RESEARCH ARTICLE

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GENETIC DIVERSITY AMONG EGYPTIAN WHEAT CULTIVARS USING SCoT AND ISSR MARKERS

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SUMMARY

ISSR and SCoT markers were used to investigate the genetic diversity and relationships among eight cultivars of Egyptian wheat (Shandweel-1, Misr-2, Sakha-93, Sakha-94, Giza-168, Giza-171, Sids-1 and Gemmiza-9). SCoT primers produced a total of 32 bands, out of which 19 (59%) were polymorphic with a mean of 3.16. ISSR primers produced 34 bands and 23 of these bands (68%) were polymorphic with a mean of 4.6. Moreover, PCR based specific primers was used for detection of *P5CS* gene in the wheat cultivars. These results indicated good sources of diversity which will help breeders to evaluate genetic diversity and potentially select economically important traits such as salinity tolerance.

Key words: Inter-simple sequence repeat (ISSR), Pyrroline-5-Carboxylate Synthetase (*P5CS*) gene, Start codon targeted (SCoT), Polymerase chain reaction (PCR), wheat, polymorphism

Key findings: This study highlighted the genetic diversity among eight Egyptian wheat cultivars based on SCoT and ISSR markers. ISSR markers exhibited higher polymorphism than SCoT markers and can be employed in wheat breeding programs to evaluate genetic diversity.

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INTRODUCTION

Wheat (*Triticum aestivum*) is one of the most important crops, being an essential component of most food industries needed for feeding billions of people worldwide (Dawlah *et al*., 2015). Egypt is the most importing country of wheat worldwide producing about 8.5 million tons per year, while the annual requirements are about 13.5 million tons (Shehata and

Mohamed, 2015). Moreover, augmentation of wheat production is necessary to match the rapidly growing population needs. This can be achieved via increasing the cultivated area and productivity.

Molecular markers provide excellent sources of diversity which help breeders to select economical traits and therefore multiply the productivity of crops (Randhawa *et al.*, 2013). It was shown that marker data about genetic relationships and diversity are very important for any breeding program to select promising cultivars (Zhang *et al*., 2015). DNA markers as SCoT and ISSR are used efficiently for studying genetic diversity of plants (Ma *et al*., 2008; Collard and Mackill, 2009; Etminan *et al*., 2016). The SCoT marker method was utilized for its simplicity, its ability to target gene sequences and for being a dominant marker system (Xiang *et al*., 2011). ISSR is another common marker method for evaluating genetic diversity and is characterized with high polymorphism and recurrence (Zhang *et al*., 2016).

The Egyptian wheat cultivars (Shandweel-1, Misr-2, Sakha-93, Sakha-94, Giza-168, Giza-171, Sids-1 and Gemmiza-9) are very important economically for Egypt, distinguished with high productivity and tolerance of some biotic and a biotic stresses. Salinity is an important a biotic stress that can decrease the productivity of crops worldwide (Sabbour *et al*., 2015). Recent studies reported that more than 6% of the world's ground is suffer from salinity (Tavakoli *et al*., 2016). Salinity harms all stages of plant growth starting from germination to harvest and can cause toxicity, nutrients uptake shortage and oxidative damage (Shrivastava and Kumar, 2015; Wu *et al*., 2014). The previous studies showed that proline accumulation occur in plants as response to salinity stress (Abou-Gabal *et al*., 2013; Tavakoli *et al*., 2016). *P5CS* is the proline synthesis gene which plays an important role in plant adaptation with stresses like salinity by scavenging free radicals, balancing cell pH and maintaining of proteins structure (Abou-Gabal *et al*., 2013; Tavakoli *et al*., 2016). The aims of this study were to evaluate the genetic diversity and relationships among eight Egyptian wheat cultivars based on SCoT and ISSR markers and to screen these cultivars for presence of *P5CS* gene.

MATERIALS AND METHODS

Wheat cultivars

The Egyptian wheat cultivars (Shandweel-1, Misr-2, Sakha-93, Sakha-94, Giza-168, Giza-171, Sids-1 and Gemmiza-9) were provided by Agriculture Research Center (ARC), Egypt. These cultivars were selected because they are highly productive, tolerant to some biotic and abiotic stresses and are recommended by the ministry of Agriculture, Egypt.

DNA isolation

The genomic DNA was isolated from the wheat grains using DNeasy plant Mini Kit (QIAGEN) following kit instructions.

PCR reactions

A. ISSR markers

PCR reactions were performed as described by Adawy *et al*. (2004) and Hussein *et al*. (2006) using five ISSR

Table 1. List of ISSR primers (Sigma, Egypt).

Primer	Sequence	Primer	Sequence
14A	5 CTC TCT CTC TCT CTC TTG 3`	HB-13	5´ GAG GAG GAG GC 3`
44A	5` CTC TCT CTC TCT CTC TAG 3`	HB-14	5´ CTC CTC CTC GC 3`
HB-12	5´ CAC CAC CAC GC 3`		

Table 2. List of SCoT primers (Sigma, Egypt).

Primer	Sequence	Primer	Sequence
SCoT 2	ACC ATG GCT ACC ACC GGC	SCoT 8	ACA ATG GCT ACC ACT GAG
SCoT 3	ACG ACA TGG CGA CCC ACA	SCoT 10	ACA ATG CTA CCA CCA AGC
SCoT 6	CAA TGG CTA CCA CTA CAG	SCoT 11	ACA ATG GCT ACC ACT ACC

primers (Table 1). The PCR conditions were carried as following; 4 min at 94º C, 45 cycles of 1 min at 94º C, 1 min at 58º C, and 2 min at 72º C and finally 72º C for 8 min. PCR products were analyzed using gel electrophoresis (1.5% agarose gel), stained with ethidium bromide and photographed under UV light.

B. SCoT markers

PCR reactions were performed as described by Collard and Mackill (2009), using six SCoT primers (Table 2). The PCR conditions were carried as following; 4 min at 94º C, then 45 cycles of 1 min at 94º C, 1 min at 58º C, and 2 min at 72º C and finally 72º C for 8 min. PCR products were analyzed using gel electrophoresis (1.5% agarose gel), stained with ethidium bromide and photographed under UV light.

C. Amplification of P5CS gene

The *P5CS* gene of the tested wheat cultivars was amplified using specific primers: *P5CS*-F 5'- GGC TGC AAT GCC ATG GAA ACT CTT-3' and *P5CS*-R 5'-ACT TGC CTT GGG TCC TCC ATA CAA-3 as described by Çelik and Atak (2012). The PCR conditions were run for 10 min at 94º C, then 45 cycles of 30 s at 94º C, 30 s at 62º C, and 1 min at 72º C and the final extension at 72º C for 5 min. PCR products were analyzed using gel electrophoresis (1.5% agarose gel), stained with ethidium bromide and photographed under UV light.

Data analysis

Bands were scored as 1 for presence and 0 for absence. The cluster analysis was done using UPGMA method based on Jaccard's similarity coefficients with SAHN module in NTSYSpc v 2.2 software (Rohlf, 2005).

RESULTS

SCoT analysis

Six SCoT primers were used to study the genetic variation among eight Egyptian cultivars (Shandweel-1, Misr-2, Sakha-93, Sakha-94, Giza-168, Giza-171, Sids-1 and Gemmiza-9). PCR produced a total of 32 bands (Figure 1 and Table 4), out of which 19 (59%) were polymorphic bands. The number of polymorphic bands ranged from 1 (SCoT 6) to 5 (SCoT 11) with a mean of 3.16. In addition,

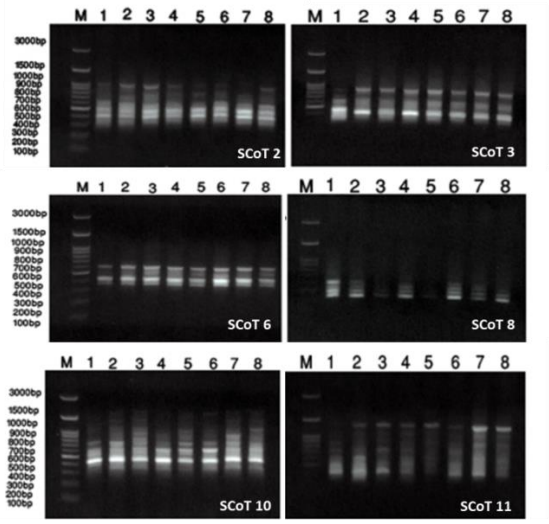

Figure 1. SCoT fingerprinting of wheat cultivars: M; DNA marker, lanes 1-8; Shandweel-1, Gemmiza-9, Sids-1, Sakha-93, Sakha-94, Misr-2, Giza-171 and Giza-168, respectively.

Table 3. The similarity matrix based on SCoT data between the eight wheat cultivars.

Cultivars	Shandweel-1	Gemmiza-9	Sids-1	Sakha-93	Sakha-94	Misr-2	Giza-171	Giza-168
Shandweel-1	1.00							
Gemmiza-9	0.68	1.00						
Sids-1	0.51	0.82	1.00					
Sakha-93	0.79	0.75	0.72	1.00				
Sakha-94	0.44	0.68	0.86	0.65	1.00			
Misr-2	0.75	0.86	0.75	0.89	0.62	1.00		
Giza-171	0.51	0.82	0.79	0.72	0.72	0.75	1.00	
Giza-168	0.55	0.86	0.82	0.68	0.75	0.72	0.89	1.00

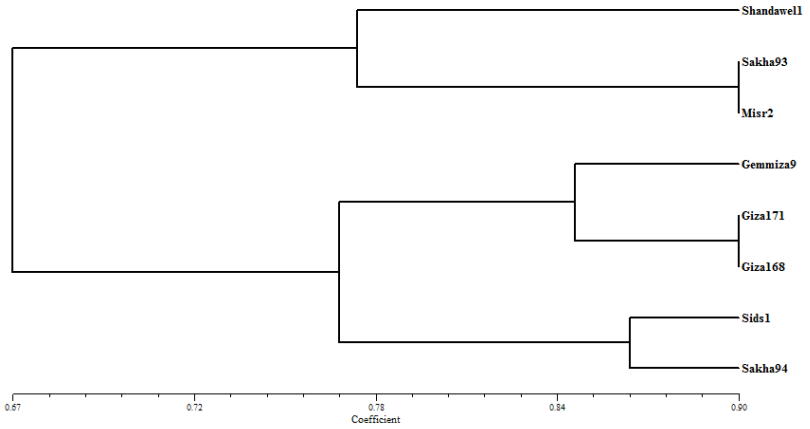

Figure 2. Dendrogram based on Jaccard's similarity coefficients scored from SCoT data using UPGMA algorithm between the eight wheat cultivars.

the top levels of polymorphism were observed with primers SCoT 11, SCoT 10, SCoT 8 and SCoT 2, respectively. The similarity matrix revealed that the highest similarity percentage was obtained between the cultivars (Giza-168 and Giza-171; and Sakha-93 and Misr-2) with 0.89, while the lowest similarity percentage was obtained between the cultivars Gemmiza-9 and Shandweel-1 with 0.44 (Table 3). The cluster analysis was done using Jaccard's similarity coefficients to study the genetic relationships among these wheat cultivars (Figure 2). The generated dendrogram divided the cultivars into two main groups, the first group contained the cultivars (Shandweel-1, Sakha-93 and Misr-2), while the second group contained (Sakha-94, Giza-168, Giza-171, Gemmiza-9 and Sids-1 cultivars).

ISSR analysis

Five ISSR primers were used to study the genetic relationships among the Egyptian wheat cultivars (Shandweel-1, Misr-2, Sakha-93, Sakha-94, Giza-168, Giza-171, Sids-1 and Gemmiza-9). PCR reactions produced a total of 34 bands (Figure3 and Table 4) and 23 of these bands (68%) were polymorphic. The number of polymorphic bands ranged from 3 (44 A) to 6 (14 A) with a mean of 4.6. The maximum polymorphism levels were obtained with primers 14A, HB12 and HB-14, respectively. The similarity matrix revealed that the highest similarity percentage was obtained between the cultivars (Giza-168 and Giza-171) with 0.96 while the lowest

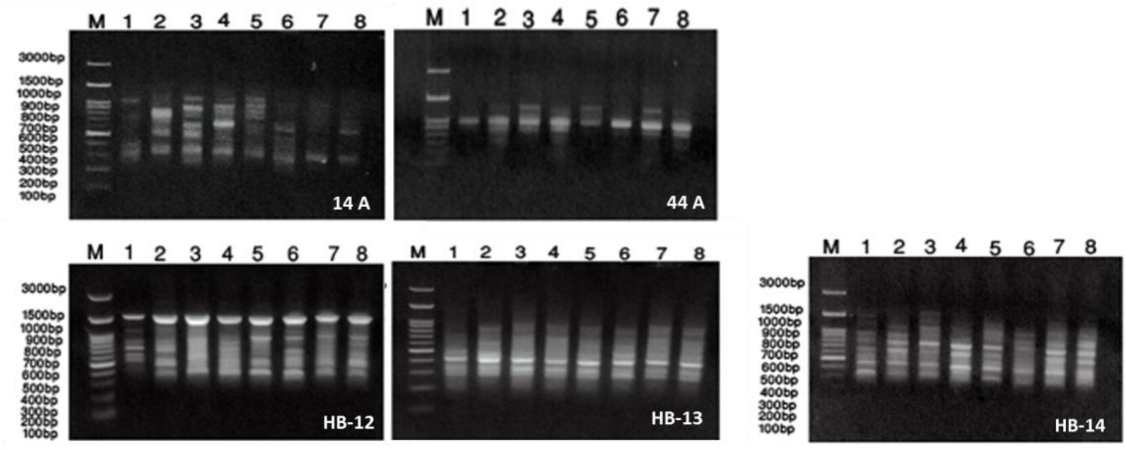

Figure 3. ISSR fingerprinting of wheat cultivars: M; DNA marker, lanes 1-8; Shandweel-1, Gemmiza-9, Sids-1, Sakha-93, Sakha-94, Misr-2, Giza-171 and Giza-168, respectively.

Table 4. SCoT and ISSR analyses of wheat cultivars.

Primers	Size of bands (bp)	Total number of bands	Number of polymorphic bands	Percentage of polymorphic bands (%)
SCoT 2	400-900	7	3	43
SCoT 3	500-900	3	2	67
SCoT 6	500-700	4	1	25
SCoT 8	500-700	4	4	100
SCoT 10	500-2000	8	4	50
SCoT 11	500-1300	6	5	83
Total		32	19	59
14 A	400-1300	7	6	85
44 A	800-1300	4	3	75
HB-12	600-1500	8	5	62
HB-13	400-1000	8	4	50
HB-14	500-1500	7	5	71
Total		34	23	68

Table 5. The similarity matrix based on ISSR data between the eight wheat cultivars.

Varieties	Shandweel-1	Gemmiza-9	Sids-1	Sakha-93	Sakha-94	Misr-2	Giza-171	Giza-168
Shandweel-1	1.00							
Gemmiza-9	0.62	1.00						
Sids-1	0.59	0.81	1.00					
Sakha-93	0.59	0.74	0.85	1.00				
Sakha-94	0.70	0.85	0.81	0.74	1.00			
Sakha-94	0.70	0.77	0.66	0.66	0.70	1.00		
Giza-171	0.66	0.81	0.77	0.70	0.81	0.81	1.00	
Giza-168	0.62	0.77	0.81	0.74	0.77	0.85	0.96	1.00

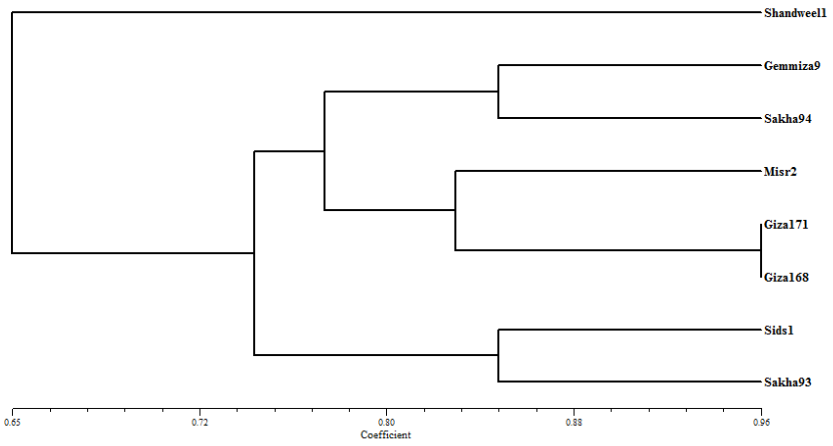

Figure 4. Dendrogram based on Jaccard's similarity coefficient computed from ISSR data using UPGMA algorithm between the eight wheat cultivars.

similarity percentage was recorded between the cultivars (Shandweel-1 and Sids-1 or Sakha-93) with value 0.59 (Table 5). The cluster analysis was done using Jaccard's similarity coefficients to study the genetic relationship among the wheat cultivars. The cluster divided the genotypes into two main groups (Figure 4), the first group contained only the Shandweel-1 cultivar, while the second group contained the remaining cultivars (Misr-2, Sakha-93, Giza-168, Gemmiza-9, Sids-1, Sakha-94 and Giza-171).

Amplification of *P5CS* gene

P5CS is the proline synthesis gene which plays an important role in plant adaptation with stresses like salinity by scavenging free radicals, balancing cell pH and maintaining of proteins structure (Abou-Gabal *et al*., 2013; Tavakoli *et al*., 2016). PCR amplification revealed the presence of fragment of 1.5 kbp in all the tested cultivars of wheat.

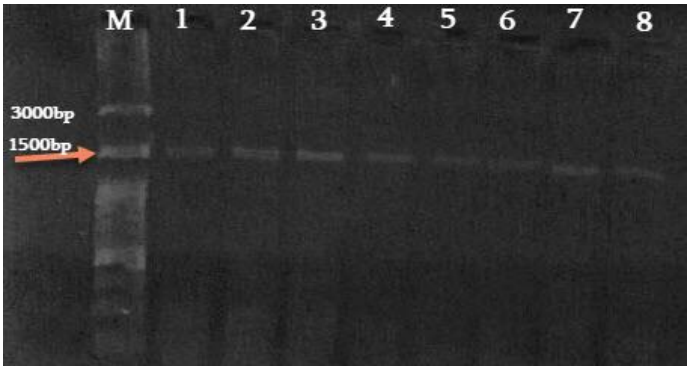

Figure 5. Amplification of *P5CS* gene in wheat cultivars: M; DNA marker, lanes 1-8; Shandweel-1, Gemmiza-9, Sids-1, Sakha-93, Sakha-94, Misr-2, Giza-171 and Giza-168, respectively.

DISCUSSION

SCoT analysis

The generated dendrogram based on SCoT markers divided the cultivars into two main groups, interestingly, the cultivars Giza-168 and Giza-171 were found together in the same group. In contrast, the cultivars Sakha-93 and Sakha-94 were classified into separate groups. These results are consistent with the observation of Xiong *et al*. (2011) who studied the polymorphism of cultivated peanut genotypes using SCoT marker and they found that not all accessions related to the same variety were classified in the same group. Furthermore, SCoT markers were utilized for evaluating the genetic relationships among 53 *Elymus sibiricus* genotypes from China. The dendrogram divided the genotypes into two main groups and three sub-groups (Zhang *et al*., 2015). These results confirm the ability of SCoT as an excellent marker system to investigate the genetic relationships among different genotypes and

obtaining new specific clustering (Etminan *et al*., 2016; Xiong *et al*., 2011). Aboulila and Mansour (2017) studied the genetic diversity among ten barley genotypes using SCoT marker, and they reported that SCoT marker is an efficient tool for obtaining new fingerprint of barley. Furthermore, Mohamed *et al*. (2017) employed SCoT markers and morphological data based on surface sculpture of grain to discriminate and identify fourteen cultivars of *T. aestivum* L. collected from different countries of North Africa. The taxonomic relationships of morphological and molecular data were consistent.

ISSR analysis

The dendrogram based on ISSR markers divided the wheat cultivars also into two main groups with some differences as compared to SCoT markers. The difference in the polymorphism and cluster analysis between the two markers is normal because each marker targets different genome sequences. The same observation is mentioned in previous studies and this highlight the importance of ISSR and SCoT markers in detection polymorphism and obtaining specific genetic relationships (Etminan *et al*., 2016; Pakseresht *et al*., 2013).

El-Assal and Gaber (2012) studied the abilities of RAPD, ISSR and SSR markers in establishing genetic relationship and distinguishing between Egyptian and Saudi wheat cultivars. They concluded that the ISSR markers gave more recurrence, polymorphism and can be used in cultivar discrimination. Furthermore, Abou-Deif *et al*. (2013) showed that the ISSR markers were highly efficient in discriminating among 20 wheat genotypes that were different in their genetic background and origin.

In conclusion, this study highlighted the genetic diversity among important Egyptian wheat cultivars using SCoT and ISSR markers. ISSR markers exhibited higher polymorphism than SCoT markers and the two markers established specific genetic relationships. It is possible that the SCoT technique could be optimized for wheat by modifying PCR conditions (e.g. lower annealing temperature). Identification of new specific markers is very important for breeders to evaluate wheat germplasm for breeding programs.

Amplification of *P5CS* gene

PCR amplification of *P5CS* gene revealed the presence of fragment of 1.5 kbp in all the tested cultivars of wheat (Figure 5). A similar fragment (1.2 kpb) was detected with amplification of *P5CS* gene in *Alhagi Maurorum* (Abou-Gabal *et al*., 2013). It's well known that the Egyptian wheat cultivar 93 is tolerant to salinity, while Sakha-94 and Gemmiza-9 are sensitive (Abd El-Samad and Mohamed, 2017; El-Hendawy *et al*., 2011; Gadallah *et al*., 2017). Furthermore, the cultivars Sids-1, Shandweel-1 and Misr-2 exhibited moderate levels of salinity tolerance (Gadallah *et al*., 2017). Further research is required to confirm if there are any polymorphisms for this marker. QPCR studies must be performed to evaluate the gene expression level of *P5CS* in a larger group of germplasm to confirm this gene has a possible association with salinity tolerance.

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