheat genotypes. The occurrence of unique alleles or rare SSR alleles provides an immense opportunity for characterization and identification of specific wheat genotypes.

Plate 2: PCR amplification obtained using primer WMC-44 Lane M= Marker 100 bp



Lane No.	Genotypes	Lane No.	Genotypes	Lane No.	Genotypes
1.	NIAW-2778	12.	NIAW-2725	23.	MACS-6222
2.	NIAW-2976	13.	NIAW-2792	24.	HD-2189
3.	NIAW-2822	14.	NIAW-2809	25.	HD-2992
4.	NIAW-2823	15.	NIAW-2837	26.	NIAW-2495
5.	NIAW-2930	16.	NIAW-2891	27.	NIAW-2539
6.	NIAW-2757	17.	NI-5439	28.	NIAW-2547
7.	NIAW-2844	18.	NIAW-1415	29.	NIAW-2613
8.	NIAW-2874	19.	NIAW-1885	30.	NIAW-2030
9.	NIAW-2892	20.	NIAW-2565	30.	NIAW-2595
10.	NIAW-2959	21.	NIAW-1994	32.	NIAW-917
11.	NIAW-2721	22.	NIAW-34	33.	NIAW-301

Plate 3: PCR amplification obtained using primer WMC-468 Lane M= Marker 100 bp



Lane No.	Genotypes	Lane No.	Genotypes	Lane No.	Genotypes
1.	NIAW-2778	12.	NIAW-2725	23.	MACS-6222
2.	NIAW-2976	13.	NIAW-2792	24.	HD-2189
3.	NIAW-2822	14.	NIAW-2809	25.	HD-2992
4.	NIAW-2823	15.	NIAW-2837	26.	NIAW-2495
5.	NIAW-2930	16.	NIAW-2891	27.	NIAW-2539
6.	NIAW-2757	17.	NI-5439	28.	NIAW-2547
7.	NIAW-2844	18.	NIAW-1415	29.	NIAW-2613
8.	NIAW-2874	19.	NIAW-1885	30.	NIAW-2030
9.	NIAW-2892	20.	NIAW-2565	30.	NIAW-2595
10.	NIAW-2959	21.	NIAW-1994	32.	NIAW-917
11.	NIAW-2721	22.	NIAW-34	33.	NIAW-301

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