Original Article

The efficiency of SCoT, ISSR, and SRAP markers for detecting genetic polymorphism among Egyptian barley genotypes

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Abstract

The results of the present study support the idea that, the tested Egyptian barley cultivars could make a useful case study for genetic diversity research. When those cultivars were examined using three distinct markers (codon targeted (SCoT), sequence-related amplified polymorphism (SRAP) and inter-simple sequence repeats (ISSR)). According to SRAP, ISSR, and SCoT analyses, respectively 85, 76, and 66 polymorphism percentages among the seven tested barley cultivars were detected. The genotype specific markers for each genotype used were determined. The data revealed that Giza127 had the highest number of unique markers, showing six specific SCoT markers, three specific SRAP markers, and one specific ISSR marker. The genetic relationships between the seven Egyptian barley cultivars are explained by the phylogenetic tree built using information gathered from various molecular markers. These gene-based molecular markers demonstrated the reliability of SCoT, ISSR, and SRAP molecular markers for identifying DNA polymorphism levels and genetic relationships in barley.

Keywords: Egyptian Barley, Molecular Marker, ISSR, SRAP, SCoT, Genetic variation.

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INTRODUCTION

Barley (Hordeum vulgare ssp. vulgare L.) is a diploid member of the Triticeae tribe. After wheat, corn, and rice, it is ranked as the fourth most significant grain produced worldwide for use in malting and brewing, human consumption, and animal feed (Morrel, 2011; Lai et al.2020) It is also referred to as a halophyte, which is a salt-tolerant plant that thrives in highly salinized soil or water (Noreen et al ,2021).Barley is also regarded to be a poor man's crop since it is easy to grow, requires little resources, and has a high degree of environmental tolerance. In ancient Egypt, a full diet was made up of materials made from barley, especially bread and beer. Considering the health advantages due to the barley's positive health effects, the need for agricultural expansion, and the decline in wheat imports, Egypt is currently looking into bringing back barley as a 30% ingredient in bread production (Mohammed et al. 2021).

Numerous molecular markers in crop species have been developed as a result of recent advances in genomics technologies. Therefore, molecular markers' development aided in our understanding of the type and degree of genetic variation in crop plants (Jan et.al., 2021). The genetic diversity of cultivars is well studied using a variety of molecular markers, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), start codon targeted (SCoT), inter-simple sequence repeats (ISSR), simple sequence repeats (SSRs), and single nucleotide polymorphism (SNP). (Jiang, 2013; Nadeem et al, 2017; Jan et al, 2021). Sequence-related amplified polymorphism (SRAP) markers, which are utilized to amplify coding portions of DNA with primers targeted to open reading frames, have been used more recently. These markers have shown to be reliable and incredibly versatile. (Robarts and Wolfe, 2014)

Recently some studies employed start codon targeted (SCoT) markers, a dominant and repeatable markers based on the short conserved region flanking the ATG start codon in plant genes. They are effectively used for assessing the genetic variability of plants and offer numerous assistances as markers of different traits, such as salinity tolerance (Gowayed and Abdelmoneim, 2021). A PCR-based molecular technique called the inter simple sequence repeat (ISSR) marker can reveal information on the genetic connections between different disease populations (Oguzet et al. 2021). (ISSR) and (SRAP) have been widely used in molecular taxonomy, gene

cloning, phylogeny, and genetic mapping because they are highly polymorphic and consistent (Gasser et al. 2006; Mutlu et al. 2008; Zhao et al. 2009).

The current study attempts to quantify and characterize the genetic variations between seven of the most significant Egyptian barley varieties, mostly for bread production. The study's findings will help to clarify the genetic differences that will increase the amount of crop that can be used to make animal and human feed.

Materials and Methods

Plant Material

Seven Barley cultivars are kindly provided by Field Crop Institute, Agricultural Research Center, Ministry of Agriculture, Egypt (Table 1).

Table 1: Names of seven barley cultivars and their characteristics

Species	Characteristics
Giza 123	Six rows, Egyptian barley variety, precocious, moderately productive in the favorable conditions and tolerant to salinity and fungi diseases. It is issued from the cross of: Giza 117 /FAO86. ARC-Egypt
Giza 126	Six rows, Egyptian barley accession, late, productive in the favorable conditions and tolerant to drought and fungi diseases. It is issued from the following cross: Baladi Bahteem/SD 729 Por 12769-BC. ARC- Egypt
Giza 127	two rows, Egyptian barley accession, precocious, high productive in the favorable conditions and tolerant to fungi diseases. It is issued from the following cross: W12291/Bags/Harmal-02. ARC-Egypt
Giza 130	Six rows, Egyptian naked barley accession, precocious, moderately productive in the favorable conditions and tolerant to drought and fungi diseases. It has been selected from the crosses ''Comp.cross'' 229//Bco.Mr./DZ02391/3/ Deir Alla 106 using the bulk method. ARC- Egypt
Giza 131	Six rows, Egyptian naked barley accession, tolerant to drought and grows well in the old irrigated lands. It has been selected from the crosses: CM67-B/CENTENO-B/3/ROW906.73/4/GLORABAR/COMEB/5/FA/CON-BAR/6/LINO
Giza 135	Six rows, Egyptian naked barley accession, late, moderately productive in the favorable conditions. It is issued from the following cross: ZARZA/BERMEJO/4/DS4931//GLORIABAR/COPAL/3/SEN/5 /AYAROS.ARC- Egypt
Giza 136	Six rows, Egyptian naked barley accession, precocious, moderately productive in the favorable conditions. It is issued from the following cross: 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. ARC- Egypt

Molecular Marker

DNA extraction

Genomic DNA was isolated from leaves of seven Barley cultivars using Cetyl Trimethyl Ammonium Bromide (CTAB) method according to Rogers and Bendich (1985) .

A) SCOT Analysis

PCR -condition

PCR reaction was performed in a total volume of 20µl containing 2µL DNA template, 10 µl master mix (Biotecke Corporation).2µlSCOT primer and the volumewas complete to 20 µl with 6µl nuclease-free water. The PCR reactions were amplified with the followingprogram: initial denaturation at 94 °C for 5 min; 35 cycle of denaturation at 94 °C for 1 min, annealing at 49–60 °Cfor 1 min. The amplified products were analyzed byelectrophoresis in 1% agarose, stained by ethidium bromide and photographed under UV light. The sequenceof the tested primers was as presented in Table2.

Table 2: Names and sequences of SCOT primers used to assess the genetic variability among seven barley cultivars

Primer	Sequence
SCOT-13	5'-ACGACATGGGGACCATCG-3'
SCOT-26	5'-ACCATGGCTACCACCGTC-3'
SCOT-31	5'-CCATGGCTACCACCGCCT-3'
SCOT-34	5'-ACCATGGCTACCACCGCA-3'
SCOT-52	5'-ACAATGGCTACCACTGCA-3'
SCOT-71	5'-CCATGGCTACCACTACCC-3'
SCOT-77	5'-CCATGGCTACCACTACCC-3'
SCOT-24	5'-CACCATGGCTACCACCAT-3'
SCOT-70	5'-ACCATGGCTACCAGCGCG-3'
SCOT-14	5'-ACGACATGGCGACCACGC-3'
SCOT-33	5'-CCATGGCTACCACCGCAG-3'
SCOT-60	5-'ACAATGGCTACCACCACA-3'
SCOT-66	5-'ACCATGGCTACCAGCGAG-3'
SCOT-36	5-'GCAACAATGGCTACCACC-3'

B) SRAP Analysis

PCR -condition

PCR reaction was accomplished for total volume of 20µl containing 2µL DNA template, 10 µl master mix. (Biotecke Corporation) , 1µl of each SRAP primer and complete volume to 20 µl with 6 µl nuclease-free water

The PCR program was initiated by denaturation at 94 C for 5 min, then five cycles of three steps: 1 min of denaturation at 94 C, 1 min of annealing at 35 C, 1 min of extension at 72 C, followed by additional 35 cycles with annealing temperature being increased to 50 C, with a final extension step of 5 min at 72 C (Li and Quiros 2001).

The amplified products were analyzed by electrophoresis in 1% agarose, stained by ethidium bromide and photographed under UV light.

Table 3: Names and sequences of SRAP primers used to assess the genetic variability among the seven barley cultivars

Primer	**Sequence**	**Primer**	**Sequence**
Me1	F:5'-TGAGTCCAAACCGGATA-3'	Em1	R:5′-GACTGCGTACGAATTAAT-3′
Me2	F:5′-TGAGTCCAAACCGGAGC-3′	Em2	R:5′-GACTGCGTACGAATTTGC-3′
Me3	F:5′-TGAGTCCAAACCGGAAT-3′	Em3	R:5′-GACTGCGTACGAATTGAC-3′
Me4	F:5'-TGAGTCCAAACCGGACC-3'	Em4	R:5′-GACTGCGTACGAATTTGA-3'
Me5	F:5′-TGAGTCCAAACCGGAAG-3′	Em6	R:5′-GACTGCGTACGAATTAAC-3′

C) ISSR Analysis

PCR -condition

The reaction volume of 20 µL containing 2µL DNA template, 10 µL master mix (Biotecke Corporation), 2µL primer and 6µL of distilled water. The PCR program was as follow : one cycle of 5 min. at 94˚C (initial denaturation) followed by 37 cycle of denaturation at 94 °C for 1 min., annealing at 52–56 °C for 1 min. and extension at 72 °C for 2 min. followed by a final extension at 72 °C for 10 min.

Then the amplified products were analyzed by electrophoresis in 1% agarose, stained by ethidium bromide and photographed under UV light.

Table 4: Names and sequences of ISSR primers used to assess the genetic variability among the seven barley cultivars

Primer	Sequence
UBC 824	(TC)8G
UBC 808	(AG)8C
UBC 810	(AG)8T
UBC 811	(GA)8C
UBC 817	(CA)8A
UBC 825	(AC)8T
UBC 834	(AG)8YT

Band scoring and cluster analysis

The SCOT, SRAP and ISSR gel images were scanned using the Gel Doc 2000 Bio-Rad system and were analyzed with Quantity One Software v 4.0.1 (BioRad Laboratories, Hercules, Co. USA). The systat ver. 7-computer program was used to calculate the pairwise differences matrix and plot the dendrogram among barley cultivars (Yang and Quiros 1993). Cluster analysis based on similarity matrices was obtained with the un-weighted pair-group method (UPGMA) using the arithmetic average to estimate the dendrogram.

Results

To investigate the genetic differences between the cultivars used, Start codon targeted (SCoT) analysis was performed. All primers used resulted in the appearance of PCR products with a variable number of bands. Ninety-two SCOT markers were detected among the seven barley cultivars of which, 61 bands were polymorphic (66.3 %). the genetic variation produced by these primers can attribute to the differences in the binding sites throughout the genome of the barley cultivars. (Fig1)

Based on the SCOT analysis, it was possible to distinguish between the seven barley genotypes. Fifteen out of the sixty-one polymorphic SCOT markers generated were found to be genotype-specific (table 6.)

The highest number of SCOT bands was detected for primers SCOT-14 (9 bands) followed by SCOT-71 and SCOT-13 and SCOT-36 (8 bands) while the lowest was scored for SCOT-31 (3 bands).

Table 5: Total number of scorable bands, polymorphism percentage, and band size of SCOT markers obtained by 14 primers

Primer	Total scorable band	Polymorphic band	Polymorphism %	Band size range
SCOT-13	**8**	**3**	**37.5**	**250-900**
SCOT-26	**6**	**3**	**50**	**350-1300**
SCOT-31	**3**	**0**	**0.0**	**350-110**
SCOT-34	**7**	**3**	**42.8**	**250-1200**
SCOT-52	**7**	**7**	**100**	**250-1500**
SCOT-71	**8**	**4**	**50**	**150-1500**
SCOT-77	**6**	**6**	**100**	**150-1200**
SCOT-24	**7**	**6**	**85.7**	**300-1200**
SCOT-70	**4**	**3**	**75**	**500-1500**
SCOT-14	**9**	**9**	**100**	**100-1400**
SCOT-33	**6**	**3**	**50**	**200-850**
SCOT-60	**6**	**4**	**66.6**	**200-750**
SCOT-66	**7**	**4**	**57.1**	**250-1300**
SCOT-36	**8**	**6**	**75**	**300-1300**
Total	**92**	**61**	**66.3**	

Table 6. The specific SCOT markers for the seven barley cultivars

Genotypes	Markers	Total marker
Giza 123	SCOT-34 (300) SCOT-52 (300)	2
Giza 126	SCOT-77 (150)	1
Giza127	SCOT-34)150(-SCOT-71)150(-SCOT-14)1400-1000-700(-SCOT-52 (250)	6
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Giza130	SCOT-26 (700)	
Giza131	SCOT-36)350(-SCOT-60 (400)	1

Giza135	SCOT-60)750(-SCOT-66)1000(-SCOT-24 (600)	2
Giza136		3
Total		**15**

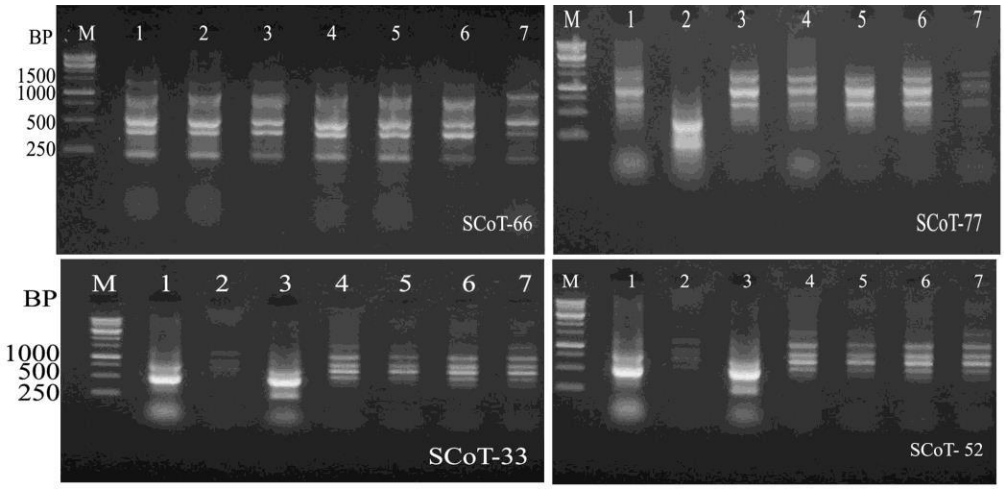

Fig.1 SCoT banding patterns among seven barley cultivars(1:7 are Giza123-Giza126-Giza127-Giza130-Giza131-Giza135-Giza136 respectively)

Nineteen different combinations of SRAP primers were used to evaluate the genetic polymorphism among the seven barley cultivars. 76 bands out of 89 bands (85%) were polymorphic. The fragment size ranged between 150 and 1250 bp approximately, the Me5-Em1 primer scored the lowest band number (1 bands) (Table 7). Table 8 shows the specific SRAP marker for different species, 4 specific markers were recorded for Giza 130 compared with Giza 126 that recorded one specific marker. (Fig,2)

Table 7 : Total number of scorable bands, polymorphism percentage, and band size of SRAP markers obtained by 19 primers

Primer	Total scorable band	Polymorphic band	Polymorphism %	Band size range
Me1-Em1	4	3	75%	250-1000
Me1-Em3	5	5	100%	250-750
Me2-Em1	5	4	80%	150-1000
Me2-Em3	4	3	75%	250-750
Me2-Em3	4	3	75%	200-700
Me2-Em4	3	2	66%	250-450
Me2-Em6	8	7	87%	150-1150
Me3-Em1	3	2	66%	450-750
Me3-Em3	5	4	80%	250-850
Me3-Em4	5	5	100%	250-750
Me3-Em6	3	1	33%	200-350
Me4-Em1	5	4	80%	250-1300
Me4-Em3	4	3	75%	400-1000
Me4-Em4	3	2	66%	350-750
Me4-Em6	7	7	100%	250-1000
Me5-Em1	1	1	100%	350
Me5-Em3	5	5	100%	400-1000
Me5-Em4	6	6	100%	200-750
Me5-Em6	9	9	100%	150-1250
Total	89	76	85%	

Table8. The specific SRAP markers for the seven barley cultivars

Genotypes	Markers	Total marker
Giza 123	Me3-Em4 (600) -Me4-Em6 (350)	2
Giza 126	Me2-Em1 (250)	1
Giza 127	Me5-Em6 (600)-Me5-Em3 (600)-Me2-Em3 (350)	3
Giza 130	Me4-Em6 (800)-Me4-Em4 (350)-Me5-Em4 (200)-Me2-Em4 (350)	4
Giza 131	Me5-Em6 (250)-Me2-Em6 (750)	2
Giza 135	Me4-Em1 (1300)-Me5-Em3 (1000)-Me3-Em3 (600)	3
Giza 136	Me2-Em6 (1000&1150)	2
Total		**17**

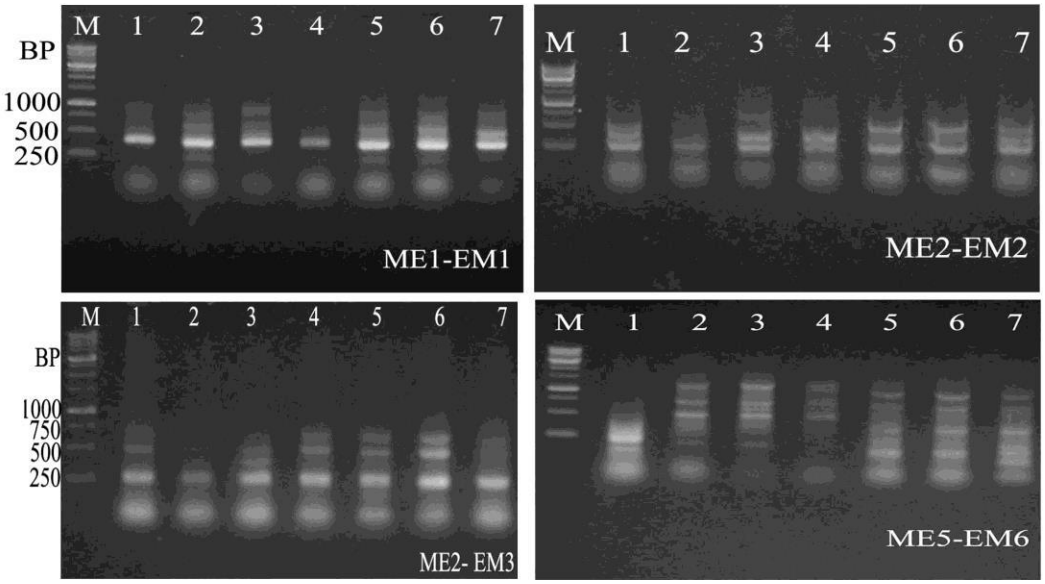

Fig.2 SRAP banding patterns among barley cultivars(1:7 are Giza123-Giza126-Giza127-Giza130-Giza131-Giza135-Giza136 respectively)

Seven ISSR markers were tested for their ability to generate ISSR banding patterns from DNA corresponding and evaluate the genetic diversity of the studied barley cultivars. 38 ISSR markers were detected among the seven barley cultivars of which, 29 bands were polymorphic (76%). Six out of the twenty-nine polymorphic ISSR markers generated were found to be genotype-specific (table 10.)The highest number of ISSR bands was found for primers UBC 817 &UBC 808(7 bands) while the lowest was scored for UBC834 (3 bands).(Fig3)

Table 9: Total number of scorable bands, polymorphism percentage, and band size of ISSR markers obtained by 7 primers

Primer	Total scorable band	Polymorphic band	Polymorphism %	Band size range
UBC 824	6	4	66%	200-750
UBC 808	7	5	71%	250-1100
UBC 810	5	5	100%	150-500
UBC 811	4	3	75%	200-450
UBC 817	7	4	57%	250-850
UBC 825	6	5	83%	400-1150
UBC 834	3	3	100%	350-500
Total	38	29	76%	

Table 10. The specific ISSR markers for the seven barley cultivars

Genotypes	Markers	Total marker
Giza 123	UBC 810 (250)	1
Giza 126	UBC 825 (400)	1
Giza 127	UBC 824 (250)	1
Giza 130	UBC 808 (600)	1
Giza 131		
Giza 135	UBC 810 (350)	1
Giza 136	UBC 817 (450)	1
Total		**6**

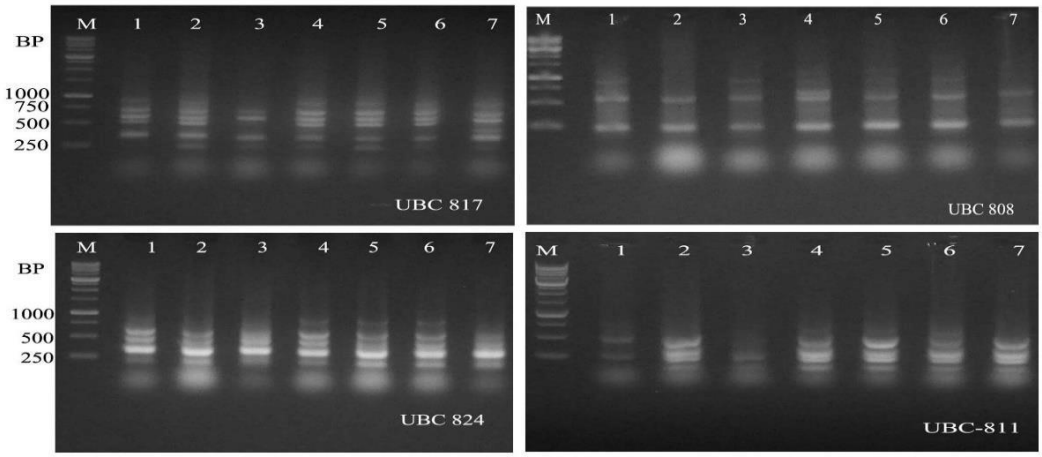

Fig.3ISSR banding patterns among barley cultivars(1:7 are Giza123-Giza126-Giza127-Giza130-Giza131-Giza135-Giza136 respevtively)

Each technique used differs in the type and amount of polymorphism detected. The polymorphism level between the three techniques varied broadly, ranging from 85% SRAP which was greater than that of ISSR (76%) and SCoT (66.3%)

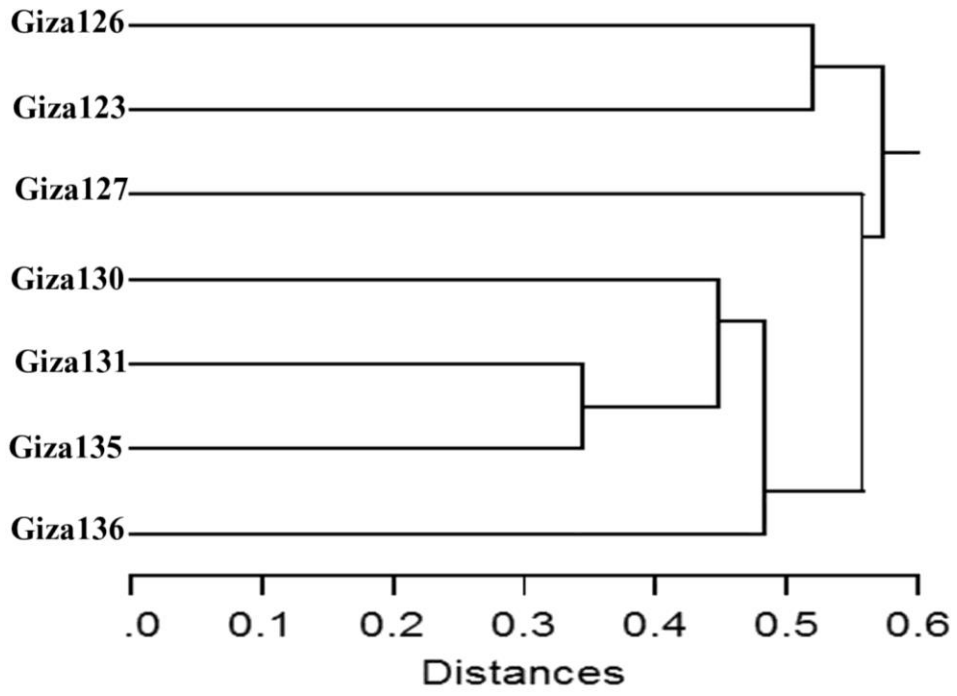

Fig.4 showed a dendrogram derived from UPGMA cluster analysis for seven Egyptian barley cultivars

Cluster analysis created a dendrogram among 7 barley cultivars which gathered the cultivars into two main clusters each group include the closest cultivars together.

Cluster one is subdivided into two sub-cluster Giza123 and Giza126. The other group is divided into sub-clusters and sub-sub clusters show the near relationship between Giza127 ,Giza136 , Giza130, Giza131 and Giza135.

The nearby relationship was between Giza123 and Giza126 also between Giza131 and Giza135 and the highest genetic distance was between cultivars Giza126 and Giza136.

Discussion

Over the course of a short period of time, countless efforts were made by researchers working in various fields to identify and select grain crops, such as barley plants, that actually exhibit some level of salt tolerance. This subsequently allows such plants to grow successfully in saline soil and domesticated desert lands and to exhibit a noticeably inflated productivity and yield at harvest. (Abdel-Hamid,2014)

The selected barley cultivars were used to test the ISSR, SCoT, and SRAP primers. In many plant species, these three marker systems (ISSR, SCoT, and SRAP) have been employed to identify cultivars and assess genetic variation (Wang.et al 2012 ,Guo et al.,2012,Lin et al.,2013).

Differences between approaches are predicted given the prevalence of genetic markers. However, these comparisons are necessary to determine which approach is best for the problems being investigated. Three systems (SRAPs, ISSRs, and SCoTs) based on polymerase chain reaction (PCR) were employed in this work.

According to a different study on 120 barley genotypes that were identified with 26 inter simple sequence repeat (ISSR) markers under heat stress and non-stress settings, in the present study, the ISSR technique revealed a high

degree of variability among the various barley cultivars. Results ISSR markers were found to be more informative in genetic diversity studies and to have greater polymorphism percentages than SCoT markers in 85 polymorphic alleles (Kumar and Agrawal ,2019 ; Ghomi et al., 2021).

The large proportion of polymorphic bands, polymorphism rate, and results that were comparable to those of earlier studies using these markers in different crop species were all advantages of this study (Amirmoradiet al. 2012;Singh et al. 2014;Heikrujamet al. 2015, Nadeem et al. 2018). The direct association between these markers and interest loci and the prevention of information loss in marker assisted selection processes utilizing prior markers are the values of these markers in plant genome analysis (Nadeem et al. 2018).

Conclusion

Finally, the results of this study support the idea that Egyptian barley varieties make a useful case study for genetic diversity research. When those cultivars were examined using three distinct markers, the findings revealed that Giza127 had the highest number of unique markers, showing six specific SCoT markers, three specific SRAP markers, and one specific ISSR marker. These gene-based molecular markers demonstrated the reliability of SCoT, ISSR, and SRAP molecular markers for identifying DNA polymorphism levels and genetic relationships in barley. Due to the low number of markers compared to SSR markers in barley, these techniques are still unsatisfactory; nonetheless, characterization of these markers' associations with important agricultural characteristics in barley can advance the strategies of marker-assisted selection.

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