10.21608/taec.2020.39905.1025 *Taeckholmia 40 (2020): 143-151*

Genetic diversity studies on seven Egyptian wheat (*Triticum aestivum L*) cultivars using Scot and ISSR polymorphism markers

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Abstract

Assessment of genetic diversity of crops by using informative molecular markers is important for their genetic improvement and conservation. Genetic diversity and relationships among seven Egyptian cultivars of wheat (*Triticum aestivum L*) (Masr1, Swiss2, Swiss4, Giza7, Giza9, Giza10 and Sakha94) were analyzed using SCoT and ISSR markers. A total of 7 SCoT and 7 ISSR primers were used to estimate genetic polymorphism among seven wheat cultivars (Masr1, Swiss2, Swiss4, Giza7, Gisa9, Giza10 and Sakha94) . DNA extraction was done using Thermo Kit according to its manufacturer's instructions followed by amplifications with ISSR and SCoT and agarose gel electrophoresis. Based on UPGMA both ISSR and SCoT markers resolved cultivars into dendrogram with three major cluster. The reproducible bands were scored for analyses polymorphic information content (PIC), Resolving power (RP) and Marker index (MI). Comparatively, two markers were effective. The average polymorphic information content (PIC), Resolving power (RP)and Marker index (MI) of SCoT were reflected relatively higher than those of ISSR. According to the present results, SCoT markers proved more informative in studying genetic diversity among seven wheat cultivars. The results demonstrated that SCoT and ISSR markers are useful for genetic diversity analysis of wheat cultivars. This information is useful for utilization in plant breeding programs.

Keywords: Inter-Simple Sequence Repeats (ISSR), Start Codon Targeted (SCoT) marker, Polymorphic Information Content (PIC), Resolving Power (RP) and Marker Index (MI).

Introduction

Cereal grains have been the principal constituent of the human diet for hundreds of centuries and have affected in human civilization (Awika, 2011) Wheat, the world's major cereal crop, accounted for constituents 31% of global cereal consumption in 1999 (FAO, 2002). Wheat represents around 37% of caloric utilization in the Middle East and North Africa region. The region is communally a net important of 58 million metric tons of cereal, making it the largest net importer region in the world (Wright and Cafiero, 2010)

Concerning Egypt, Bread wheat (*Triticum aestivum L.*) is one of the most important crops and its local production is about 8 million tons, conversely; it covers less than 60% of local consumption (FAO., 2009). Therefore, wheat productivity must be enhanced in Egypt, (Abdel-Razek, 2013). Essential objective of Egyptian Government is filling gap between wheat production and consumption (Abd El-Mohsen *et al*.,2012). consequently, using of plant breeding program is very important for production of promising wheat harvest. Cultivation of crops with different genetic background is an effective strategy for maintenance and to reduce genetic weakness in crop enhancement (Smale *et al*., 2002; Mardi *et al*., 2011). Crops tolerance to biotic and abiotic stress needs strong genetic diversity.

Received on 02/10/2020 and accepted on 04/12/2020 Edited by Dr. Ashraf Soliman

(Talebi *et al*., 2012). Therefore, knowledge of the genetic diversity in crops is important in crop breeding programs (Soleimani *et al.*, 2002)

Molecular markers are useful accompaniments to morphological and molecular characterization because they are abundant independent of environmental effects and allow cultivar identification in the early steps of development (Chen *et al.*, 2009; Yu and Pauls 1993., Biswas *et al*., 2014 and Cabo *et al*., 2014). Recently, Collard and Mackill (2009) described a simple and novel DNA marker technique Start Codon Targeted (SCoT) Polymorphism. This marker technique uses 18-mer single primer in PCR and an annealing temperature of at 50C, and PCR products are resolved using standard agarose gel electrophoresis. The primers are very easy to design based on the preserved region surrounding the translation initiation codon, ATG (Joshi *et al*., 1997; Milbourne *et al*., 1997; Sawant *et al*., 1999) without the requisites of genomic sequence information. Inter-simple sequence repeats (ISSR) marker is identified by means of repeaters' anchored primers that are amplified between SSRs and has also been used to analyses the genetic diversity among different species of plants. Furthermore, this technique, due to high repeatability and polymorphism, as well as highly informative, is suitable for assessing genetic diversity in different crops (Bornet *et al*., 2001, Moradkhani *et al.*, 2012 and 2015, Al-kordy *et al.*, 2013 and Abdel-Lateif *et al.*, 2018)). Thus, the main goal of the present study was to determine the genetic variability in the used 7 cultivars (Masr1, Swiss2, Swiss4, Giza7, Giza9, Giza10 and Sakha94) of Egyptian wheat using SCoT and ISSR markers.

Material & Methods:

Seven Egyptian cultivars of wheat grains were obtained from Agricultural Genetic Engineering Research Institute (AGERI) and cultivated in the garden of botany department of science collage, Ain Shams University. The seedling leaves were used for DNA extraction.

Genomic DNA extraction:

The genomic DNA was extracted from the seedling leaves by (Thermo Scientific Gene JET Plant Mini Kit) Agarose gel electrophoresis definite that the DNA was of high molecular weight with no degradation.

SCoT& ISSR analysis:

Seven SCoT & ISSR primers were used in this study (Tables 1 & 2). The PCR amplification was performed in a 25 µl reaction volume containing about 3µl (10 ng/ µl) genomic DNA, 1 µl primer ,1.5 mMMgCl2, 0.2 mM of each dNTP, 0.4 µM of a primer; 50 ng genomic DNA and 2U of Taq DNA polymerase. The thermal cycler was programmed with an initial step of 5 min at 94°C; the amplification reaction was carried out using 40 cycles of 40 seconds at 94° C, an annealing step of 1 min at a specific annealing temperature for each primer (Tables1 & 2) and an elongation step of 1min at 72°C; and finally, a 7 min extension at 72° C. All PCR amplification products were separated on 1.5% agarose gel in TBA, stained with ethidium-bromide.

Data analysis:

In order to measure the informativeness of the markers to differentiate between genotypes, polymorphism information content (PIC), marker index (MI) and resolving power (RP) were calculated. PIC was calculated according to the formula of (Anderson *et al.*, 1993), MI was determined according to (Varshney *et al*., 2007). The RP of each primer was calculated according to (Prevost and Wilkinson, 1999).

Results

SCoT analysis:

SCoT analysis with seven primers of 7 Egyptian cultivars of bread wheat were done as shown in (Fig. 4). The number of total bands, the polymorphism, and % of polymorphism was presented in (Table 1). The total number of bands was 96 fragments and the number of bands by each primer varied from 7 to 28 fragments. Scot46 amplified the highest number of bands (28 bands), while Scot28 primer produced the lowest number of bands (7 bands). All the primers used were found to produce polymorphic bands. The percent of polymorphism revealed by the different primers ranged from 100% for Scot (7, 14, 24, 28, 35 & 46) primer to 90% for Scot11 primer. PIC values ranged from 0.40 (Scot24) to 0.62 (SCoT.35), with an average value of 0.52 per primer (Table 1). The of Resolving power of SCoT marker is 42.5.MI of SCoT primer were at the (Table, 1)

Cluster analysis:

Based on UPGMA clustering algorithm generating from obtained SCoT database, the seven cultivars were grouped into 3 clusters (Fig. 1). Cluster I consisted of two clades the first contain the cultivar swiss2 and the second divided to two subclades the cultivars swiss4 in the first and Giza7 in the second. Cluster II is divided into two clades the cultivar Giza9 is in the first and the second subgroup is divided into two subclades the cultivars Giza10 is in the first subclade and cultivars Sakha 94 is in the second. Cluster III contains only cultivars Masr1.

ISSR analysis:

ISSR analysis with seven primers of 7 Egyptian cultivars of bread wheat were done as shown in (Fig. 4). The number of total bands, the polymorphism, and % of polymorphism was presented in Table 2. The total number of bands was 54 and the number of bands by each primer varied from 5 to 16. the highest number of bands (16 bands) is in ISSR5, while ISSR 2 & 6 primer produced the lowest number of bands (5 bands) The polymorphism percentage revealed by the different primers ranged from 100% for ISSR1 & 6 primer to 50% for ISSR4 primer. PIC values ranged from 0.21 (ISSR2) to 0.71 (ISSR 1 & 6), with an average value of 0.42 per primer (Table 2). The Resolving power (RP) of ISSR marker is 19.3. Marker index (MI) of ISSR primer were at table (2).

Table (1). Data of SCoT primers and the extent of polymorphism.

p. no.	Primer ID.	Seq5\ -------3\	ANT	M	Po	Un	TNB	P (%)	PIC	RP	MI
1	Scot7	ACAATGGCTACCACTGAC	54	0	9	8	16	100	0.48	3.44	7.68
2	Scot11	ACAATGGCTACCACTACC	54	1	5	4	10	90	0.43	3.22	3.87
3	Scot14	ACCATGGCTACCAGCGCG	60	0	7	7	14	100	0.48	4.34	5.32
4	Scot24	CCATGGCTACCACCGCAG	80	1	2	12	15	100	0.40	5.04	5.6
5	Scot28	CAACAATGGCTACCACCA	54	1	4	2	7	100	0.60	2.24	3.42
6	Scot35	AACCATGGCTACCACCAC	56	1	2	13	16	100	0.62	4.92	6.3
7	Scot46	ACCATGGCTACCACCGCC	60	0	4	16	28	100	0.52	3.22	8.4
	total			4	32	70	96	97.8		42.5	
	average								0.50		

(ANT) annealing temperature, (NMB) number of monomorphic bands, (NPB) number of polymorphic bands, (TNB) total number of bands, (NUB) number of unique bands unique band (PPB), percentage of polymorphic bands, (PIC) polymorphism information content, (RP) resolving power, (MI) marker index

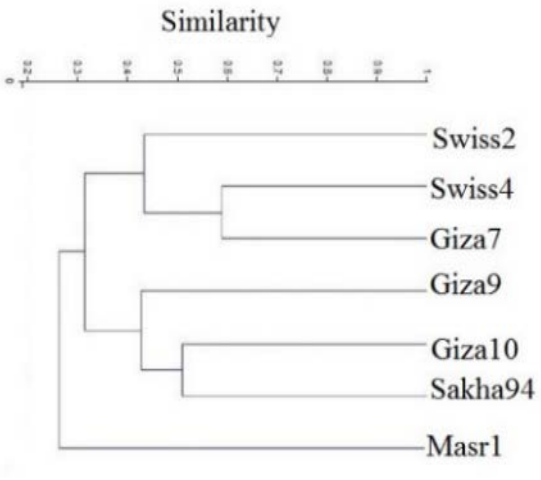

Fig.1. Dendrogram of 7 wheat cultivars resulting from the UPGMA cluster analysis based on Jaccard's similarity coefficients obtained from 7 SCoT marker.

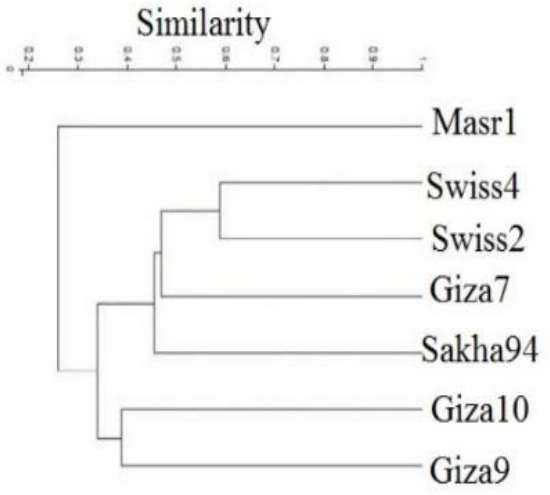

Fig. 2. Dendrogram of 7 wheat cultivars resulting from the UPGMA cluster analysis based on Jaccard's similarity coefficients obtained from ISSR marker

Cluster analysis:

The seven cultivars were divided into three cluster. The first cluster contain Masr1 cultivar and the second were divided into three clades, the first clade were divided into two subgroups one subclade contains Swiss4 cultivar while swiss2 cultivar were found in the second subgroup. The second clade contains only Giza7 cultivar and Sakha94 cultivar were found in clade three. The third cluster were divided into two clades, the first clade contains cultivar Giza10 while the second contains Giza9 cultivar.

Table (2). Data of ISSR primers and the extent of polymorphism.

p. no.	Primer ID.	Seq5\ -------3\	ANT	NMB	NPB	NUB	TNB	PPB	PIC	RP	MI
1	Issr1	AGAGAGAGAGAGAGAGYC	55	0	2	8	10	100	0.39	3.68	3.9
2	Issr2	AGAGAGAGAGAGAGAGYG	55	2	2	1	5	60	0.21	1.54	0.63
3	Issr3	ACACACACACACACACYT	53	1	1	4	6	83	0.71	1.54	3.55
4	Issr4	ACACACACACACACACYG	55	3	0	3	6	50	0.41	2.52	1.23
5	Issr6	CGCGATAGATAGATAGATA	52	0	7	9	16	100	0.22	5.5	3.3
6	Issr7	GACGATAGATAGATAGATA	50	1	2	2	5	80	0.53	2.28	2.65
7	Issr9	GATAGATAGATAGATAGC	48	1	2	3	6	83	0.48	2.28	2.4
	Total			8	14	30	54	85.7		19.3	
	Average								0.42	2.76	

(ANT) annealing temperature, (NMB) number of monomorphic bands, (NPB) number of polymorphic bands, (TNB) total number of bands, (NUB) number of unique bands unique band (PPB), percentage of polymorphic bands, (PIC) polymorphism information content, (RP) Resolving power, (MI) marker index

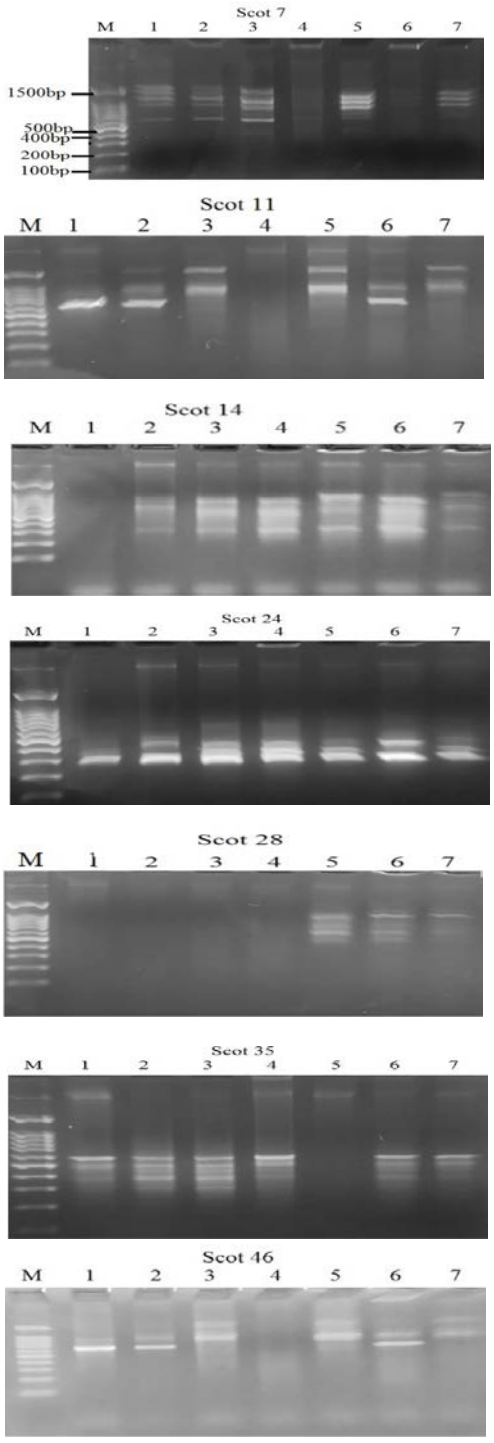

Fig.3 Amplification profiles of seven wheat cultivars DNA samples using 7 Scot primers M= DNA ladder1= Masr1 2=Swiss2 3=Swiss4 4= Giza7 5= Giza9 6= Giza 10 7= Sakha94

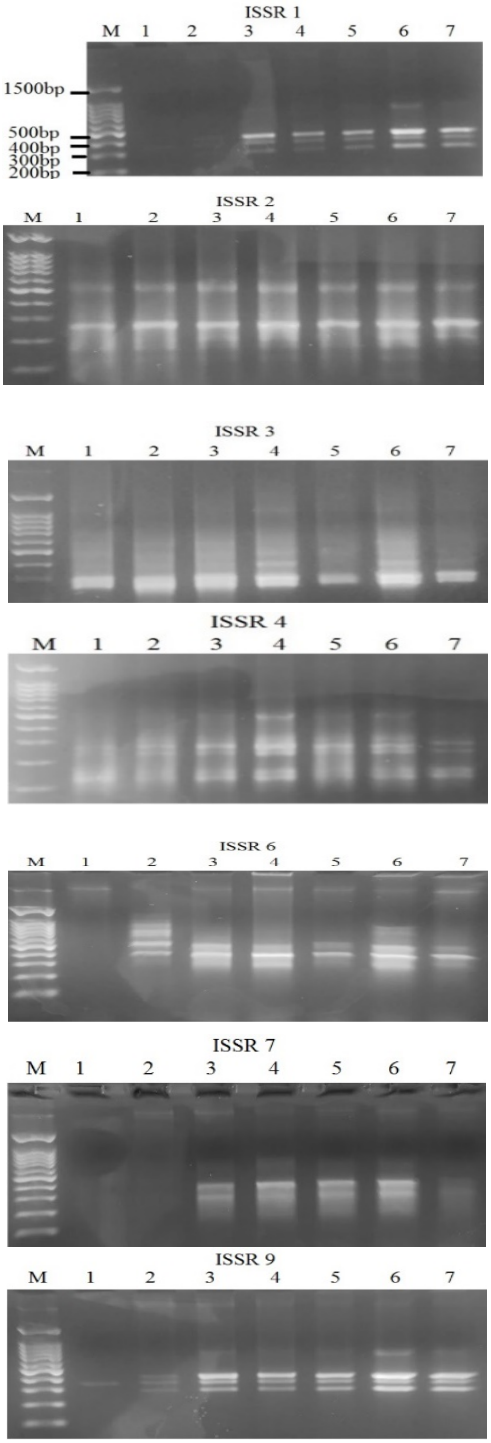

Fig.4 Amplification profiles of seven wheat cultivars DNA samples using 7 ISSR primers M= DNA ladder1= Masr1 2=Swiss2 3=Swiss4 4= Giza7 5= Giza9 6= Giza 10 7= Sakha94

Discussion

SCoT and ISSR markers are valuable in genetic diversity studies because of their high resolving power (Guo.*et al*., 2012, Hamidi *et al*., 2014, Cao *et al*., 2006 and Sofalian *et al*., 2009). These markers are efficient and inexpensive analytical methods to evaluate genetic variations for many plants (Xiong *et al*., 2011, Gorji *et al*.,2011, Moradkhani *et al*.,2015 Etminan *et al*.,2016, Pour-Aboughadareh *et al*., 2017 and 2018). In the present study, the efficiency of both SCoT and ISSR markers is assessed through parameters such PIC, polymorphism percentage, RP and MI. Parameters such as PIC have been used usually for evaluating the informative potential of ISSR markers in different cultivated genotypes (Gomes *et al*., 2009 and Tatikonda *et al*.,2009).Also, the average PIC value per primer of ISSR is equivalent to that found by (Zamanifard *et al*.,2015, and Hamidi *et al*.,2014). using SCoT markers in wheat germplasm. In the present study, we compared genetic diversity of wheat cultivars by using SCoT markers of functional domains of well characterized plant genes and ISSR markers (Collard and Mackill., 2009, Abdel-Lateif *et al*., 2018). Several authors also reported that these marker techniques were able to provide more reliable diversity information (Amirmoradi *et al*., 2012 and Li *et al*., 2013 and Poczai *et al*., 2013) and are useful as tools for studying the genetic diversity of different plant germplasm. At present study showed high genetic diversity among the seven wheat cultivars and this can be useful for utilization in breeding programmed (Tanya *et al*.,2011). The MI, which can be a common measure of efficiency in discovering polymorphism (Khodadadi *et al*., 2011), was different in two marker systems (Table 1 & 2). Our study revealed that RP of SCoT primers is higher than ISSR primers. Genetic corrosion in cultivated wheat provides a good motivation for evaluating genetic diversity among different cultivars (Moradkhani *et al*., 2012, Abdel-Lateif *et al*., 2018), as well as determines the possible for improving efficiency of plant materials which eventually may result in improved food production (El-Assal and Gaber, 2012).

Conclusion

Information about the degree and distribution of genetic variation and relationships among breeding materials has a significant effect on crop improvement. In the present study, SCoT marker, like ISSR marker, was an efficient technique to estimate the genetic variation. Additionally, the large polymorphic fragment percentage, PIC, RP&MI indicate the power of SCoT marker in fingerprinting and diversity analyses. Also, our results revealed a high genetic variation among tested cultivars, which can be used in wheat breeding program and development of new cultivars.

Acknowledgment

I would like to thank Prof. Dr. Mohamed Mohamadein Mokhtar professor of microbiology and molecular biology, The Regional Center for Mycology and Biotechnology, Al-Azhar University for his valuable support during this study.

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