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Research Article

Evaluation of genetic diversity in drought tolerant and sensitive varieties of wheat using ISSR markers

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Abstract
Drought stress is one of the major constraints that greatly limit the productivity of wheat worldwide. Deciphering the molecular basis of drought tolerance can help in combating drought stress. Therefore, the present study was undertaken to determine genetic diversity among six drought tolerant and six drought sensitive varieties of wheat. Out of 15 ISSR primers used, 14 primers gave reproducible banding pattern. These 14 primers generated 191 amplified products out of which 93 were polymorphic. Overall size of PCR amplified products ranged between 400 bp and 4000 bp. Based on similarity matrix data, the value of similarity coefficient ranged from 0.70 to 0.94. Cluster analysis discriminated the wheat varieties into two major clusters, one consisted of drought tolerant varieties and the other had all drought sensitive varieties. The present study showed that ISSR markers provide a valuable tool to study genetic variability in wheat varieties.

Keywords
Wheat, Drought tolerant, ISSR markers, Genetic diversity, Cluster analysis

Introduction

Wheat is a major source of energy, protein and dietary fiber in human nutrition since decades. As the world water supply is declining, drought stress has reached at an alarming stage and is a major threat to world food security. Wheat is grown under rainfed conditions in many places around the world and this rainfed crop commonly suffers from drought resulting in considerable yield loss (Rana *et al*., 2013). Exposure to drought stress is further enhanced by climate changes due to global warming as wheat growing areas of the world are exposed to intense heat waves. Most of the times, drought and high temperature occur simultaneously causing significant yield loss (Lott *et al*., 2011; Hossain *et al*., 2012). The combined effect of drought and high temperature on the physiological growth and yield was significantly higher than the individual effects (Shah and Paulsen, 2003; Sharma and Kaur, 2009; Grigorova *et al*., 2011). The effect of drought stress is more pronounced in arid and semi-arid areas. Drought stress reduces crop growth rate and results in significant yield loss regardless of the growth stage at which it occurs, such as tillering, booting, earing, anthesis and grain development stages (Bilal *et al.*, 2015).

However, the range of yield reduction due to water stress during growth phase of wheat plant is highly depending on the variety. Hence, developing varieties that possess drought tolerance is one of the major priorities of wheat improvement programmes. Determination of genetic diversity for various crop plants is one of the important objectives in any plant breeding program as it helps in selecting diverse parental combinations and enhances selection efficiency. The assessment of crop germplasm based on phenotypic and biochemical markers like isoenzymes, frequently lack the resolving power needed to identify individual varieties and is influenced by environmental conditions. However, selection based on DNA markers overcomes the problems of conventional methods. Among the various DNA markers, ISSR markers have been successfully used in genetic diversity analysis and other molecular studies in wheat (Najaphy *et al.*, 2011; Katakpara *et al.*, 2016). Also, ISSR markers have the potential for identifying trait specific unique alleles (Goyal *et al.*, 2015a). The present study was conducted to detect the genetic diversity among 12 wheat varieties varying for drought tolerance using ISSR markers.

Materials and Methods

The present investigation was carried out on 12 Indian bread wheat (*Triticum aestivum* L.) varieties procured from Indian Institute of Wheat and Barley

Research (IIWBR), Karnal (Table 1). Out of these, six varieties were drought tolerant and six were drought sensitive. The seeds of these varieties were raised in small pots. Genomic DNA was isolated from leaves of 2-3 week-old plants using cetyl trimethyl ammonium bromide (CTAB) method as given by Murray and Thompson (1980) and modified by Saghai-Maroof *et al*. (1984). The quality and quantity DNA was checked using spectrophotometer. PCR amplification was carried out in mY-CYCLER (programmable thermal cycler from BIORAD™ INTERNATIONAL). PCR reaction was conducted in 20 µl of reaction mix containing 25 ng of template DNA, 200 µM of each dNTPs, 1X PCR buffer, 0.3 µM primers, 2.0 mM $MgCl_2$ and 1.0 U Taq DNA polymerase. PCR amplification was performed with initial denaturation of 94ºC for 4 min followed by 35 cycles of denaturation at 94ºC for 1 min, annealing at 36 to 65ºC (depending on primer) for 1 min and extension at 72ºC for 2 min. Final extension was carried out at 72ºC for 10 min followed by a 4ºC hold until recovery. Amplified DNA fragments were separated by 1.5% agarose gel electrophoresis and visualized by staining with ethidium bromide. PCR amplification products were viewed under UV light and photographed using gel documentation system.

ISSR amplification profiles were scored visually, based on presence (taken as 1) or absence (taken as 0) of bands for each wheat variety. The 0/1 matrix was used to calculate the similarity matrix using 'Simqual' subprogram of numerical taxonomy and multivariate analysis system program (NTSYS-pc) version 2.02 software (Rohlf, 1998). Dendrogram was constructed based on the similarity matrix using unweighted pair group method with arithmetic average (UPGMA) sub-programme of NTSYS-pc that showing the genetic relationships between varieties. Two dimensional principal component analysis (PCA) based clustering was done using EIGEN algorithm of NTSYS-pc software. Effective multiplex ratio (EMR) is the product of the fraction of polymorphic bands and the number of polymorphic bands for an individual marker (Powell *et al.,* 1996).

Results and Discussion

A total of 15 ISSR markers were used to study the genetic diversity of 12 wheat varieties varying for drought tolerance. ISSR primers used in this study were composed of di-, tri-, tetra- and penta-nucleotide repeat sequences. Out of 15 ISSR markers, 14 markers that gave scorable and reproducible banding pattern were used for genetic diversity analysis. The banding profile of 12 wheat varieties using ISSR 26 marker is shown in Fig.1. ISSR markers have been proved useful in genetic variability studies of wheat varieties because of their high reproducibility and great power for the detection of polymorphism (Goyal *et al.*, 2015a,b; Najaphy *et al*., 2011). A total of 191 alleles were amplified using 14 ISSR primers, out of which 93 were polymorphic. The number of polymorphic alleles per primer ranged from 1 (ISSR 13) to 11 (ISSR 12 and ISSR 23) with an average of 6.64 alleles per primer (Table 2). Different workers reported different efficiencies of ISSR markers for detecting DNA polymorphism. Najaphy *et al.* (2011) reported 6.9 polymorphic alleles per primer, which is in agreement with the present study. Katakpara *et al.* (2016) reported only 3.16 polymorphic alleles per primer while Etminan *et al.* (2016) reported 10.2 polymorphic alleles per primer. This variation in polymorphic alleles generated per primer depends on genetic relatedness of the varieties under study and also on the primers used.

The polymorphism percentage ranged from 10% to 71.4%, giving an average percentage polymorphism of 48.7%. Goyal *et al.* (2015a) and Katakpara *et al.* (2016) obtained similar results and average percentage polymorphism reported was 52.97% and 51.93%, respectively. High levels of polymorphism have been reported by Zhu *et al.* (2011), Sadigova *et al.* (2014) and Etminan *et al.* (2016), which may be due to selection of wide range of pedigrees or wild relatives of wheat varieties. In the present investigation, size of amplified DNA fragments varied from approx. 400 bp to 4000 bp (Table 2).

ISSR similarity indices of different varieties ranged from 0.70 to 0.94 with average similarity value of 0.85, indicating a high level of genetic similarity among the varieties studied. Maximum similarity value of 0.94 was observed between varieties MP3173 (DT3) and HW2004 (DT4). Wheat varieties, HI1500 (DT1) and GW273 (DS2) were found to be genetically diverse with similarity value of 0.70. These genetically diverse varieties can be used in future breeding programmes for development of drought tolerant wheat varieties. Dendrogram was constructed (Fig. 2) using similarity matrix values as determined from ISSR analysis using UPGMA (unweighted pair group method of arithmetic averages) subprogram of NTSYSpc version 2.02 programme. ISSR markers based analysis of dendrogram shows outgrouping of variety DT1 at similarity coefficient of 0.74. Rest of varieties was divided into two cluster-I and cluster-II at a similarity

coefficient of 0.85. Cluster I is having two drought sensitive varieties while cluster II is having rest of the varieties. At a similarity coefficient of 0.855, cluster II is further divided into two subclusters. Subcluster I is having all drought sensitive varieties while sub cluster II is having all drought tolerant ones. Separate grouping of drought tolerant and sensitive varieties indicates that despite of high average genetic similarity (0.85) among all varieties; drought tolerance in these varieties can be dissected using ISSR markers.

Two dimensional principal component analysis (2D-PCA) was performed to understand the genetic distance among drought tolerant and sensitive varieties of wheat. Two-dimensional PCA clearly showed that drought tolerant varieties formed one group while sensitive varieties formed separate group (Fig. 3). One variety, DT1 is outgrouped as was evident from the dendrogram.

This study showed that ISSR markers are an important tool in agricultural research as these are invaluable in assessing genetic diversity even among closely related varieties. Based on ISSR analysis, the varieties used in this study were found to be highly similar with 85% genetic similarity. Despite high similarity, ISSR based cluster tree analysis was able to differentiate the varieties into separate clusters based on their drought tolerance. Thus, ISSR markers have the capability to dissect drought tolerance trait at molecular level and can be useful in future breeding programmes.

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Table 1. Details of wheat varieties used in the present study

Drought tolerant		Drought sensitive	
Variety	**Variety code**	**Variety**	**Variety code**
HI1500	DT1	WH 331	DS1
HI1531	DT2	GW273	DS2
MP3173	DT3	GW322	DS3
HW2004	DT4	GW190	DS4
JW17	DT5	WL410	DS5
C306	DT6	DL803-3	DS6

Table 2. Primers details and genetic diversity parameters of ISSR markers generated from wheat varieties

Primer	Primer sequence	Tm (°C)	Average band size (bp)	TB	PB	PP (%)	EMR
ISSR 1	GAGAGAGAGAGAGAGAC	50	500-4000	14	8	57.14	4.57
ISSR 3	CACCACCACGC	42	630-2100	18	7	38.80	2.72
ISSR 6	GGGTGGGGTGGGGTG	50	600-3200	7	5	71.40	3.57
ISSR 11	CTCTCTCTCTCTCTCTG	47	700-2900	14	7	50.00	3.50
ISSR 12	CTCCTCCTCGC	40	430-2850	22	11	50.00	5.50
ISSR 13	GTGGTGGTGG	36	550-2500	10	1	10.00	0.10
ISSR 15	CTCTCTCTCTCTCTCTAC	42	850-3200	10	7	70.00	4.90
ISSR 18	GAGAGAGAGAGAGAGAYA	47	520-2350	7	2	28.50	0.57
ISSR 20	CTCTCTCTCTCTCTCTG	47	700-3050	11	6	54.50	3.27
ISSR 22	GAGAGAGAGAGAGAGAAC	51	600-1650	14	8	57.10	4.57
ISSR 23	CTCTCTCTCTCTCTCTTT	48	900-4000	17	11	64.70	7.12
ISSR 25	GATAGATAGACAGACA	38	850-3200	17	4	23.50	0.94
ISSR 26	GGGGGTGGGGTGGGGT	62	580-2100	16	6	37.50	2.25
ISSR 27	GTCACCACCACCACCACCACCAC	65	400-1550	14	10	71.40	7.14
Mean				**13.64**	**6.64**	**48.90**	**3.62**

Tm- annealing temperature; TB- total number of bands; PB- polymorphic bands; PP-percentage polymorphism; EMR- effective multiplex ratio

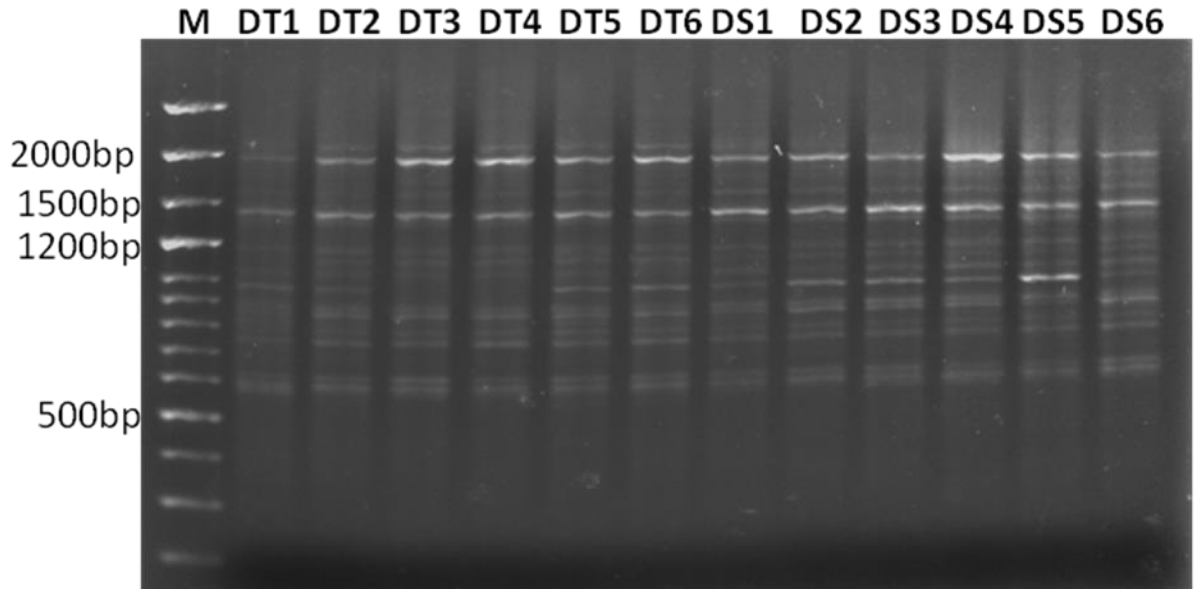

Fig. 1. DNA amplification profiles of 12 wheat varieties using ISSR 26 primer
(Lane M: 100 bp DNA ladder; DT1 to DT6: drought tolerant wheat varieties; DS1 to DS6: drought sensitive wheat varieties)

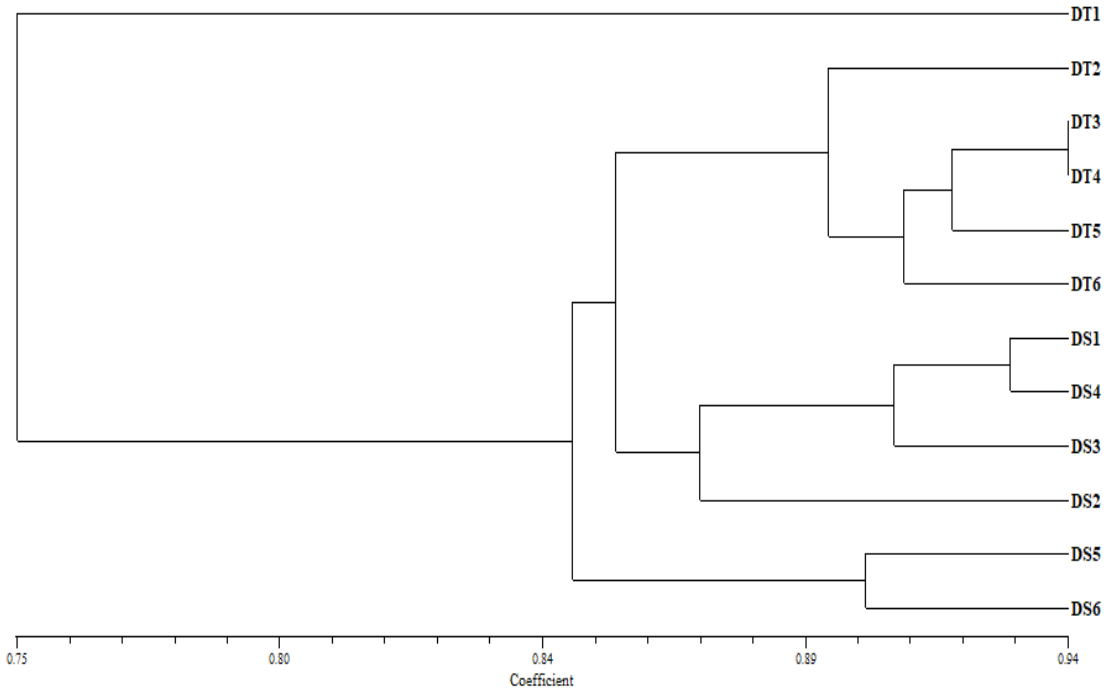

Fig. 2. Dendogram depicting the genetic relationship among 12 wheat varieties based on the ISSR markers data

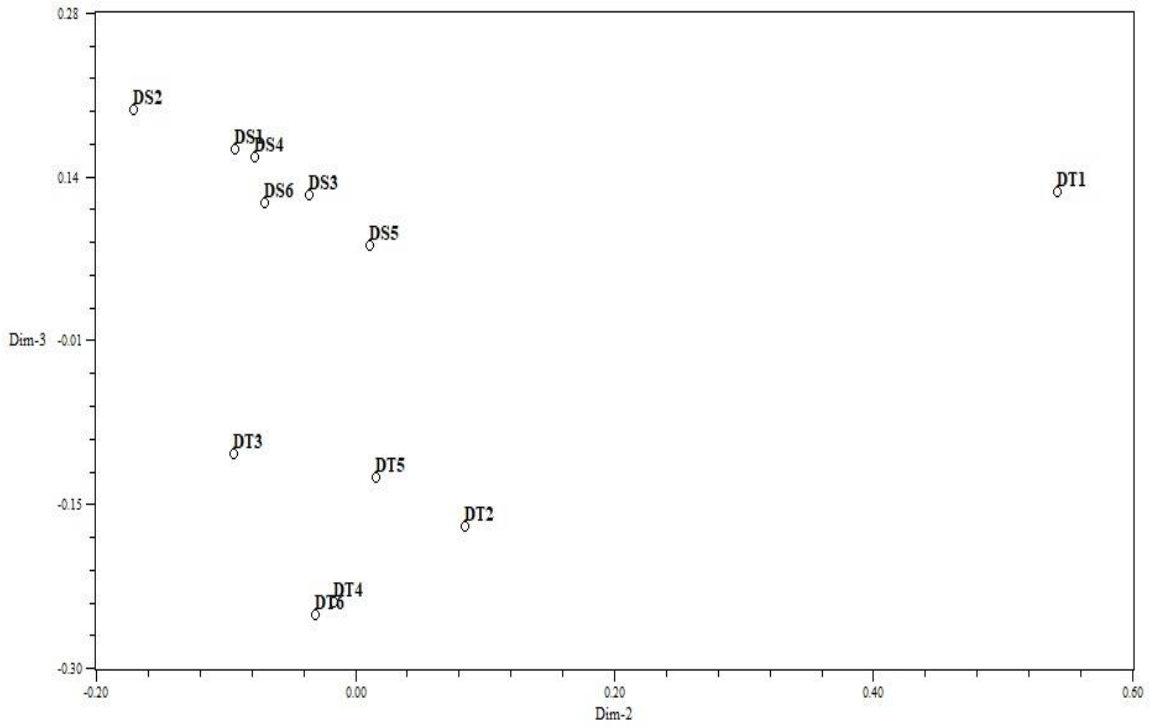

Fig. 3. Two dimensional principal component analysis (PCA) based clustering of 12 wheat varieties based on the ISSR markers data