98

Egypt. J. Agron. **Vol. 47,** No. 4, pp: 1315 - 1325 (2025)

Egyptian Journal of Agronomy

http://agro.journals.ekb.eg/

Genetic Diversity Analysis in Barley by ISSR and SCoT Markers

Walaa Abdel-Kader Ramadan, Mahmoud Hussien Abou-Deif*, Fatma El-Sayed Mahmoud and **Rashad Mahmoud Shoaib**

Genetics and Cytology Department, Biotechnology Institute, National Research Centre, 12622 Dokki, Giza, Egypt

ESTIMATING genetic diversity is crucial for managing genetic resources, protecting species in their natural environment, and selecting suitable parental combinations, all of which impact the genomic improvement of crop species. This study aimed to estimate the genetic diversity and relationships among ten Egyptian barley cultivars using molecular genetic markers, inter-simple sequence repeats (ISSRs), and start codon targeted (SCoT). The amplification results obtained by PCR analysis for the studied cultivars, using 12 ISSR and 10 SCoT primers, identified each of the ten cultivars. The total number of markers observed among the ten barley genotypes, based on ISSR analysis, was 953 bands in 126 loci, of which 76 loci were polymorphic, resulting in a polymorphism rate of 60.31%. The two primers ISSR-1 and ISSR-15 distinguished the cultivars in a unique banding pattern for each. The total number of markers detected among the ten cultivars based on SCoT analysis was 864 bands in 121 loci, of which 72 loci were polymorphic (59.50%). The primer SCoT-34 distinguished the barley cultivars into unique banding patterns. The genetic similarity index and dendrogram tree were performed using ISSR and SCoT amplified fragments for the studied cultivars. The values of similarity exhibited considerable differences between the barley cultivars. The similarity ranged from 72 to 94%. The results indicated that two molecular markers, ISSR and SCoT, are efficient in analyzing the genetic diversity in barley cultivars.

Keywords: Barley, PCR analysis, Molecular genetic markers, Banding pattern, Similarity index, Dendrogram tree.

Introduction

Assessment of genetic diversity is essential for the management of genetic resources. Estimating the genetic diversity of barley genotypes provides valuable information for selecting parents to develop improved barley germplasm for advantageous traits. For cloning competitive allele-specific PCR markers in barley, specific alleles/bands for gene-based markers can be used (Ghaffari *et al.*, 2014; Shakhatreh *et al*., 2016). Molecular genetic markers are heritable and widely distributed across the genome; therefore, they have various applications in genotyping and plant taxonomy (Danilevicz *et al.*, 2021). Molecular markers have been used in plants to analyze genetic variation as they effectively link genotypic and phenotypic variation (Varshney *et al.*, 2005; Grover and Sharma, 2016). The development of functional markers located in or near interest genes has become simpler due to the expansion of genomic databases (Andersen and Lübberstedt, 2003). Molecular genetic markers are powerful tools for analyzing variation between plant genomes, constructing linkage maps, and selecting parental genotypes for breeding programs. They also have various applications, including phylogenetics, conservation, developmental biology, forensics, disease testing, and paternity assessment (Appleby *et al.*, 2009; Kalendar *et al.*, 2011; Edwards *et al.*, 2014).

The molecular markers ISSRs and SCoT have been used to evaluate genetic diversity, identify cultivars, protect biodiversity, identify markers associated with specific traits, fingerprint DNA in different species, evolution, molecular ecology, phylogenetics, gene tagging, and genome mapping (Iruela *et al.*, 2002; Reddy *et al.*, 2002; Shen *et al.*, 2006 and Wang *et al.*, 2012). Due to their high polymorphism and repeatability across the barley genome, ISSRs have proven to be appropriate markers for evaluating inbreeding and genetic diversity (Adhikari *et al.*, 2017). Tanyolac (2003) and Rahimi *et al.* (2014) used ISSR markers to determine the genetic diversity in barley collections. Shayan *et al.* (2019) investigated the genetic diversity among 28 barley genotypes using 14 ISSR primers, of which eleven primers generated a total of 559 polymorphic bands of 80-3000 bp with polymorphic information content between 0.116 and 0.252. Yigider *et al.* (2024) studied the diversity and structure of 20 lines and four varieties of barley by using 16 ISSR primer pairs, generating a total of 172

*Corresponding author email: aboudeif@yahoo.com
Received: 18/04/2025; Accepted: 12/10/2025
DOI: 10.21608/AGRO.2025.372784.1656

alleles in 24 genotypes. The results indicated that the average values of Nei's genetic diversity and polymorphism information content (PIC) were 0.34 and 0.4062, respectively. Cluster analysis grouped barley genotypes into two groups. The expected heterozygosity ranged from 0.2152 to 0.3867, with a mean of 0.3057.

The molecular markers of SCoT are developed based on conserved short regions flanking to ATG translation start codon in the plant genome. SCoT markers are joined to functional genes; thus, the amplicons can be converted to gene-targeted marker systems (Collard and Mackill, 2009; Xiong *et al*., 2011). SCoT markers can be used in gene isolation and to evaluate genetic diversity (Gorji *et al*., 2011). Hamidi *et al.* (2014) compared ISSR and SCoT markers for fingerprinting 40 varieties of bread wheat. Their results suggested that the efficiency of ISSR and SCoT markers was relatively the same in fingerprinting and detecting polymorphism for genotypes, but SCoT analysis was more effective. Drine *et al*. (2016) studied the genetic variability of eight barley genotypes of various origins and pedigrees using nine ISSR primers. They found that ISSR markers revealed 72.2% polymorphic bands and cluster analysis discriminated the genotypes according to their genetic background and geographical origin.

Habiba *et al.* (2021) used ten SCoT primers to assess molecular diversity for ten barley lines. They found that the SCoT primers generated reproducible and reliable amplicons, which displayed polymorphism 66.67% to 100%. Güngör *et al.* (2022) screened 54 barley cultivars from Turkey and Bulgaria with four SCoT markers to evaluate population structure and genetic diversity. They found 92 polymorphic bands, which the genetic diversity and statistics index analysis indicated that SCoT is a powerful marker for genetic diversity analysis. Shaban *et al*. (2022) used ISSR and SCoT markers to estimate the genetic diversity in ten wheat genotypes. Seven SCoT and seven ISSR primers synthesized a total of 112 amplified DNA fragments, resulting in 61 and 51 bands, respectively. They reported that the percentage of polymorphism was 93.4% and 78.4% for SCoT and ISSR, respectively.

Abaza *et al*. (2022) characterized the genetic variations between seven Egyptian barley varieties using two distinct markers, ISSR and SCoT, which detected 76% and 66% polymorphism, respectively. Ghonaim *et al*. (2023) evaluated ten genotypes of barley using SCoT and ISSR techniques. They found that ten SCoT primers displayed a total of 94 fragments with sizes ranging from 1800 to 100 base pairs; 65 were polymorphic (62.18%). A total of 54 amplified bands with molecular sizes ranging from 2200 to 200 bp were produced using seven ISSR primers; 23 of them were polymorphic bands (40.9%). Tahir *et al*. (2023) investigated the diversity of 59 barley accessions using 44 ISSR and 12 SCoT markers. The two markers produced 255 and 101 polymorphic bands, respectively. The genetic diversity was 0.77 and 0.81, and the mean values of polymorphism were 0.74 and 0.80 for ISSR and SCoT markers, respectively. Ramadan *et al*. (2025) used molecular markers ISSR and SCoT to evaluate the genetic diversity in eighteen Egyptian faba bean genotypes. They revealed 86.2% and 92.8% polymorphisms, respectively. The Cluster analysis based on the two markers detected the genetic variability and heterogeneity within the studied genotypes.

The objectives of this study were to estimate the genetic diversity and relationships among ten Egyptian barley cultivars using molecular genetic markers, ISSRs, and SCoT.

Materials and Methods

Plant Materials

Ten Egyptian cultivars of barley (*Hordeum vulgare* L.) from different genetic origins were used in this study, named G123, G124, G126, G129, G130, G131, G132, G133, G134, and G2000 (Table 1). The cultivars were supplied by the Barley Department, Agricultural Research Center, Giza, Egypt.

Nucleic Acid Extraction

Ten grains were germinated on wet filter papers for two weeks from each of the ten barley cultivars. Then, genomic DNA was extracted from 0.5 g of fresh leaves following the Dellaporta method (Dellaporta *et al*., 1983).

PCR- ISSR and SCoT Amplifications

The genomic DNA and 12 ISSR primers were used to produce ISSR markers by the PCR technique. The primers were selected from the set of the Biotechnology Laboratory, University of British Columbia, Canada (Table 2). Ten SCoT primers (Table 3) were selected from the previous studies by Joshi *et al.* (1997) and Sawant *et al*. (1999) to produce SCoT markers. PCR analysis was performed in a reaction mixture of 25 μL, 2 μL DNA, 1 μL primer, 0.5 μL dNTPs mix, 2 μL $MgCl_2$, 5 units Taq polymerase (0.5 μL), 5 μL PCR buffer, and 14 μL H_2O. Amplification was carried out at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 34-61°C (varied for each primer) for 45 sec, and elongation at 72°C for 2 min. The final extension was 7 min at 72°C. The PCR-ISSR and SCoT amplifications were detected by a 1.2% agarose gel stained with red-safe staining, photographed under UV light, and scanned by a Gel-Documentation system.

Data Analysis

The data were analyzed using Bio-Rad Model 620 Software Programs, USA. Nei-Li's similarity index (Nei and Li, 1979) was used to evaluate genetic similarity. Pairwise comparisons between cultivars, based on the proportion of shared bands produced by the primers, were calculated using the Dice similarity coefficients using the program Free Tree (Pavlicek *et al*., 1999). A dendrogram displaying the genetic relationships between cultivars, based on the unweighted pair-group method with arithmetic averages (UPGMA), was constructed using the Tree View program (Page, 1996).

Table 1. Names and pedigrees of the ten cultivars of barley.

No.	Name	Pedigree
1	G123	Giza117/FAO86
2	G124	Giza117/Bahteem52//Giza118/FAO86
3	G126	Baladi Bahteem/SD729-Por12762-BC
4	G129	Deir Alla106/Cel//AS46/Aths*2
5	G130	Comp.cross229//Bco Mr/ DZ02391/3/Deir Alla106
6	G131	CM67-B/CENTENO//CAM-B/3/ROW906.73/4/GLORIA-BAR/COME-B/5/FALCON-161 LINO
7	G132	Rihane-05//As46/Aths*2Aths/Lignee686
8	G133	CarboxGusto
9	G134	Alanda-01/4/W12291/3Api/CM67//L2966-69
10	G2000	Giza117/Bahteem52//Giza118/FAO86/3/Baladi16/Gem

Table 2. List of 12 ISSR primers and their nucleotide sequences.

Primer	Sequence (5’-3’)	Primer	Sequence (5’-3’)
ISSR-1	ACGAACACACACACACAC	ISSR-13	GAGAGAGAGAGAGAGACC
ISSR-3	GGATGGATGGATGGAT	ISSR-14	AGAGAGAGAGAGAGAGT
ISSR-4	AGAGAGAGAGAGAGAGCT	ISSR-15	ACACACACACACACACCC
ISSR-5	ACACACACACACACACC	ISSR-23	CTCTCTCTCTCTCTCTAC
ISSR-6	GAGAGAGAGAGAGAGAAC	ISSR-25	CACACACACACACACAAG
ISSR-11	ACACACACACACACACCA	ISSR-28	TCTCTCTCTCTCTCTCG

Table 3. List of ten SCoT primers and their nucleotide sequences.

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
SCoT-1	CAACAATGGCTACCACCA	SCoT-22	AACCATGGCTACCACCAC
SCoT-12	ACGACATGGCGACCAACG	SCoT-29	CCATGGCTACCACCGGCC
SCoT-13	ACGACATGGCGACCATCG	SCoT-30	CCATGGCTACCACCGGCG
SCoT-18	ACCATGGCTACCACCGCC	SCoT-31	CCATGGCTACCACCGCCT
SCoT-19	ACCATGGCTACCACCGGC	SCoT-34	ACCATGGCTACCACCGCA

Results

Identification of Barley cultivars by ISSR Analysis

All ISSR primers exhibited distinct band patterns and were utilized for band scoring, following genetic similarity and cluster analyses. The total number of ISSR markers observed among the ten barley cultivars using 12 primers was 953 bands (Table 4) of 126 loci, of which 76 loci were polymorphic with a polymorphism of 60.31% (Table 5). The number of polymorphic bands produced per primer ranged from two (ISSR-13) to 13 bands (ISSR-11), with product sizes ranging from 121 bp to 1770 bp.

The cultivar G133 gave the highest number of PCR-amplified fragments (105 fragments) by using all primers, while cultivar G132 gave the lowest number (81 fragments), as shown in Fig. 1 and Table 4. Different numbers of amplified fragments were scored in the other cultivars. Primer ISSR-11 presented the highest number for all studied cultivars (116 fragments), while primer ISSR-5 showed the lowest number (54 fragments). An ISSR-specific marker of MW 882 bp was detected in the electrophoretic pattern of cultivar G131 using primer ISSR-5. In addition, another specific ISSR marker of 1770 bp was detected in the pattern of cultivar G129 using primer ISSR-11. These two bands can be used as molecular genetic markers to characterize and identify the two cultivars G131 and G129.

ISSR analysis using primer ISSR-1 revealed that a specific ISSR marker of 722 bp was only present in the two cultivars, G132 and G133. Primer ISSR-14 displayed a fragment of 488 bp in the cultivars G129 and G130. Primer ISSR-23 showed a specific fragment of 1299 bp in the patterns of cultivars

G126 and G130, while it showed another band of 809 bp in these cultivars. The fragments of 1251 bp and 1000 bp were detected only in the two cultivars G132 and G133 by using primer ISSR-25. One specific fragment of 1000 bp was found in the two cultivars G131 and G132, while a fragment of 515 bp was found in the two cultivars G130 and G131 by using primer ISSR-28.

Table 5 presents the polymorphism detected by the 12 ISSR primers used for the identification of barley cultivars. Primer ISSR-11 gave the highest number of polymorphic loci in all cultivars (13 fragments) with 86.66% polymorphism, while primer ISSR-13 showed the lowest number (2 fragments) with 22.22% polymorphism. The ISSR markers of the primer ISSR-1 characterized nine barley cultivars into nine patterns; each cultivar appeared in a unique pattern, except that G130 and G131 showed the same pattern. Primer ISSR-15 distinguished the ten cultivars into nine patterns; only cultivar G132 did not manifest any bands by using this primer. Primer ISSR-4 identified the ten cultivars into seven patterns, in which the three cultivars G124, G130, and G133 were presented in the same pattern, and the two cultivars G123 and G132 manifested the same pattern. Primer ISSR-11 distinguished the ten cultivars in eight patterns, and cultivars G123 and G126 showed the same pattern. Primer ISSR-14 identified ten cultivars in seven patterns, of which the three cultivars G124, G132, and G134 showed the same pattern, and the two cultivars G123 and G133 were similar. Primer ISSR-23 identified ten cultivars in seven patterns, and the two cultivars G123 and G124 presented the same pattern, while G131 and G132 displayed the same pattern. Primer ISSR-25 identified ten cultivars in seven patterns, in which the two cultivars G131 and G2000 had the same pattern, while G132 and G133 showed the same pattern. Primer ISSR-28 identified ten cultivars in seven patterns. The three cultivars G123, G124, and G126 shared the same pattern, while G133 and G2000 exhibited the same pattern. Therefore, two primers (ISSR-1 and ISSR-15) out of the twelve used in PCR-ISSR analysis successfully distinguished the ten cultivars through a unique banding pattern for each cultivar.

Identification of Barley Cultivars by SCoT Analysis

All SCoT primers exhibited distinct and reliable band patterns (Fig. 2). The total number of markers (amplified bands) detected among the ten barley cultivars based on SCoT analysis using ten primers was 864 bands (Table 6) in 121 loci, of which 72 loci were polymorphic with a polymorphism of 59.50% (Table 7). The number of polymorphic bands produced per primer ranged from three bands (SCoT-18) to ten bands (SCoT-34), and the size ranged from 155 to 1766 bp. The cultivar G131 presented the highest number of PCR-amplified fragments (91 fragments) by using all primers, while cultivar G126 gave the lowest number (81 fragments). Primer SCoT-13 gave the highest number of amplified bands (110 fragments) for all cultivars under study, while primer SCoT-12 showed the lowest number of fragments (56 fragments). Six specific markers with different molecular weights were presented in six barley cultivars; SCoT-1 (802 bp), SCoT-22 (1419 bp), SCoT-29 (1100 bp), SCoT-30 (1322 bp), SCoT-30 (708 bp) and SCoT-31 (813 bp) in the cultivars G133, G130, G126, G124, G130 and G129, respectively. These six bands can be used as molecular genetic markers for identifying these cultivars. SCoT analysis using primer SCoT-1 illustrated a specific marker of 955 bp in the patterns of the two cultivars G123 and G124. The two cultivars, G131 and G2000, showed a band of 705 bp in their patterns using SCoT-19; the primer also produced a band of 450 bp in the patterns of the two cultivars, G129 and G133. Two bands of 1244 bp and 682 bp were only presented in the patterns of the two cultivars, G130 and G134, by using SCoT-34. The ten SCoT primers revealed polymorphism used for the identification of barley cultivars, as shown in Table 7. Primer SCoT-34 gave the highest number of polymorphic loci in all cultivars (10 fragments) with 76.92% polymorphism, while primer SCoT-18 showed the lowest number of polymorphic loci (3 fragments) with 37.50% polymorphism. Primer SCoT-1 characterized nine barley cultivars into eight patterns, in which each cultivar appeared in a unique pattern, except G131 and G134 showed the same pattern, while cultivar G126 did not manifest any band. Primer SCoT-13 distinguished the ten cultivars in eight patterns, and the three cultivars G123, G124, and G126 gave the same pattern. Primer SCoT-19 identified the ten cultivars into eight patterns, and the two cultivars, G123 and G126, were presented in the same pattern, while the cultivar G130 did not give any band. Primer SCoT-29 distinguished the ten cultivars into nine patterns, and the two cultivars, G129 and G130, showed the same pattern. Primer SCoT-30 identified ten cultivars in nine patterns, and the two cultivars G123 and G126 showed the same pattern. Primer SCoT-34 identified ten cultivars in ten patterns; each cultivar gave different pattern. Therefore, primer SCoT-34 out of ten used in PCR-SCoT analysis succeeded in distinguishing the ten barley cultivars in a unique banding pattern for each cultivar.

Genetic Similarity

The genetic similarity and dendrogram tree were performed using Nei-Li's similarity index based on ISSR and SCoT markers for the ten barley cultivars, as shown in Table 8 and Fig. 3. The values of similarity exhibited substantial differences, ranging from 72 to 94%, with an average of 83%. Some distinctive cultivars gave high values of genetic similarity with others, for example, G123 and G124 (94%), G123 and G126 (88%), and G123 and G129 (88%). However, some cultivars showed slightly low genetic similarity, such as G126 and G132 (72%), G126 and G134 (76%), and G132 and G134 (76%). The dendrogram showed that the ten cultivars could be divided into two main clusters; one of them has only one cultivar, G132. Another cluster was separated into two sub-clusters; the first sub-cluster contained one cultivar, G130, while the second contained eight other cultivars divided into two groups. The first group included the two cultivars G134 and G2000, with a similarity of 82%. The second group was divided into two subgroups. The first sub-group contained the two cultivars G131 and G133 with a similarity of 85%, while the second sub-group included the remaining four cultivars G123, G124, G126, and G129 with a similarity of 94% for G123 and G124, 88% for G123 and G126, and 88% for G123 and G129.

Table 4. Total bands produced from each primer of ISSR for the barley cultivars and all amplified fragments in each variety.

Varieties	Primers												
	ISSR-1	ISSR-3	ISSR-4	ISSR-5	ISSR-6	ISSR-11	ISSR-13	ISSR-14	ISSR-15	ISSR-23	ISSR-25	ISSR-28	Total bands
G123	10	7	10	6	7	12	8	8	11	6	9	7	101
G124	8	8	11	6	7	13	8	9	10	6	10	7	103
G126	7	8	9	6	7	12	8	9	13	6	5	7	97
G129	9	7	9	6	7	13	9	7	13	3	9	6	98
G130	9	0	11	4	6	14	7	8	11	8	8	7	93
G131	9	7	8	5	7	12	9	8	8	5	7	7	92
G132	11	6	10	5	8	4	8	9	0	5	11	4	81
G133	11	7	11	6	7	14	9	8	12	6	11	3	105
G134	8	7	9	5	1	12	7	9	7	8	7	6	86
G2000	11	8	8	5	8	10	7	10	13	7	7	3	97
Total bands	93	65	96	54	65	116	80	85	98	60	84	57	953

Table 5. The polymorphic loci and specific band values in barley cultivars as revealed by ISSR markers.

Primer	loci	range sizes of loci	polymorphic bands	Monomorphic bands	Polymorphism (%)	No of specific bands	Total bands in all genotypes
ISSR-1	12	222-1100	7	5	58.33	0	93
ISSR-3	8	267-1570	3	5	37.5	0	65
ISSR-4	11	142-911	5	6	45.45	0	96
ISSR-5	7	154-1103	3	4	42.85	1	54
ISSR-6	8	155-596	7	1	87.5	0	65
ISSR-11	15	185-1770	13	2	86.66	1	116
ISSR-13	9	150-1313	2	7	22.22	0	80
ISSR-14	11	152-928	5	6	45.45	0	85
ISSR-15	14	121-1711	9	5	64.28	0	98
ISSR-23	10	251-1452	8	2	80	0	60
ISSR-25	12	163-1599	8	4	66.66	0	84
ISSR-28	9	267-1309	6	3	66.66	0	57
Total	126	121-1770	76	50	60.31	2	953

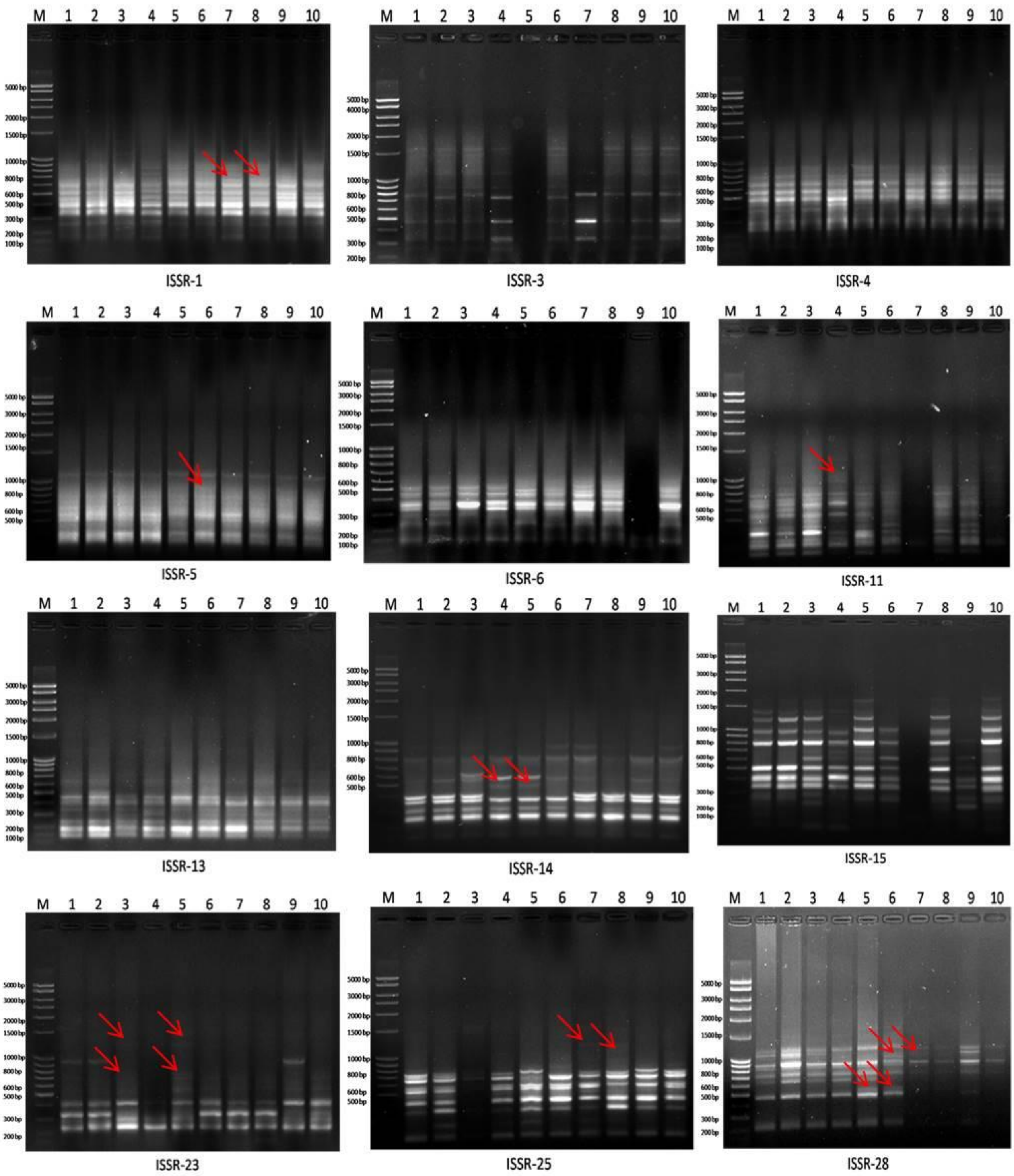

Fig. 1. Electrophoretic profiles of PCR-DNA products by using 12 ISSR primers with ten barley cultivars 1-10: G123, G124, G126, G129, G130, G131, G132, G133, G134, and G2000. M: DNA ladder markers ranged in size from 5000 to 100 bp.

Table 6. **Total bands produced from each primer of SCoT for the barley cultivars and all amplified fragments in each cultivar.**

Varieties	Primers									
	SCoT-1	SCoT-12	SCoT-13	SCoT-18	SCoT-19	SCoT-22	SCoT-29	SCoT-30	SCoT-31	SCoT-34	Total bands
G123	9	6	11	7	12	10	11	9	8	7	90
G124	10	3	11	7	10	10	13	9	8	7	88
G126	0	6	11	8	12	8	14	9	7	6	81
G129	10	7	9	7	12	9	10	8	10	6	88
G130	9	5	12	7	0	11	10	11	9	11	85
G131	10	6	11	7	13	8	9	10	9	8	91
G132	9	7	10	7	5	11	9	9	9	9	85
G133	11	6	11	7	10	11	10	11	0	7	84
G134	10	4	13	6	11	10	10	6	9	9	88
G2000	10	6	11	5	12	11	9	8	3	9	84
Total bands	88	56	110	68	97	99	105	90	72	79	864

Table 7. **The polymorphic markers loci and specific band values in barley cultivars as revealed by SCoT analysis.**

Primer	Loci	Range sizes of loci	Polymorphic bands	Monomorphic bands	Polymorphism (%)	No. of specific bands	Total bands in all genotypes
SCoT-1	14	155-1422	7	7	50	1	88
SCot-12	7	217-1350	4	3	57.14	0	56
SCot-13	13	188-1350	7	6	53.84	0	110
SCoT-18	8	222-777	3	5	37.5	0	68
SCoT-19	14	159-1766	9	5	64.28	0	97
SCoT-22	14	178-1594	7	7	50	1	99
SCoT-29	14	155-1433	9	5	64.28	1	105
SCoT-30	14	251-1550	9	5	64.28	2	90
SCoT-31	10	190-1355	7	3	70	1	72
SCoT-34	13	211-1423	10	3	76.92	0	79
Total	121	155-1766	72	49	59.50	6	864

Table 8. Percentages of genetic similarity for the ten barley cultivars based on ISSR and SCoT banding patterns.

	G 123	G 124	G 126	G 129	G 130	G 131	G 132	G 133	G 134	G 2000
G123	1									
G124	0.94	1								
G126	0.88	0.87	1							
G129	0.88	0.87	0.83	1						
G130	0.80	0.80	0.77	0.80	1					
G131	0.86	0.85	0.82	0.84	0.82	1				
G132	0.80	0.80	0.72	0.78	0.77	0.82	1			
G133	0.84	0.85	0.80	0.81	0.80	0.85	0.80	1		
G134	0.83	0.83	0.76	0.80	0.77	0.83	0.76	0.77	1	
G2000	0.83	0.82	0.78	0.80	0.77	0.83	0.80	0.84	0.82	1

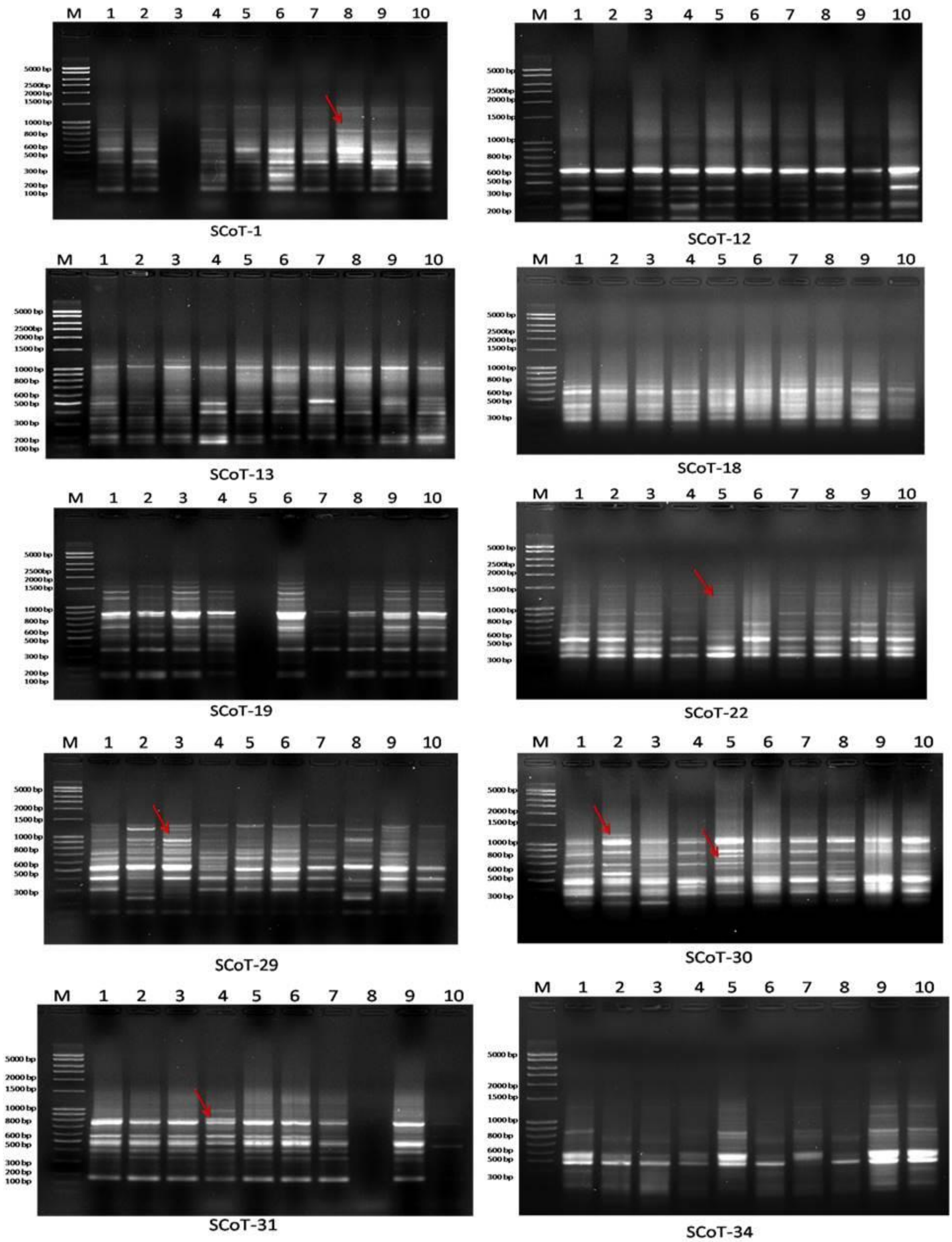

Fig. 2. Electrophoretic profiles of PCR-DNA products by using ten primers of SCoT with barley cultivars 1-10: G123, G124, G126, G129, G130, G131, G132, G133, G134, and G2000. M: DNA ladder markers ranged in size from 5000 to 100 bp.

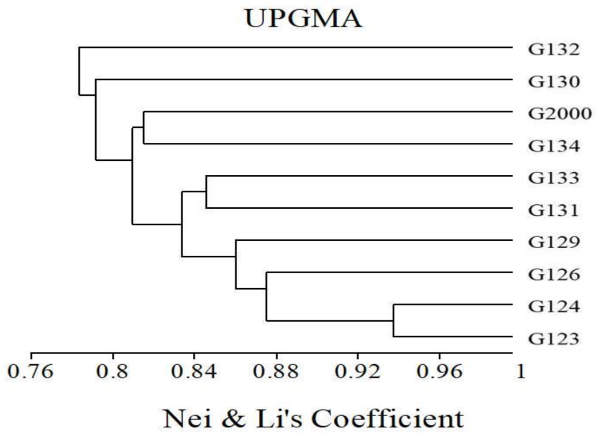

Fig. 3. Dendrogram representing the genetic relationships among the ten barley cultivars via UPGMA cluster analysis using ISSR and SCoT markers.

Discussion

In this study, two different molecular markers, ISSR and SCoT, were used in genetic diversity analyses of ten Egyptian barley cultivars. The results indicated that the two molecular markers revealed high polymorphism and were efficient in analyzing the genetic diversity in barley cultivars, which distinguished and identified each of them in a unique banding pattern. The two primers (ISSR-1 and ISSR-15) out of the twelve used in PCR analysis successfully distinguished the ten cultivars through a distinctive banding pattern. Additionally, primer SCoT-34 identified the ten barley cultivars through a unique banding pattern for each cultivar. On the other hand, some cultivars of barley revealed similarity in banding patterns and also showed similarities in their pedigrees. The percentages of genetic similarity showed that the two cultivars, G123 and G124, displayed a high value of genetic similarity (94%), and the dendrogram presents them in a single sub-group. In addition, the two cultivars, G124 and G126, showed a high value of genetic similarity (87%) and were grouped in a sub-group of the dendrogram. The Study of genetic diversity and relationships between cultivars enables the selection of the best parents for the development of improved barley germplasm for advantageous traits. The specific bands of ISSR and SCoT produced from PCR analysis in this study can be used as molecular genetic markers for identifying cultivars of good performance. Molecular genetic markers are heritable and widely distributed across the genome; therefore, they have many applications in genotyping and plant taxonomy.

The results in the study coincided with those reported by several investigators who used molecular markers in their studies for genetic analyses. Adhikari *et al.* (2017) indicated that ISSR markers have proven suitable markers for inbreeding and genetic diversity studies in barley, compared to other types of molecular markers, due to their high polymorphism and repeatability across the genome. Tanyolac (2003) and Rahimi *et al.* (2014) reported that ISSRs as genetic markers measured genetic diversity in various barley varieties. Shayan *et al.* (2019) investigated the genetic diversity among 28 barley genotypes using 14 ISSR primers. A total of 559 polymorphic bands with 80-3000 bp were generated by 11 primers, with which polymorphic information was between 0.116 and 0.252. Yigider *et al.* (2024) studied the diversity and structure of 20 lines and four varieties by using 16 ISSR primer pairs; their results indicated that the average values of Nei's genetic diversity and polymorphism information content (PIC) were 0.34 and 0.4062, respectively. Cluster analysis using ISSR markers grouped barley genotypes into two groups, and the expected heterozygosity ranged from 0.2152 to 0.3867, with a mean of 0.3057.

SCoT marker is useful because it is derived from the region of the genome associated with functional genes (Seyedimoradi *et al*., 2016; Tomar and Malik, 2016; Nadeem *et al*., 2017; Shekhawat *et al*., 2018). Nadeem *et al*. (2017) reported that, in the analysis of plant genomes, the SCoT marker is useful due to its direct link with the target locus, which prevents loss of information in a marker-assisted selection program. Habiba *et al.* (2021) used ten SCoT primers to assess molecular diversity for ten barley lines. They found that the SCoT primers generated reproducible and reliable amplicons, revealing 66.67% to 100% polymorphism. Güngör *et al.* (2022) screened 54 barley cultivars from Turkey and Bulgaria with four SCoT markers to evaluate population structure and genetic diversity. They found 92 polymorphic bands for the SCoT marker, which the genetic diversity and statistics index analyses indicated that SCoT is a powerful marker in genetic diversity analysis. Tahir *et al*. (2023) investigated the diversity of 59 barley accessions using ISSR and SCoT markers. They found a total of 391 amplified polymorphic bands were generated using 44 ISSR and 12 SCoT primers revealed average values of gene diversity 0.77 and 0.81, respectively.

Conclusion

The genetic variation and relationship information between plant resources significantly affect crop improvement. In the studied barley cultivars, ISSR and SCoT markers were efficient in estimating genetic variation, polymorphic fragments percentage, and specific bands. The percentages of genetic similarity and the dendrogram analyses indicated that some cultivars, such as G132 and G130, have low similarity with the cultivars G123 and G124, which can be used in a hybridization program. This indicates the power of these markers in fingerprinting and characterization of each barley cultivar in a unique pattern that can be used in barley breeding programs. Therefore, to obtain improvement and development of new cultivars with high yield performance and resistance to abiotic stresses, we should focus on finding more genetic information.

Consent for Publication:

All authors declare their consent for publication.

Authors' Contributions:

The manuscript was edited and revised by all authors.

Competing Interests:

The authors declare that they have no competing interests.

References

Abaza, N.O., Yousief, S.S., Moghaieb, R.E.A. (2022) The efficiency of SCoT, ISSR, and SRAP markers for detecting genetic polymorphism among Egyptian barley genotypes. *Journal of Pharmaceutical Negative Results*, 13(3):1851-1862.

Adhikari, S., Saha, S., Biswas, A., Rana, T.S., Bandyopadhyay, T.K., Ghosh, P. (2017) Application of molecular markers in plant genome analysis: a review. *Nucleus*, 60:283-297.

Andersen, J.R., Lübberstedt, T. (2003) Functional markers in plants. *Trends in Plant Science*, 8:554–560.

Appleby, N., Edwards, D., Batley, J. (2009) New technologies for ultra-high throughput genotyping in plants. *Methods in Molecular Biology*, 513:19-39.

Collard, B.C.Y., Mackill, D.J. (2009) Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter*, 27:86-93.

Danilevicz, M.F., Fernandez, C.G.T., Marsh, J.I., Bayer, P.E., Edwards, D. (2021) High-throughput genotyping technologies in plant taxonomy. *Methods in Molecular Biology*, 2222:149-166. DOI:10.1007/978-1-0716-0997-2-9.

Dellaporta, S.L., Wood, J., Hicks, J.B. (1983) A plant DNA minipreparation. *Plant Molecular Biology*, 1:19-21.

Drine, S., Guasmi, F., Ben Ali, S., Triki, T., Boussorra, F., Ferchichi, A. (2016) Genetic diversity analysis of different barley (*Hordeum vulgare* L.) genotypes from arid and humid regions using ISSR and RAPD markers. *Journal of New Science, Agriculture and Biotechnology*, 34(2):1930-1939.

Edwards, D., Zander, M., Dalton-Morgan, J., Batley, J. (2014) New technologies for ultrahigh-throughput genotyping in plant taxonomy. *Methods in Molecular Biology*, 1115:151-175.

Ghonaim, M.M., Attya, A.M., Aly, H.G., Mohamed, H.I., Ahmed, A.A., Omran, A.A.A. (2023) Agro-morphological, biochemical, and molecular markers of barley genotypes grown under salinity stress conditions. *BMC Plant Biology*, 23(1):526. DOI:10.1186/s12870-023-04550-y

Ghaffari, P., Talebi, R., Keshavarz, F. (2014) Genetic diversity and geographical differentiation of Iranian landrace, cultivars and exotic chickpea lines as revealed by morphological and microsatellite markers. *Physiology and Molecular Biology of Plants*, 20(2):225–233.

Gorji, A.M., Poczai, P., Polgar, Z., Taller, J. (2011) Efficiency of arbitrarily amplified dominant markers (SCOT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. *American Journal of Potato Research*, 88:226-237.

Grover, A., Sharma, P.C. (2016) Development and use of molecular markers: past and present. *Critical Reviews in Biotechnology*, 36:290-302.

Güngör, H., İlhan, E., Kasapoğlu, A.G., Filiz, E., Hossein-Pour, A., Valchev, D., Haliloğlu, K., Dumlupınar, Z. (2022) Genetic diversity and population structure of barley cultivars released in Turkey and Bulgaria using iPBS-retrotransposon and SCoT markers. *Journal of Agricultural Sciences*, 28(2):239–250.

Habiba, R.M., Bashasha, J., Haffez, S.H., Abo Leilah, A.A.A. (2021) Assessment of genetic diversity using SCoT markers and some morphological traits in ten lines of barley (*Hordeum vulgare* L.). *Assiut Journal of Agricultural Sciences*, 52(4):53-65.

Hamidi, H., Talebi, R., Keshavarz, F. (2014) Comparative efficiency of functional gene-based markers, start codon targeted polymorphism (SCoT) and conserved DNA derived polymorphism (CDDP) with ISSR markers for diagnostic fingerprinting in wheat (*Triticum aestivum* L.). *Cereal Research Communications*. 42(4):558–567.

Iruela, M., Rubio, J., Cubero, J.I., Gil, J., Millan, T. (2002) Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. *Theoretical and Applied Genetics*, 104:643–651

Joshi, C., Zhou, H., Huang, X., Chiang, V.L. (1997) Context sequences of translation initiation codon in plants. *Plant Molecular Biology*, 35:993-1001.

Kalendar, R., Flavell, A.J., Ellis, T.H.N., Sjakste, T., Moisy, C., Schulman, A.H. (2011) Analysis of plant diversity with retrotransposon-based molecular markers. *Heredity*, 106:520-530.

Nadeem, M.A., Nawaz, M.A., Shahid, M.Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G., Baloch, F.S. (2017) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, 32(2):261-285. DOI:10.1080/13102818.2017.1400401

Nei, M., Li, W.H. (1979) Mathematical model for studying genetic variations in terms of restriction endonucleases. *Proceedings of National Academy of Sciences*, 76:5269-5273.

Page, R.D.M. (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, 12:357-358.

Pavlicek, A., Hrda, S., Flegr, J. (1999) FreeTree-freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of the genus *Frenkelia*. *Folia Biologica* (Praha), 45:97-109.

Rahimi, M., Hervan, I.M., Valizadeh, M., Kajori, F.D., Ebrahimpour, F. (2014) Genetic diversity among wild and cultivated barley by ISSR marker. *Bulletin of Environment, Pharmacology and Life Sciences*, 3(10):57–62.

Ramadan, W.A., Baheeg, M.A., Mahmoud, F.E., Zayed, E.M. (2025) Estimation of genetic variation of Faba bean genotypes using agronomic traits, biochemical, ISSRs, and SCOTs markers. *Egyptian Journal of Agronomy*, 47(3):419-432.

Reddy, M.P., Sarla, N., Siddiq, E.A. (2002) Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, 128:9-17.

Sawant, S.V., Singh, P.K., Gupta, S.K., Madnala, R., Tuli, R. (1999) Conserved nucleotide sequences in highly expressed genes in plants. *Journal of Genetics*, 78:123–131.

Seyedimoradi, H., Talebi, R., Fayaz, F. (2016) Geographical diversity pattern in Iranian landrace durum wheat (*Triticum turgidum*) accessions using start codon targeted polymorphism and conserved DNA-derived polymorphism markers. *Environmental and Experimental Biology*, 14:63-68.

Shaban, A.S., Arab, S.A., Basuoni, M.M., Abozahra, M.S., Abdelkawy, A.M., Mohamed, M.M. (2022) SCoT, ISSR and SDS-PAGE investigation of genetic diversity in several Egyptian wheat genotypes under normal and drought conditions. *International Journal of Agron*omy, 2022:1-14.

Shakhatreh, Y., Baum, M., Haddad, N., Alrababah, M., Ceccarelli, S. (2016) Assessment of genetic diversity among Jordanian wild barley (*Hordeum spontaneum*) genotypes revealed by SSR markers. *Genetic Resources and Crop Evolution*, 63(5):813–822.

Shayan, S., Vahed, M.M., Mohammadi, S.A., Ghassemi-Golezani, K., Sadeghpour, F., Yousefi, A. (2020) Genetic diversity and grouping of winter barley genotypes for root characteristics and ISSR markers. *Journal of Plant Production*, 43(3):323-336.

Shen, J., Ding, X., Liu, D., Deng, G., He, J., Li, X., Tang, F., Chu, B. (2006) 'Inter simple sequence repeats (ISSR) molecular fingerprinting markers for authenticating populations of *Dendrobium officinale* 'Kimura et Migo'. *Biological & Pharmaceutical Bulletin*, 29:420-422.

Shekhawat, J.K., Rai, M.K., Shekhawat, N.S., Kataria, V. (2018) Exploring genetic variability in *Prosopis cineraria* using two gene-targeted CAAT box-derived polymorphism (CBDP) and start-codon targeted (SCoT) polymorphism markers. *Molecular Biology Reports*, 45:2359-2367.

Tahir, N., Lateef, D., Rasul, K., Rahim, D., Mustafa, K., Sleman, S., Mirza, A., Aziz, R. (2023) Assessment of genetic variation and population structure in Iraqi barley accessions using ISSR, CDDP, and SCoT markers. *Czech Journal of Genetics and Plant Breeding*, 4:1-12. DOI:10.17221/112/2022-CJGPB

Tanyolac, B. (2003) Inter-simple sequence repeat (ISSR) and RAPD variation among wild barley (*Hordeum vulgare* subsp. *spontaneum*) populations from west Turkey. *Genetic Resources and Crop Evolution*, 50(6):611–614.

Tomar, P., Malik, C.P. (2016) Genetic diversity assessment in *Trachyspermum ammi* L. Sprague using CDDP and CBDP markers. *The Journal of Plant Science Research*, 32:27-36.

Varshney, R.K., Graner, A., Sorrells, M.E. (2005) Genic microsatellite markers in plants: features and applications. *Trends in Biotechnology*, 23:48–55.

Wang, X., Yang, R., Feng, S., Hou, X., Zhang, Y., Li, Y., Ren, Y. (2012) Genetic variation in *Rheum palmatum* and *Rheum tanguticum* (*Polygonaceae*), two medicinally and endemic species in China, using ISSR markers. *PLOS One*, 7: e51667.

Xiong, F., Zhong, R., Han, Z., Jiang, J., He, L., Zhuang, W., Tang, R. (2011) Start codon-targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) genotypes. *Molecular Biological Reports*, 38:3487-3494.

Yigider, E., Akgun. I., Yuksel, S. (2024) Determination of genetic diversity with ISSR assay among barley genotypes. *Iranian Journal of Science*, 48:289-299