ASSESSMENT OF GENETIC DIVERSITY AMONG SOME EGYTIAN BARLEY CULTIVARS BASED ON SCoT AND ISSR MARKERS

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The world's population increases dramatically and it's expected to reach nine billion people by 2050. In Egypt, about two million people are added each year to the population (Shalaby *et al*., 2011). In addition, 97% of Egyptian land is desert and with limited water resources (Abdalla *et al*., 2023). Hence, it's obligatory to face the growing population by increasing the cultivated area with strategic crops as wheat and barley and also expansion in reclamation of desert lands.

Barley is considered one of the premier and most important cereal crops for its earliness and tolerance of each drought and salinity (Mariey *et al*., 2018; Habiba *et al*., 2021and Güngör *et al*., 2022). The cultivated area of barley around the world is estimated with 46.9 million hectares giving about 141 million tons annually (FAO, 2020). El-Khalifa *et al*. (2022) stated that barley is indispensable crop in Egypt and occupy 76.9 % of newly reclaimed area. The productivity of barley in Egypt reached 108,000 tons in 2019 and it's expected to increase more and more in the future (Mohamed *et al*., 2021). Barley can be utilized as animal feeding, for manufacturing of bread and many healthy foods (El-Seidy *et al*., 2019; Kumar *et al*., 2020; Aly *et al*., 2021). One of the suggested solutions for overcoming wheat shortage in Egypt is addition barley to wheat flour to obtain bread (Abdalla *et al*., 2023).

The plant breeding sciences depend on the genetic diversity for selection of promising lines and cultivars for doing the crosses (Cieplak *et al*., 2021). DNA markers supply plant breeders with broad level of diversity to select desirable traits, and therefore improvement the productivity of crops (Randhawa *et al*., 2013; Zhang *et al*., 2015). ISSR (Inter-simple sequence repeats) is an smart, dominant marker and is distinguished with high distinction and repetition (Zhang *et al*.,

2016; Cieplak *et al*., 2021). Moreover, the SCoT (Start Codon Targeted) is also dominant marker and able to target preserved sequences next to the ATG start codon (Xiong *et al*., 2011; Cieplak *et al*., 2021). Previous study indicated that coupling of different molecular markers is better for exploitation of genetic divergence in analyzed species (Cieplak *et al*., 2021).

SCoT and ISSR markers are employed efficiently for evaluating the genetic variations within plant species (Ma *et al*., 2008; Collard and Mackill, 2009; Etminan *et al*., 2016; Abdel-lateif and Hewedy, 2018; Güngör *et al*., 2022; Abaza *et al*., 2022).

The barley cultivars (Giza 123, 124, 126, 132, 135 and 136) are very indispensable for Egypt, distinguished with tolerance of some stresses as drought and salinity. The current study aimed to characterize the genetic relatedness between six significant Egyptian barley cultivars using SCoT and ISSR markers.

MATERIALS AND METHODS

Barley cultivars

The Egyptian barley cultivars (Giza 123, 124, 126, 132, 135 and 136) were provided by Department of Crops, Faculty of Agriculture, Menoufia University, Egypt (Table 1).

DNA isolation

Genomic DNA was isolated from young leaves following the instructions of DNeasy Plant Mini Kit (QIAGEN, Germany).

SCoT and ISSR Reactions

Twelve primers (six of each SCoT and ISSR primers) were selected for PCR reactions as shown in Table (2). The amplification reactions were carried out in 20 µl reaction volume containing 10 µl Master Mix (sigma), 2 µl primer (10pcmol), 2 µl template DNA (10ng) and 6 µl dH_2O, according to Ibrahim *et al*. (2019).

PCR conditions

PCR amplification was carried in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems), programmed for one cycle at 94ºC for 5 min followed by amplification 40 cycles of 94ºC for 45 s, 48ºC for 50s and 72ºC for 1 min. The reaction was finally stored at 72ºC for 7 min. PCR products were separated in a 1.5% agarose gel at 95 volts and photographed under UV light.

Data analysis

The clear bands were visually scored as either present (1) or absent (0). Dice's similarity matrix coefficients were then calculated between genotypes using UPGMA method. The phylogenetic analysis was performed according to Euclidean similarity index using the PAST software Version 1.91 (Hammer *et al*., 2001).

RESULTS AND DISCUSSION

SCoT analysis

The genetic divergence among six Egyptian barley cultivars (Giza 123, 124, 126, 132, 135 and 136) was investigated using six SCoT primers (Fig.1). A total of 56 bands were obtained, out of which 22 (39%) were polymorphic (Table 4). The polymorphic bands ranged from 2 (SCoT-03, SCoT- 05 and SCoT- 06) to 8 (SCoT-04). In addition, the maximum levels of polymorphism were recorded with primers SCoT- 04, SCoT- 01, SCoT- 05, SCoT- 03, SCoT- 02 and SCoT- 06 with 57, 50, 50, 29, 25 and 22 %, respectively. The similarity matrix revealed that the highest similarity value was obtained between the cultivars Giza 123 and Giza 132 with 0.97, while the lowest similarity value was obtained between the cultivars Giza 132 and Giza 136 with 0.81 (Table 3). The cluster analysis classified the cultivars into two main divisions, the first division included the cultivars (Giza 123, 124, 126, 132 and 135), while the second division contained only the cultivar Giza 136 (Fig. 2).

Previous studies employed SCoT markers for exploring the genetic divergence within barley varieties and exhibited about 66.67 to 100% polymorphism (Habiba *et al*., 2021; Güngör *et al*., 2022; Abaza *et al*., 2022). Moreover, Al-Khayri *et al*. (2022) employed each RAPD, ISSR and SCoT markers to check the genetic divergence of five Kalanchoe genotypes, they indicated that SCoT and RAPD markers exhibited high polymorphism among the tested genotypes. Similarly, Zhang *et al*. (2016) tested the efficiency of three markers (ISSR, SCoT, and EST-SSR) for highlighting the genetic diversity among thirty-four switchgrass (*Panicum virgatum* L.) genotypes. They reported that SCoT was better than ISSR and EST-SSR.

These results are in line with the observations of Aboulila and Mansour (2017) and Mohamed *et al*. (2017), who studied the genetic diversity among each barley and wheat genotypes using SCoT marker, and they concluded that SCoT marker is an efficient tool for obtaining new relationships.

ISSR analysis

Six ISSR primers examined the genetic variation among the tested Egyptian barley cultivars (Fig. 3). A total of 66 bands were scored, out of which 33 (50 %) were polymorphic (Table 4). The polymorphic bands ranged from 4 (ISSR-02 and ISSR-04) to 8 (ISSR- 01). In addition, the high percentages of polymorphism were obtained with primers ISSR-05, ISSR-03, ISSR-04, ISSR-01, ISSR-02 and ISSR-06 with 78, 56, 56, 53, 33 and 33 %, respectively. The similarity matrix showed that the highest similarity percentage was recorded between the cultivars Giza 123 and Giza 124 with 0.94, while the lowest similarity percentage was scored between the cultivars Giza 123 and Giza 135 with 0.75 (Table 5). The phylogenetic tree divided the culti-

vars into two main clusters, the first cluster included Giza 123, 124, 126 and 132 cultivars, while the second cluster involved Giza 135 and 136 cultivars (Fig. 4). ISSR markers were stated to be efficient tool to explore the genetic divergence within the Egyptian and the Tunisian barley varieties (Guasmi *et al*., 2012; Abaza *et al*., 2022; Shaban *et al*., 2022). Moreover, El-Sherbeny *et al*. (2020) coupled the phenotypic criteria with ISSR for evaluation of genetic variation between twenty-six bread wheat genotypes. ISSR primers exhibited 87 bands, 43 were polymorphic and the mean of polymorphism reached 46.97%.

The incompatibility in the phylogenetic analysis between the two markers is expected because each marker targets diverse genome sequences. The same observation is mentioned in previous studies, and this highlight the importance of ISSR and SCoT markers in detection of polymorphism and obtaining new specific cluster (Pakseresht *et al*., 2013; Etminan *et al*., 2016). Guasmi *et al*. (2012) used RAPD and ISSR to examine the genetic divergence among 80 Tunisian barley varieties and the cluster analysis of the two markers was asymmetric. In general, it can be concluded that ISSR markers can exhibit higher polymorphism than SCoT markers. These finding concurs with previous reports indicating that the ISSR markers gave more recurrence, diversity and can be used to discriminate among genotypes (Abdel-lateif and Hewedy, 2018; El-Assal and Gaber, 2012; Abou-Deif *et al*., 2013).

SUMMARY

DNA molecular markers (ISSR and SCoT) were exploited to investigate the genetic distinction and relationships among six cultivars of Egyptian barley (Giza 123, 124, 126, 132, 135 and 136). SCoT primers outputted a total of 56 bands, 22 bands were polymorphic (39%). In addition, ISSR primers exhibited 66 bands and 33 of these bands were polymorphic (50%). The two markers were proved to be efficient tools in exploiting the genetic divergence among the tested cultivars and establishing specific phylogenetic relationships. Generally, the ISSR gave more polymorphism than SCoT and can be used in cultivar discrimination.

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Table (1): Barley cultivars, their pedigree and characteristics.

Name	origin	Pedigree	Characteristics
Giza 123	**ARC, Egypt**	Giza 117 /FAO86	Tolerant to salinity and fungal diseases.
Giza 124		Giza 117/ Bahteem 52// Giza	Tolerance to fungal diseases and high temperature
Giza 126		Baladi Bahteem/SD 729 Por 12769-BC.	Tolerant to drought and funal diseases
Giza 132		Rihane-05//As46/Aths*2" Aths/	Tolerance to fungal diseases
Giza 135		ZAR-ZA/BERMEJO/4/DS4931//GLORIABAR/ COPAL/3/SEN/5 /AYAROS.	Naked barley with moderate production
Giza 136		PLAISANT/7/CLN-B/LIGEE640/3/S.P B//GLORIAAR/COME B/5/FALCONBAR/6/LINOCLN-B/A/S.P-/LIGNEE640/3/S.P-B//GLORIA BAR/COME B/5/FALCONBAR/6/LINO	Naked barley with moderate production and tolerant to salinity

Table (2): List of ISSR primers (Sigma, Egypt).

Primer Name	Sequence
SCoT-1	5'-ACGAC**ATG**GCGACCACGC-3'
SCoT-2	5'-ACC**ATG**GCTACCACCGGC-3'
SCoT-3	5'-ACGAC**ATG**GCGACCCACA-3'
SCoT-4	5'-ACC**ATG**GCTACCACCGCA-3'
SCoT-5	5'-CA**ATG**GCTACCACTAGCG-3'
SCoT-6	5'-CA**ATG**GCTACCACTACAG-3'
ISSR-1	5'-AGAGAGAGAGAGAGAGC-3'
ISSR-2	5'-AGAGAGAGAGAGAGAGG-3'
ISSR- 3	5'-ACACACACACACACACT-3'
ISSR- 4	5'-ACACACACACACACACG-3'
ISSR -5	5'-GTGTGTGTGTGTGTGTG-3'
ISSR -6	5'-CGCGATAGATAGATAGATA-3'

Table (3): The similarity percentages based on SCoT data among six barley cultivars.

	Giza 123	Giza 124	Giza 126	Giza 132	Giza 135	Giza 136
Giza 123	1.00					
Giza 124	0.95	1.00				
Giza 1126	0.88	0.93	1.00			
Giza 132	0.97	0.94	0.89	1.00		
Giza 135	0.91	0.91	0.87	0.92	1.00	
Giza 136	0.82	0.82	0.84	0.81	0.89	1.00

Table (4): SCOT and ISSR analyses of barley cultivars; Total Number of Bands (TB), Monomorphic Bands (MB), Polymorphic Bands (PB), Percentage of Polymorphism (%P), Frequency (F) and Polymorphism Information Content (PIC).

Primer	TB	MB	PB	% P	F	PIC	Fragment size
SCoT-01	10	5	5	50	0.80	0.27	170-550
SCoT-02	12	9	3	25	0.93	0.12	160-2000
SCoT-03	7	5	2	29	0.79	0.28	300-1350
SCoT-04	14	6	8	57	0.76	0.30	170-1700
SCoT-05	4	2	2	50	0.67	0.35	170-650
SCoT-06	9	7	2	22	0.85	0.22	170-710
ISSR-01	15	7	8	53	0.73	0.31	210-1100
ISSR-02	12	8	4	33	0.89	0.18	220-850
ISSR-03	9	4	5	56	0.67	0.35	140-790
ISSR-04	9	4	5	56	0.67	0.35	190-770
ISSR-05	9	2	7	78	0.57	0.37	180-570
ISSR-06	12	8	4	33	0.78	0.29	200-1100
Total	**122**	**67**	**55**	**-**	**-**	**-**	**-**
Average	**10.2**	**5.6**	**4.6**	**45**	**0.76**	**0.28**	**-**

Table(5): The similarity percentages based on ISSR data among six barley cultivars.

		Giza 123	Giza 124	Giza 126	Giza 132	Giza 135	Giza 136
Giza 123	**1**	1.00					
Giza 124	**2**	0.94	1				
Giza 1126	**3**	0.87	0.89	1			
Giza 132	**4**	0.93	0.91	0.87	1		
Giza 135	**5**	0.75	0.80	0.77	0.77	1	
Giza 136	**6**	0.76	0.76	0.77	0.78	0.92	1

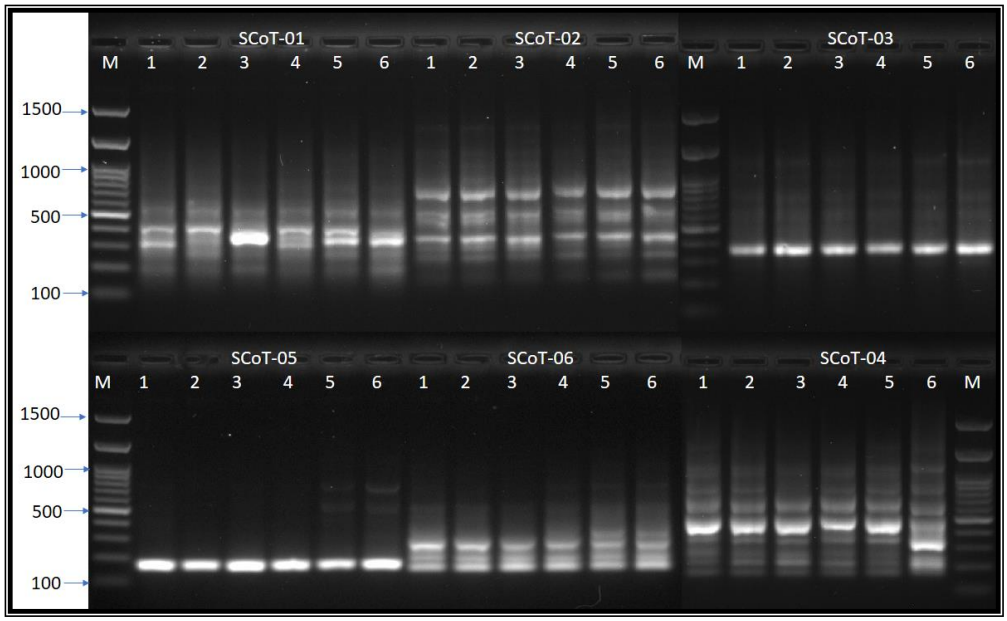

Fig. (1): SCoT banding profiles of barley cultivars: M; DNA marker, lanes 1-6; Giza 123, Giza 124, Giza 126, Giza 132, Giza 135, and Giza 136, respectively.

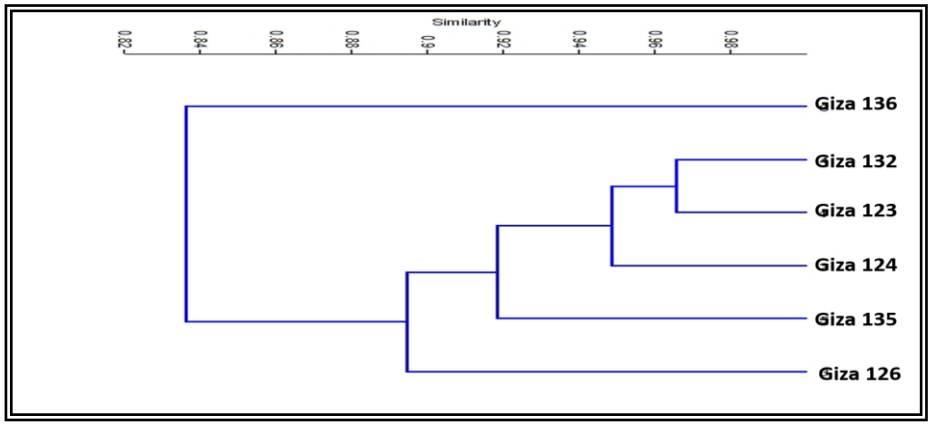

Fig. (2): The cluster analysis based on SCoT data using UPGMA method among six barley cultivars.

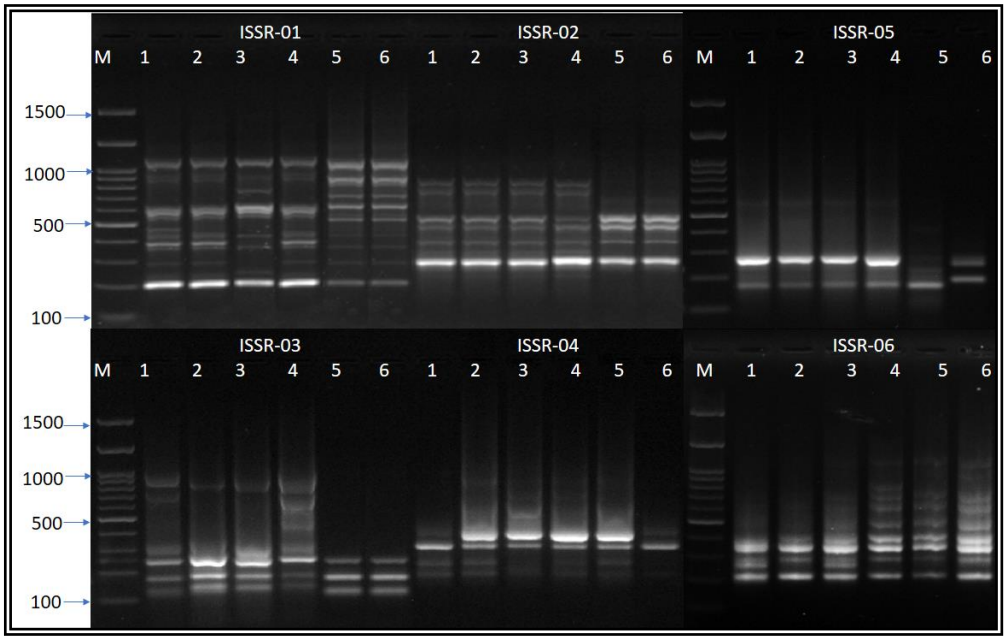

Fig. (3): ISSR banding profiles of barley cultivars: M; DNA marker, lanes 1-6; Giza 123, Giza 124, Giza 126, Giza 132, Giza 135, and Giza 136, respectively.

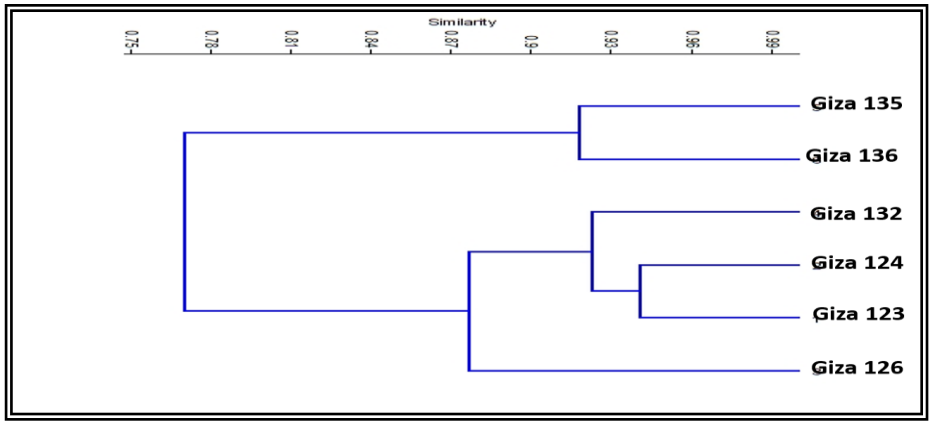

Fig. (4): The cluster analysis computed from ISSR data using UPGMA method among six barley cultivars.