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Assessment of the tissue-culture induced variation in durum wheat (*Triticum turgidum*) under normal and osmotic stress conditions

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ABSTRACT: Durum wheat (*Triticum turgidum* L. var. durum) is a major crop with a high protein content that mainly used for the production of pasta and spaghetti. The induction of somaclonal variation is a way for widening the genetic variation. DNA-based markers are able to detect the somaclonal variation in plants. In the present study, six ISSR primers were used to investigate the genetic variation among different callus subcultures derived from embryo culture of durum wheat on MS medium supplemented with various concentration of mannitol. The results showed levels of genetic variation among samples and also, revealed that ISSR technique is a simple and suitable method for assessment of tissue culture induced variation in durum wheat.

Keywords: Durum wheat, somaclonal variation, ISSR, mannitol

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. durum) is a tetraploid (AABB, 2n = 4x = 28) species of wheat with high protein content and superior cooking quality (Dexter et al., 1990; Von Buren, 2001). Durum wheat is a major crop in the Mediterranean basin of West Asia, North Africa, and Southern Europe that mainly used for the production of pasta and spaghetti (Moragues *et al.*, 2006).

The plant tissue culture includes various techniques used for callus induction, micropropagation, production of secondary metabolites and preservation of plants. Callus induction is an experimental method through which mass of cells is produced from an explants tissue. The callus tissue, as a source of totipotent cells can be utilized to regenerate plantlets by organogenesis or by embryogenesis or to extract some valuable secondary metabolites (Vyas and Dixit, 1999). Plant tissue culture as a technique of growing explants isolated from the mother plant is a suitable approach to prepare sufficient amount of plant materials within a short span of time in large scale and enhance the natural levels of in vitro production of valuable compounds. (Sung 2006; Pande *et al.*, 2013). It has been also enabled to increase the knowledge in many areas of biology and molecular plant breeding. (Pande *et al.*, 2013).

The induction of somaclonal variation is one of the methods for widening the genetic bases of the crops. Somaclonal variation has been related to growth regulators, cultivar variability, age of cultivars in culture, ploidy level, explant source, and another culture conditions (Skirvin *et al.*, 1994). Considering somaclonal variation occurs mainly as the result of mutations, appropriate techniques should be served for detection of occurred mutations in in vitro culture procedures. Although there are many techniques for detection of occurred variations, molecular markers are considered as one of the most appropriate tools due to their high accuracy and low performing time for evaluation of many samples (Esmaeili *et al.*, 2014). It has been shown that DNA-based molecular markers are able to detect the occurred somaclonal variations in plant tissues effectively. RAPD analysis has been used to detect the somaclonal variations (Jin *et al.*, 2008). Esmaeili *et al.*, (2014) also estimated the genetic variation among eighteen callus samples derived from different explants, media and subcultures using ISSR primers. The results of this study, revealed that ISSR technique can be used to detect somaclonal variation in P. major (Esmaeili *et al.*, 2014). According to Poorjabar *et al*, (2009), extend of genetic variations is related to the number of performed subcultures and also hormonal composition of culture medium (Poorjabar *et al.*, 2009). The present study was undertaken to investigate the genetic variation among different callus subcultures derived from embryo culture of durum wheat on MS medium, supplemented with various concentration of mannitol as the osmotic stress agent using ISSR molecular markers.

MATERIALS AND METHODS

The experiments of the present study were conducted in the Tissue Culture and Genomics Laboratory, Kermanshah branch, Islamic Azad University, Kermanshah, Iran.

A. Plant Material and Surface Sterilization

The mature seeds of durum wheat were put under running tap water for a day and then were sterilized by dipping in 70 % Ethanol for 1 min, followed by rinsing with sterile water. The seeds were then dipping in 2.5% (v/v) sodium hypochlorite solution for 20 min with constant shaking, followed by three rinses with sterile distilled water. The embryos were isolated from sterile seeds and then were cultured on MS (Murashige and Skoog, 1962) medium.

B. Media and culture conditions

MS (Murashige and Skoog, 1962) medium with 30 g L^{-1} of sucrose was used as the basal medium and was solidified using 7 g L^{-1} of agar. The MS medium was supplemented with 10 mg L^{-1} 2,4-D as an over dosage of plant regulator and different concentrations of Mannitol as an osmotic stress agent(Table 1). The pH of the medium was adjusted to 5.6 - 5.8 before adding agar, then the culture medium was autoclaved at 121°C for 20 min. All the cultures were maintained in growth room at 25±2°C, 16 h light/8 h dark photoperiod.

Table1: The number of subcultures and mannitol concentrations in callus culture.

Mannitol concentration(gr/l)	Number of subcultures	Treatment code
-	-	1(control)
0	0	2
0	1	3
0	2	4
0	3	5
20	2	6
40	2	7

C. DNA extraction

Genomic DNA were isolated from one normal plant as the control treatment and six callus samples derived from different number of subcultures on MS medium, supplemented with various concentration of mannitol, by CTAB method (Saghai-Maroof *et al.*, 1984) with some modifications. The quality and quantity of extracted DNA were tested on 0.8% agarose gel electrophoresis.

D. ISSR analysis

six ISSR primers were used to amplify the genomic DNA of all seven samples. PCR amplifications were performed in 20 µl reaction volume containing: 2 µl of genomic DNA, 1.2 µl of primer, 0.4 µl dNTPs Mix, 1.5 µl Mgcl2, 0.3 µl Taq DNA polymerase (5 unit/ µl), 2 µl PCR buffer and 12.6 µl double distilled water. PCR amplification was carried out with 4 min initial denaturing (to activate Taq DNA polymerase), followed by 35 cycles of denaturing at 94°C for 30 s, annealing (considering Tm of primers) for 45s and extension at 72°C for 2 min.

This was followed by a final extension stage for 7 min at 72°C. Amplification reaction products were separated on1.5% agarose gels at 100 V until the loading dye reached to the bottom of the gel. After electrophoresis, staining performed by ethidium bromide and photographed with gel documentation.

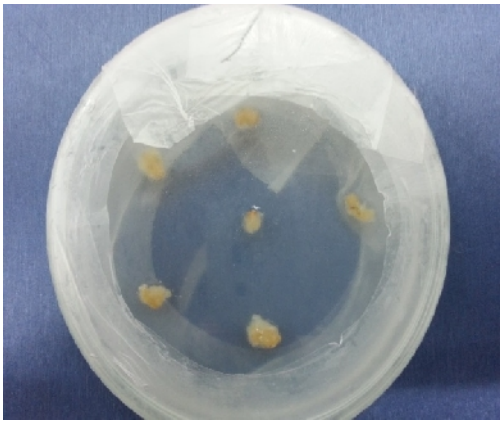

Fig. 1. Callus induction from embryo explants on MS medium supplemented with 2,4-D and Mannitol.

RESULTS AND DISCUSSION

The six ISSR primers generated 45 polymorphic bands with an average of 7.5 polymorphic fragments per primer. The highest and the lowest number of polymorphic bands per assay were 10 and 4 bands, respectively (Table 2). The results showed genetic variation among different callus subcultures derived from embryo culture of durum wheat on MS medium supplemented with various concentration of mannitol. Since our experiment was performed starting from a single individual gynotype, the polymorphism in banding pattern reveal conspicuous somaclonal variation in samples. The polymorphism information content (PIC) values for ISSR primers in the present assessment ranged from 0.35 to 0.42. The average of PIC index was 0.38, that showed a good efficiency of primers to separate individuals.

A summary of the MI calculated based on the PIC and polymorphic bands for each primer, is reported in Table 2. The MI values for ISSR primers ranged between 1.48 and 3.78 for UBC-844 and IS-9 respectively. Genetic similarities for ISSR data were calculated using the Dice similarity index, according to Nei and Li (1979).

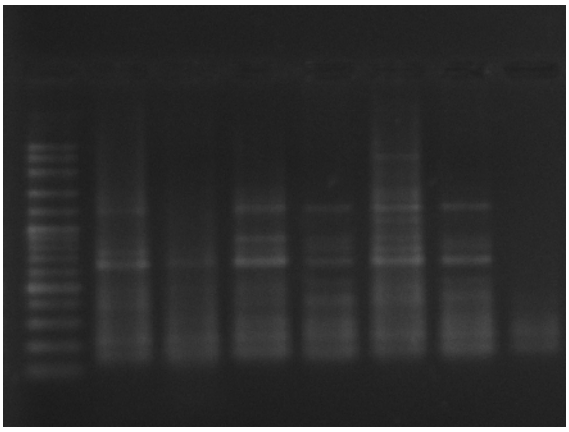

Fig. 2. ISSR banding profile generated by primer IS-9.

Table 2: Primers used for ISSR amplification with the number of bands, PIC and MI per primer.

Primer code	Primer sequences(5'-3')	No. of bands scored	No. of polymorphic bands	PIC	MI
IS-1	ACACACACACACACACYA	5	5	0.41	2.05
IS-3	GAGAGAGAGAGAGAGAYC	11	7	0.38	2.66
IS-5	AGAGAGAGAGAGAGAGC	13	10	0.35	3.5
IS- 9	CTCTCTCTCTCTCTCTG	9	9	0.42	3.78
IS-16	DBDACACACACACACACA	11	10	0.36	3.6
UBC-844	CTCTCTCTCTCTCTCTRC	5	4	0.37	1.48

Estimates of genetic similarities of ISSR markers based on the 45 polymorphic fragments among 7 samples ranged from 0.12 (1 and 2) to 0.68 (3 and 4) with an average of 0.45. Karaca and Izbirak (2008), in analysis of genetic diversity in Turkish durum wheat cultivars using ISSR markers reported 57.9% for average of polymorphism. (Karaca and Izbirak, 2008). To express the results of cluster analyses based on ISSR data, the dendrogram was constructed using the un weighted pair-group method with arithmetic averages(UPGMA).

The denderogram derived from molecular data, classified all seven samples into two separated groups(Fig.3). Genetic relationship among seven samples was also visualized by performing principle coordinate analysis (PCoA) based on ISSR data (Fig. 4). The Principal Component Analysis (PCA) results showed a good congruency with cluster analysis and supported the clustering pattern of UPGMA dendrogram.

The results showed levels of genetic variation among samples and also, revealed that ISSR markers can indicate the tissue-culture induced variation in durum wheat which has lead to the suggestion that they can be used for assessment of somaclonal variation. These results also showed that ISSR technique is a simple, informative and suitable approach for assessment of tissue-culture induced variation in durum wheat.

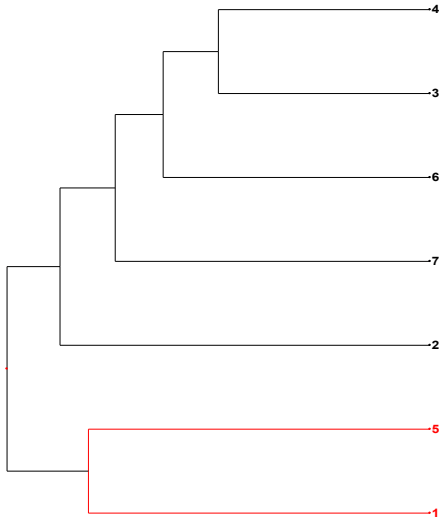

Fig. 3. Dendrogram generated with hierarchical UPGMA cluster analysis based on ISSR data.

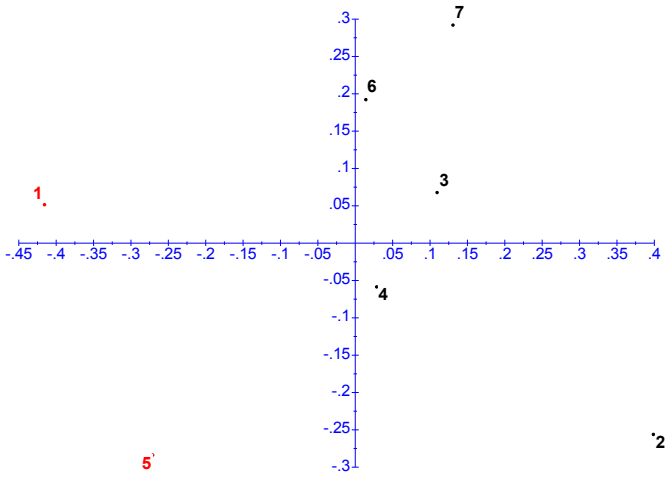

Fig. 4. Plot of the first and second coordinate in seven samples according to ISSR markers.

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