International Journal of Science, Environment
and Technology, Vol. 5, No 4, 2016, 2459 – 2466

ISSN 2278-3687 (O)
2277-663X (P)

MOLECULAR CHARACTERIZATION OF A FEW WHEAT GENOTYPES USING SIMPLE SEQUENCE REPEAT MARKERS

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Abstract: Genetic characterization of plant genotypes at molecular level is considered very much important as it is free from environmental factors. Recent advancement in the field of molecular markers has made the genetic characterization of genotypes rapid, reliable and reproducible. In this study, we have characterized 10 wheat genotypes at molecular level using 12 simple sequence repeat (SSR) markers. Individual distinctness of the genotypes has become evident from the dendrogram prepared on the basis of allelic diversity revealed by the molecular markers. Among the 12 SSR markers used, 2 have been observed to be monomorphic, whereas the rest of the markers revealed polymorphic information content values ranging from 0.17 to 0.50. Hence, we advocate the use of these SSR markers for characterization of wheat genotypes at molecular level, in general.
Keywords: Dendrogram; Genetic characterization; Molecular marker; Polymerase chain reaction; Simple Sequence Repeat.

Introduction

Common hexaploid wheat (*Triticum aestivum* L.) is a common grass belonging to *Poaceae* family, the produce of which is highly important for feeding the world population. Artificial selection during plant breeding has been rendered as a major reason behind the loss of a huge amount of variability in different cultivated crops, including wheat (Allard, 1996). Naturally, the genetic improvement of a crop like wheat requires collection of diversified genotypes and their proper documentation through characterization. As phenotypes are influenced by environment, characterization of genotypes on the basis of genotypes in considered most useful. The recent advancement in the field of molecular markers has made a significant contribution in this aspect. Owing to their independence from environmental factors, different DNA-based molecular markers have been found to be useful in wheat breeding (Gupta et al., 1999). Among the different DNA-based molecular markers, simple sequence repeat (SSR) markers are generally preferred the most. Microsatellites/ SSRs are simple sequence repeats of only a few (mostly 1-6) base pairs with conserved flanking regions. These polymerase chain reaction (PCR)-based markers are highly reproducible and

*Received July 14, 2016 * Published Aug 2, 2016 * www.ijset.net*

co-dominant in nature. They are abundant in plant genome (Condit and Hubbel, 1991), which makes them a versatile marker system. Several SSRs have been identified and mapped in wheat genome (Song et al., 2005), and have been explored for studying the genetic diversity in wheat (Hammer et al., 2000; Börner et al., 2000; Huang et al., 2002).

In the present study, we have attempted to characterize 10 wheat genotypes using 12 SSR markers. Through analysis of the amplification profiles generated by these 12 SSR markers, all the 10 wheat genotypes could be distinguished from each other at molecular level. The polymorphic information content (PIC) values of the used SSR markers were calculated to evaluate their usefulness in studying genetic diversity in wheat. Hence, the present study paves the way for the adoption of molecular breeding programme, involving these 10 wheat genotypes.

Materials and Methods

Plant materials

In the present study, 10 wheat genotypes were used. Among them, 7 (i.e., PBW343, C306, HUW234, HD2733, DBW14, HD2967 and HD2985) are released varieties and 3 (i.e., BRW934, BRW3723 and BRW3708) are promising genotypes identified at Bihar Agricultural University, Sabour. All the seed materials of the aforementioned genotypes were obtained from Wheat Section, Bihar Agricultural University, Sabour.

Isolation of genomic DNA and polymerase chain reaction

Seeds of the aforementioned wheat genotypes were germinated in plug trays containing a mixture of soil and vermicompost (1:1, w/w). Genomic DNA was isolated from the young leaves of the germinated seedling by SDS-potassium acetate method, as described elsewhere (Hosaka, 2004), with minor modifications. The isolated genomic DNA was dissolved in 50 µl of sterile water and 2 µl of the same was used to set up a polymerase chain reaction (PCR).

PCR was carried out in total 12 µl volume, containing 2 µl genomic DNA, 1.2 µl of 10X PCR buffer (Xcelris), 0.1 mMdNTP mix (Xcelris), 0.4 µM of each forward and reverse primers and 1 U of *Taq* DNA polymerase (Xcelris). PCR amplification was carried out in an automated thermal cycler (Veriti 96 well thermal cycler, Applied Biosystems) using the thermal profile consisting of an initial denaturation at 94 ˚C for 4 min followed by 35 cycles of 30 s at 94 ˚C, 40 s at appropriate annealing temperature, 30 s at 72 ˚C, and ended with final extension at 72 ˚C for 10 min followed by hold at 4 ˚C for 2 min. Following amplification, the amplicons were separated through 2.5 % (w/v) agarose gel electrophoresis in presence of

ethidium bromide and visualized in a gel documentation system (Bangalore Genei). The sequences of the forward and reverse primers along with their respective annealing temperatures used in this study are mentioned in Table 1.

Diversity analysis

The amplification profiles were analysed, where the presence of an allele was indicated by 1 and absence of the same was indicated by 0. This 1/0 matrix was used to analyse similarity of the genotypes at molecular level on the basis of Jaccard's similarity co-efficient values using the Dendro UPGMA web-programme (http://genomes.urv.cat/UPGMA/). Dendrogram was prepared on the basis of the similarity values using Phylogeny.fr web-programme (http://phylogeny.lirmm.fr/phylo_cgi/index.cgi). Polymorphic information content (PIC) values for the markers were calculated, as described previously (Chattopadhyay et al., 2008), by the formula:

$$PIC = \frac{1}{n} \Sigma 2F\ (1 - F)$$

where, n = number of alleles generated by a marker, and, F = proportion of a particular allele among the genotypes.

Table 1: Details of the primers used in the present study

SSR Marker	Forward//Reverse primer sequence	Annealing temperature used
Xgwm 149-4B	CATTGTTTTCTGCCTCTAGCC// CTAGCATCGAACCTGAACAAG	55 °C
Xgwm 140-1B	ATGGAGATATTTGGCCTACAAC// CTTGACTTCAAGGCGTGACA	55 °C
Xgwm 293-5A	TACTGGTTCACATTGGTGCG// TCGCCATCACTCGTTCAAG	55 °C
Xgwm 160-4A	TTCAATTCAGTCTTGGCTTGG// CTGCAGGAAAAAAAGTACACCC	60 °C
Xgwm 60-7A	TGTCCTACACGGACCACGT// GCATTGACAGATGCACACG	60 °C
Xgwm 63-7A	TCGACCTGATCGCCCCTA// CGCCCTGGGTGATGAATAGT	60 °C
Xgwm 577-7B	ATGGCATAATTTGGTGAAATTG// TGTTTCAAGCCCAACTTCTATT	55 °C
Xgwm 349-2D	GGCTTCCAGAAAACAACAGG// ATCGGTGCGTACCATCCTAC	55 °C
Xgwm 190-5D	GTGCTTGCTGAGCTATGAGTC// GTGCCACGTGGTACCTTTG	60 °C
WMS 0935-2B	GTCCGCCACCTCCTCTG// GAGCACACCCTGTTGCG	60 °C
Xgwm 626-6B	GATCTAAAATGTTATTTTCTCTC// TGACTATCAGCTAAACGTGT	50 °C
WMS 0745-7B	TATGAATTCAGGTAATATTCA// GTCATGTGATGTTTTTTTCA	50 °C

Results and discussion

In the present study, we attempted to characterize 10 wheat genotypes at molecular level, using 12 simple sequence repeat (SSR) markers. Through these 12 SSR markers, a total of 31 alleles were generated in the 10 test genotypes. As evident from the amplification profiles of all these markers (Figure 1), 2 markers (i.e., Xgwm293-5A and Xgwm 190-5D) were found to be monomorphic among the test genotypes. The marker Xgwm 293-5A amplified 2 alleles of 141 bp and 201 bp size, which were found to be present in all the test genotypes (Figure 1.C). On the other hand, all the test genotypes were found to contain the allele of 211 bp size, amplified by the marker Xgwm 190-5D (Figure 1.I).

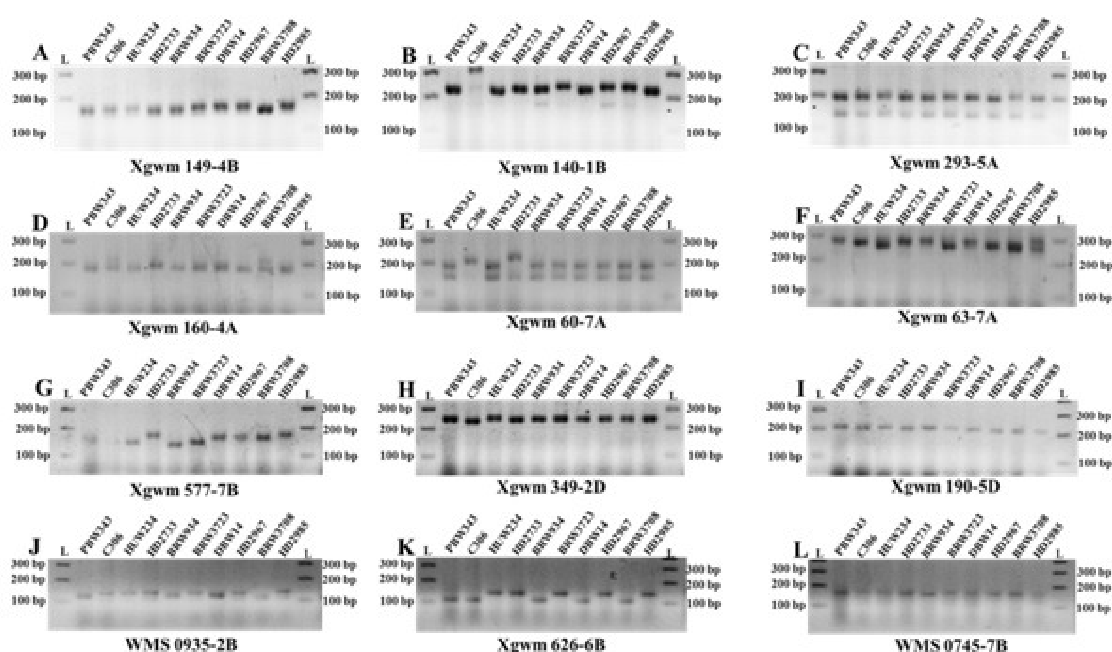

Figure 1: Inverted images of ethidium bromide stained 2.5% agarose gels showing allelic diversity of 10 wheat genotypes revealed by 12 SSR markers.

Amplification results along with polymorphic information content (PIC) values of all these SSR markers are summarised in Table 2.

Table 2: Summary of performance of the SSR markers used in the present study

Marker	No. of alleles generated	Size range	Difference	PIC
Xgwm 149-4B	2	161 bp - 173 bp	12 bp	0.48
Xgwm 140-1B	4	237 bp - 331 bp	94 bp	0.33
Xgwm 293-5A	2	141 bp - 201 bp	60 bp	0
Xgwm 160-4A	3	173 bp - 191 bp	18 bp	0.36
Xgwm 60-7A	4	151 bp - 226 bp	75 bp	**0.17**
Xgwm 63-7A	3	255 bp - 274 bp	19 bp	0.43
Xgwm 577-7B	4	133 bp - 171 bp	38 bp	0.35
Xgwm 349-2D	2	234 bp - 243 bp	9 bp	0.32
Xgwm 190-5D	1	211 bp	-	0
WMS 0935-2B	2	121 bp - 142 bp	21 bp	0.48
Xgwm 626-6B	2	105 bp - 137 bp	32 bp	**0.50**
WMS 0745-7B	2	156 bp - 165 bp	9 bp	0.18

The highest and lowest PIC values are indicated by bold, underlining

In this study, the lowest PIC value (0.17) was recorded in case of the marker Xgwm 60-7A, which generated 4 alleles, ranging from size 151 bp - 226 bp.On the other hand, highest PIC value (0.50) was recorded for the marker Xgwm 626-6B, which generated 2 easily distinguishable alleles of 105 bp and 137 bp size (Figure 1.K). Apart from these, the markers Xgwm 149-4B, Xgwm 160-4A, Xgwm 63-7A, Xgwm 577-7B and WMS 0935-2B were found to be useful in the present study, owing to their high PIC values (Table 2).

Dendrogram, generated on the basis of presence and absence of the different alleles generated by the 12 SSR markers, indicated that all the test genotypes could be distinguished from each other at molecular level. From the Jaccard's similarity co-efficient values, the genotypes HD2967 and HD2985 were found to be most closely related (Jaccard's similarity co-efficient value = 0.706, Table 3). On the other hand, the genotypes BRW934 and HD2985 were observed to be most distantly related (Jaccard's similarity co-efficient value = 0.261, Table 3). As per the dendrogram, the test genotypes were distributed in 2 major clusters (Figure 2), where the first cluster contained 6 genotypes (HD2985, HD2967, BRW 3723, HUW234, HD2733 and C306).

Table 3: Jaccard's similarity co-efficient values between the test genotypes on the basis of amplification profiles using 12 SSR markers

	PBW343	C306	HUW234	HD2733	BRW934	BRW3723	DBW14	HD2967	BRW3708	HD2985
PBW343	1	0.4	0.556	0.273	0.474	0.333	0.647	0.333	0.556	0.381
C306		1	0.474	0.474	0.474	0.474	0.474	0.333	0.4	0.318
HUW234			1	0.4	0.333	0.647	0.4	0.474	0.4	0.526
HD2733				1	0.4	0.4	0.333	0.474	0.4	0.45
BRW934					1	0.333	0.556	0.333	0.556	**0.261**
BRW3723						1	0.474	0.647	0.4	0.611
DBW14							1	0.474	0.556	0.45
HD2967								1	0.647	**0.706**
BRW3708									1	0.45
HD2985										1

The highest and lowest Jaccard's similarity co-efficient values are indicated by bold, underlining

The rest 4 genotypes (BRW934, BRW3708, DBW14 and PBW343) were found to constitute the second cluster.

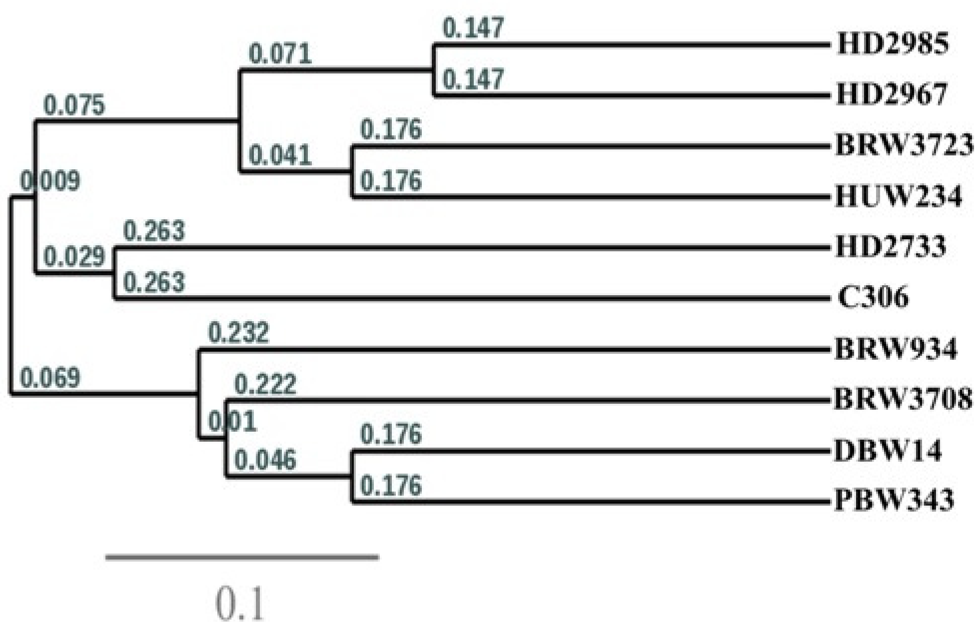

Figure 2: Dendrogram of 10 wheat genotypes developed on the basis of polymorphism revealed by the 12 SSR markers. Branch length values are also indicated.

Conclusion

Identification of distinctness of a genotype is very much important in plant breeding research. With the advent of robust molecular markers in wheat, like SSR, characterization of a genotype at molecular level has become easy, cost-effective and reliable. Different SSR markers have already been used in recent past for genetic characterization in wheat and related species (Ijaz and Khan, 2009; Islam et al., 2012; Sehgal et al., 2012). Here, we have characterized 10 wheat genotypes at molecular level, using 12 SSR markers. Out of these 12 markers, 2 were found to be monomorphic, whereas rest of the 10 markers have revealed polymorphism among the test genotypes. On the basis of the dendrogram, generated using the allelic diversity of the test genotypes revealed by these molecular markers, all the test genotypes have been found to be distinguished from each other.

Acknowledgements

The authors thank Mr. Deepak Baranwal for providing the primers for the present study. The authors also thank Dr. P.K. Singh, Chairman, Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur, Bihar for providing help and valuable suggestions throughout the study. Supports provided by Dr. A.K. Singh, Dr. Awadhesh Kumar Pal and Mr. Sudhir Kumar and are also acknowledged. This article bears the BAU COMMUNICATION NO: 124/2016.

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