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RESEARCH **P**APER

Assessing genetic diversity in bread wheat using inter simple sequence repeat (ISSR) markers

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A major effort of a plant breeder is the constant improvement of the best available genotypes for further enhancement in their yield potential either directly or through improvement of various factors which contribute indirectly to high yield. Genetic diversity of wheat cultivars is very important in reducing genetic vulnerability during plant breeding efforts. In order to estimate the genetic diversity, molecular markers provide excellent tools. The aim of this study was to molecularly characterize the fifty wheat accessions to assess phylogenetic relationship and mutual genetic distances through the use of 10 ISSR markers and 50 accessions of wheat. The dendrogram separated genotypes into two clusters I and II comprising of 49 and 1 accessions, respectively. The allelic polymorphism information content (PIC) value ranged from 0.147 to 0.467 with an average of 0.287. The similarity co-efficient ranged from .41 to .89. Significant correlation of microsatellite genetic distance was tested by mantel test (r= 0.77557). Results shows that high level of polymorphism among the wheat accessions. Cluster analysis suggested that ISSR markers were efficient tools for estimating intra-specific genetic diversity in wheat and this molecular marker could differentiate the accessions obtained from different locations. ISSRs have been successfully used to estimate the extent of genetic diversity at inter- and intra-specific level in a wide range of crops. The genetic relationships estimated by the polymorphism of ISSR markers revealed greater level of genetic variability in wheat accessions of wide adaptability and applicability.

Key words : Bread wheat, Genetic diversity, ISSR markers, Wheat accessions

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INTRODUCTION

Bread wheat (*Triticum aestivum* L. em. Thell), an allohexaploid (2n=6x=42), is the premier food crop of worldwide importance. It is also a crop where conventional plant breeding has paid rich dividend, as epitomized by the "green revolution". Genetic diversity provides means of specific identification of species and strains. It describes genetic distance between genotypes and polygenic relationships are well traced through it and thus, help in searching out genetic relationship among different genotypes. It will also be helpful in selection of better parent, therefore, effective and efficient utilization of germplasm is needed. Knowledge of genetic diversity in a crop species is fundamental to its improvement. Evaluation of genetic diversity levels among adapted, elite germplasm can provide predictive estimates of genetic variation

Applying genetic markers and recognition of polymorphic nucleotide sequences dispersed throughout the genome have provided new possibility for evaluating genetic diversity and determining of inter- and intra-species genetic relationships (Gostimsky *et al*., 2005). Genetic markers that are located in close proximity to genes (*i.e.* tightly linked) may be referred as gene 'tags'. Such markers themselves do not affect the phenotype of

the traits of interest because they are located only near or 'linked' to genes controlling the trait.

Several PCR based molecular markers are available for investigation of genetic diversity. SSR (Tautz, 1989), RAPD (Williams *et al*., 1990), AFLP (Vos *et al*., 1995) and ISSR (Zietkiewicz *et al*., 1994) were the most important of them. The major limitations of these methods were low reproducibility of RAPD markers, high cost of AFLP and need to know the flanking sequences to design specific primers for SSR markers. ISSR markers overcome most of these limitations (Reddy *et al*., 2002). Easy handling, reliability and high information level are the salient features of ISSR markers that justify the utility of these primers in DNA fingerprinting of wheat genetic analysis and germplasm management.

RESEARCH METHODOLOGY

A total of fifty accessions of wheat germplasm was taken and evaluated during *Rabi* 2009-10 and 2010-11 at N. E. Borlaug Crop Research Centre, Pantnagar. Diversity at molecular level was studied at Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture and Technology, Pantnagar using 10 ISSR markers.

DNA extraction :

Total genomic DNA was extracted from leaf tissue per each accession. Young leaves from fifteen day old plants were cut used as samples for DNA extraction. Cetyl trimethyl ammonium bromide (CTAB) method as described by Saghai-Maroof *et al*. (1984) and quantification was done in a Dynaquant™ 200 fluorimeter (Hoefer Instruments. USA)).

Data analysis :

The ISSR markers were scored for the presence (1) or absence (0) of amplified bands of microsatellites for each of 50 wheat accessions.

The amplification products were viewed under UV light and photographs were saved for the experimental evaluation. The amplification products were scored separately for each primer. The bands were scored for the presence or absence by binary coding *i.e.*, assigning a value of 1 for presence and 0 for absence in a lane (Hartigan, 1975). Molecular size (bp) of amplified DNA fragment was determined by the DNA ladder marker which was used in the wells of agarose gel.

DNA fragment analysis was performed using the NTSYS-PC (Numerical Taxonomy System, version 2.11 W) software (Rohlf, 1992). The SIMQUAL programme was used to calculate Jaccard's co-efficient, a common estimator of genetic identity and similarity matrices based on Jaccard co-efficient were calculated.

RESEARCH FINDINGS AND ANALYSIS

In the present study 10 ISSR primers yielded a total of 28 amplified fragments (100-1200 bp in size) ranging from 2 to 4 polymorphic fragments per primer. Table 1 comprised of 10 ISSR primers.

The number of alleles per locus ranged from two for UBC 845, UBC 848, and UBC 852 primers. Where as number of alleles per locus ranged from three for UBC 812, UBC 823, UBC 826, UBC 840, UBC 853 and UBC 855, whereas number of alleles per locus ranged from four was UBC 834 primer with an average of 3 alleles per locus. None of the primers yielded any unique band in 50 germplasm lines of wheat. The data scored from

Table 1: Characteristics of ISSR primers

Sr. No.	Operon code	Sequence 5'- 3'	GC content (%)
1.	UBC 812	GAGAGAGAGAGAGAGAA	47.05
2.	UBC 823	TCTCTCTCTCTCTCTCC	52.94
3.	UBC 826	ACACACACACACACACC	52.94
4.	UBC 834	AGAGAGAGAGAGAGAGYT	47.22
5.	UBC 840	GAGAGAGAGAGAGAGAYT	47.22
6.	UBC 845	CTCTCTCTCTCTCTCTRG	51.83
7.	UBC 848	CACACACACACACACARG	52.83
8.	UBC 852	TCTCTCTCTCTCTCTCRA	46.27
9.	UBC 853	TCTCTCTCTCTCTCTCRT	47.2
10.	UBC 855	ACACACACACACACACYT	47.2

Where, R= (A, G); Y=(C, T)

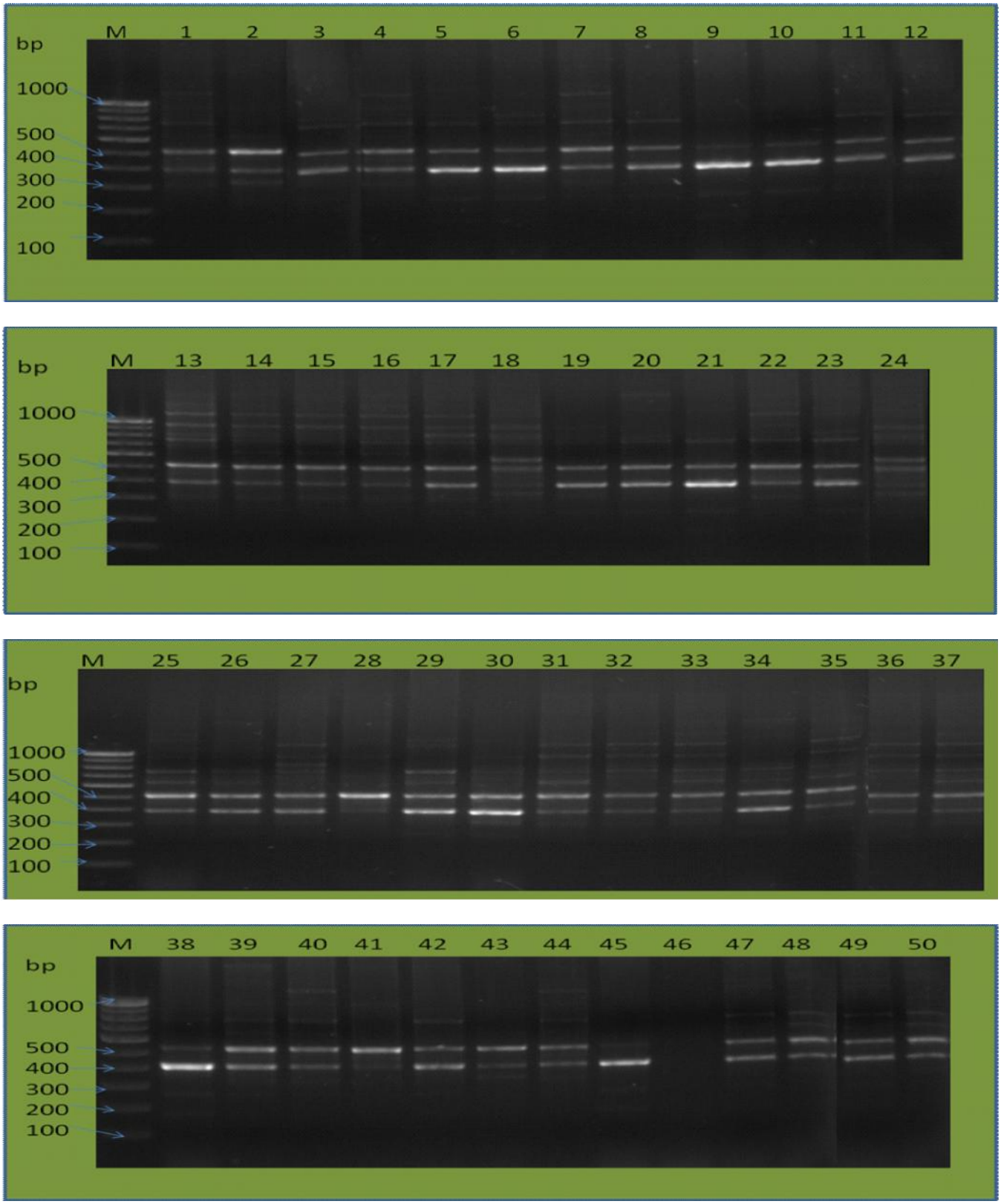

Fig.1: The inter simple sequence repeat (ISSR) profile of 50 wheat accessions of with Primer UBC 840. Lane M denotes DNA ladder and 1 to 50 digits denote 50 accessions of wheat

the ISSR analysis of wheat accession using ISSR primers were used to generate pairwise matrix based on Jaccard's similarity co-efficient. The similarity co-efficient ranged from 0.41 to 0.89 (*i.e.*,41 to 89% similarity). The allelic polymorphism information content (PIC) value ranged from 0.147 for the UBC 848 to 0.467 for the UBC 834 with an average of 0.287 for the UBC 826. Jaccard's similarity co-efficient ranged from 41to 89 per cent among the paired accessions. Significant correlation of microsatellite genetic distance tested by mantel test (r= 0.77557). Genetically diverse groups were formed based on the dendrogram. The difference in the genotypes may be attributed to different geographical locations of the place of release of accession and different ploidy levels. Percentage polymorphism ranged from 50 per cent (UBC 848) to a maximum of 100 per cent (UBC 845), with an average of 70 per cent polymorphism. Only two out of 10 primers showed more than 80 per cent polymorphism.

The values of similarity co-efficients varied from 29 per cent between accession IC 75315 and IC 78988, followed by 32 per cent between accession IC 78984 and IC 534808, followed by 34 per cent between accession IC 532477 and IC 78188 to a maximum of 88 per cent between accession IC 532239 and IC 66550 followed by followed by 85 per cent between accession IC 532239 and IC 104614, followed by 83 per cent between accession IC 532921 and IC 534802. The similarity co-effiecients between most of the accession lies between 45 to 65 per cent similarity co-effiecient that give indication that the accession are more or less similar to each other.

The primer sequences composed of GA repeats present the low level of polymorphism in plants (Akkaya *et al*. (1992). UBC 812 and UBC 840 belong to this group and other eight primers belong to different groups other than GA group. They have shown different levels of polymorphism.

Based on data achieved by ISSR-PCR, cluster analysis performed to generate dendrogram (Fig. 2). The dendrogram separated genotypes into two cluster I and II comprising of 49 and 1 accession, respectively. These major clusters showed 42 per cent similarity to each other. Cluster I comprised of two subgroups I1 and I2. I2 comprised of 1 accessions *viz.*, IC, 78988. Cluster I1 comprised of two subgroups 11A and I1B at the demarcation of approximately 47 per cent similarity. Subgroup I1A and I1B comprised of 47 and 1 accessions, respectively.

The cluster II comprised of only one accession, IC 78984 with similarity co-efficient of 42 per cent. The

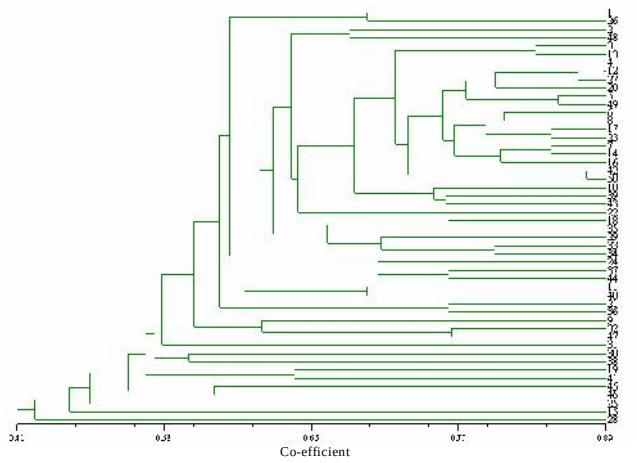

Fig. 2: Dendrogram depicting the classification of 50 wheat accession constructed through UPGMA method and based on ISSR marker. The scale at the bottom is Jaccard's co-efficient of genetic similarty

results show that ISSR markers are efficient tools in revealing the genetic diversity. ISSR targets a subset of SSRs and amplifies the region between two closely spaced and oppositely oriented SSRs. The ISSR molecular markers are semi-arbitrary. Single forward primers with 16-18 nucleotide length comprises repetitive units and anchors 2-4 arbitrary nucleotides at the 3' or 5' end. This method did not required the information about genomic sequences and, therefore, by means of these primers high level of polymorphism could be realized (Zietkiewicz *et al*., 1994). ISSR markers were successfully used for estimating of genetic diversity in main crops for instance maize (Kantety *et al*., 1995), wheat (Nagoaka and Ogihara, 1997), rice (Blair *et al*., 1999) and barley (Brantestam *et al*., 2004 and Hou *et al*., 2005).

Genetically diverse groups were formed based on the dendrogram. The difference in the genotypes may be attributed to different geographical locations of the place of release of varieties and different ploidy levels. The evolution leading to adaptation to different agro-ecological conditions may mutate the SSR sequences, which lead to differences in the ISSR amplification pattern. Similar types of studies in wheat were performed by Pasqualone *et al*. (2000); El-Maati *et al.* (2004) and Carvalho *et al*. (2005). ISSR studies in shisham were conducted by Arif *et al*. (2009).

Conclusion :

ISSR markers are highly polymorphic and repeatable and are being used in revealing the genetic diversity at intra-specific level. The clusters, groups and subgroups

formed in case of fifty wheat accessions, which are indigenous collections from different locations across the country suggest that ISSRs are potential molecular markers are studying the genetic diversity.

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