



GENETIC VARIATION AMONG *ZEA MAYS* GENOTYPES USING START CODON TARGETED MARKER POLYMORPHISM

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SUMMARY

Maize (*Zea mays* L.) is considered as one of the most important cereals after wheat and rice for nutritional value and industrial use. Molecular markers have been frequently used by breeders for studying genetic variation and selecting suitable promising genotypes for hybridization programs. The current study was conducted with the aim to determine the genetic variation among 10 synthetic local and exotic maize genotypes (SNH-8605, IPA-5012, Sarah, Buhooth-106, IPA-5011, Biotech Bag, DKC-5783, 3078, 89-May-70, and Pio-3751) using 11 start codon targeted (SCoT) markers. These primers produced a total of 627 fragments across 10 maize genotypes, in which 56 were found to be polymorphic with an average of 5.09 polymorphic fragments per primer, and the number of amplified fragments ranged from 16 (SCoT-63) to 117 (SCoT-44). Among all the studied primers, primer SCoT44 produced the highest and lowest molecular size amplification with unique bands, i.e., 1670 and 102 bp, respectively. The highest discriminatory value and polymorphic bands were produced by primers SCoT-44 and SCoT-12. Primer SCoT-29 produced the highest value for efficiency and polymorphism. The highest numbers of monomorphic bands were produced by primers SCoT-9, SCoT-30, and SCoT-36. Primer SCoT-12 provided a unique fingerprint for seven maize genotypes. The lowest genetic distance (0.167) was observed between maize genotypes Sarah (local) and IPA-5011 (local), whereas the highest value for genetic distance (0.5066) was obtained between two other genotypes, i.e., DKC-5783 (USA) and 3078 (Russia). The variation in genetic distance among genotypes was correlated to the geographical origins of the genotypes. A UPGMA dendrogram was constructed for 10 genotypes based on SCoT markers. In the phylogenetic tree, the maize genotypes with the most closely related geographical origins were arranged in the same cluster. Present results could serve as a guideline for the selection of promising maize genotypes for future breeding programs to obtain the optimum heterosis in maize.

Keywords: SCoT molecular markers, genetic variation, cluster analysis, phylogenetic tree, *Zea mays* L.

Key findings: SCoT markers were found to be capable of revealing genetic variation among maize germplasm. Cluster and genetic distance analyses among maize genotypes were associated with their geographical origins and pedigree.

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INTRODUCTION

Maize (*Zea mays* L.) is known as the “queen of cereals” and considered as one of the world’s most important cereals after wheat and rice (Dhaka *et al.*, 2010; Ahmad *et al.*, 2011; Iqbal *et al.*, 2015; Vivodik *et al.*, 2016). The maize plant is monoecious and protandrous, and hot dry weather usually accelerates pollen shedding (Poehlman, 1977). It is an annual short-day plant and belongs to family Poaceae and tribe Maydeae. It is grown at an altitude from sea level to 3300 m above sea level and from 500 N to 400 S latitude in temperate, sub-tropical, and tropical regions of the world (Iqbal, 2009). It is used as a staple food by human beings in the majority of developing countries and worldwide and is also processed into many food byproducts (Eo and Jo, 2017). Maize is also used in diverse industrial feed products and as silage for feeding livestock and as a substrate for biogas stations (Ranum *et al.*, 2014). It has achieved a remarkable place in agricultural industry due to its wide utilization. Worldwide maize yields are estimated to be 4000 industrial products (Sprague *et al.*, 1988).

For the identification of superior genotypes, genetic variation is required in breeding populations. However, this is usually also a problem with the crossing of individuals with a broad genetic base

for the creation of genetic variation followed by selection (Ali *et al.*, 2019). Another reason is that many genes control quantitative traits, and finding a genotype with desirable genes at all loci is difficult. To overcome this issue, selection should be collectively based on parental genotype performance for targeted traits and then proceed to make crosses (Ali *et al.*, 2018). Sometimes, the expression of one trait (one gene) is modified by the manifestation of other traits (other genes). In addition, gene linkage blocks are difficult to break up. Therefore, in the present era of molecular breeding, conventional breeding has a sustainable base. It is a well-known fact that the application of molecular markers must be certified through conventional breeding (Ali *et al.*, 2017).

The exploitation of genetic variability in the germplasm of any crop species is considered the key point for making further genetic improvement in economically important traits. The production and evolution of high-yielding and well-adopted cultivars with desirable characters generally remain the prime objective of plant breeding programs. In maize, a greater magnitude of genetic variability has been reported, which indicates the potential for genetic improvement (Wattoo *et al.*, 2009).

Previous studies reported variation in maize germplasm by using

morphological characters (Shrestha, 2019; Sultana, 2019), biochemical markers (Nerling *et al.*, 2018), and molecular markers (Sharma *et al.*, 2018; Adu *et al.*, 2019). However, preference was given to molecular markers because morphological and biochemical markers are subject to the effects of genotype by environment interactions (Kumar, 1999; Kumar *et al.*, 2009). Among molecular markers, start codon targeted (SCoT) markers are the dominant markers developed by Collard and Mackill (2009) from transcribed and conserved regions flanking the initiation codon sequences of genes and thus may be linked to gene function (Luo *et al.*, 2014). These molecular primers are an advanced tool and possess high resolution and polymorphism (Gorji *et al.*, 2011). They may be used directly in marker-assisted breeding programs (Mulpuri *et al.*, 2013) and in the application study of genetic diversity in crop plants, i.e., date palm (AL-Qurainy *et al.*, 2015), summer squash (Xanthopoulou *et al.*, 2015), durum wheat (Etminan *et al.*, 2016), grape variety discrimination (Miro *et al.*, 2017), and pistachio (Baghizadeha and Dehghan, 2018).

In maize germplasm, SCoT markers have been used to study cultivar genetic relationships, polymorphism, and genetic diversity (Vivodik *et al.*, 2016, Vivodik *et al.*, 2017, Sadek and Ibrahim, 2018). Genetic uniformity in maize hybrids and insufficient genetic diversity are major problems due to the low availability of diverse germplasm and preventing environmental damage resulting in low yield because the selection of an improved maize genotype mainly depends on available genetic variability within the breeding

material (Bauer *et al.*, 2007). In Iraq, maize production declined sharply during 2006 to 2014 due to the decreased supply of irrigation water, which discouraged farmers from choosing maize as a summer crop (AL-Niemi *et al.*, 2019). Therefore, it is earnestly required that breeders should take some necessary measures to promote the maize crop and to raise the efficiency of its production through the creation of new cultivars characterized by drought resistance with high production potential. The enhanced and enlarged genetic variation in maize germplasm can help in the selection of new maize genotypes through hybridization (AL-Nuaimi *et al.*, 2019). In the present study, 11 SCoT primers were used to detect polymorphism among selected local and exotic maize genotypes.

MATERIALS AND METHODS

Maize germplasm and procedure

Ten synthetic local and exotic maize genotypes were collected from certified sources, including four local genotypes (IPA-5012, Sarah, Buhooth-106, and IPA-5011); one genotype each from Turkey (SNH-8605) and Russia (3078); and four genotypes from the USA (Biotech Bag, DKC-5783, 89-May-70, and Pio-3751). The selected genotypes have diverse morphological and yield traits, including plant height, days to tasseling and silking, cobs (number, covering, shape, length, and size), and location of lower cobs from the soil surface (AL-Badeiry, 2013). Sowing was made during 2018 in the Coated Agriculture Project, Department of Biology, Faculty of Science, University of Kufa, Iraq. Six-

week-old seedlings were used for genomic DNA extraction.

Total genomic DNA extraction

Genomic DNA from the selected maize genotypes was extracted using a Genomic DNA Mini Kit, Geneaid Biotech. Ltd. The evaluation of the concentration and purity of isolated DNA were performed using a Biodrop apparatus.

Polymerase chain reaction (PCR) and amplification

For amplification process, 11 SCoT primers developed by Collard and Mackill (2009) were selected for the present study. Their names and sequences are provided in Table 1. Each 20 µl amplification reaction contained 8 µl of template DNA, 5 µl of Maxime PCR Pre-Mix Kit (i-Taq) containing (i-TaqTM DNA Polymerase (5U/µl): 2.5 U, dNTPs 2.5 mM each, reaction buffer (10x): 1x, gel loading buffer: 1x), and 5 µl of primer. The final reaction volume of 20 µl was completed with deionized distilled water. Amplification was performed in a programmed thermocycler (Agilent Technology Surecycler 8800, Malaysia) with the following program (94 °C for 3 min; 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min; and a final extension at 72 °C for 5 min (Vivodik *et al.*, 2016). The separation of amplified products was performed in 2% agarose in 1× TBE buffer stained using ethidium bromide and photographed using UV-Transilluminator, Cleaver Scientific, UK.

Statistical analysis

The results of agarose gel electrophoresis were scored for data analysis. The presence of a product was identified as (1) and absence as (0). The data were later entered into PAST statistic vital program, Version 62.1 (Hammer *et al.*, 2001). A dendrogram was constructed based on genetic distance using the Unweighted Pair-Group Method with Arithmetical Average (UPGMA). Polymorphism, primer efficiency, and discriminatory value were calculated in accordance with Hunter and Gaston (1988) and Graham and McNicol (1995).

RESULTS

In the present study, 10 synthetic local and exotic maize genotypes originating from different countries were studied. Maize genotypes with diverse morphological and yield traits, i.e., plant height, days to tasseling and silking, cobs (number, covering, shape, length, and size) and location of lower cobs from the soil surface, were used as established by Al-Badeiry (2013) (Table 2). The high diversity for these morphological traits among the selected maize genotypes provided a basis for wide differences at the molecular level.

Total genomic DNA was extracted from the selected maize genotypes. The quantity and quality of the DNA were further checked on agarose gel. High-quality DNA with the concentration of 76.81 µg/ml and purity of 1.9 was obtained from all samples. The genomic DNA from all

Table 1. SCoT Primers sequences (5'-3').

SCoT primers	Primer sequence (5'-3')	SCoT primers	Primer sequence (5'-3')
SCoT-29	CCATGGCTACCACCGGCC	SCoT-54	ACAATGGCTACCACCAGC
SCoT-36	GCAACAATGGCTACCACC	SCoT-9	CAACAATGGCTACCAGCA
SCoT-6	CAACAATGGCTACCACGC	SCoT-40	CAATGGCTACCACTACAG
SCoT-44	CAATGGCTACCATTAGCC	SCoT-60	ACAATGGCTACCACCACA
SCoT-30	CCATGGCTACCACCGGCG	SCoT-63	ACCATGGCTACCACGGGC
SCoT-12	ACGACATGGCGACCAACG	-	-

Table 2. Morphological characteristics of studied maize genotypes.

Genotypes	Plant height (cm)	Days to tasseling	Days to silking	Cob covering	No. of Cobs	Lower cob height (cm)	Cob shape	Cob length (cm)	Cob size
Buhooth-106	196	50	59	Closed	1.8	63	Cylindrical	22	Large
IPA-5012	149	47	53	Closed	1.6	56	Cylindrical	13	Small
IPA-5011	192	50	53	Closed	2	40	Cylindrical	12	Small
Sarah	195	47	52	Closed	1.2	82	Cylindrical	13	Small
SNH-8605	179	52	57	Closed	1.6	54	Cylindrical	18	Medium
3078	138	55	60	Closed	1.2	62	Conical	11	Small
Pio-3751	210	47	52	Medium	1.8	79	Cylindrical	16	Medium
DKC-5783	219	48	51	Closed	1.8	97	Cylindrical	24	Large
89-May-70	196	48	52	Closed	1.6	93	Conical	23	Large
Biotech Bag	200	48	51	Closed	2	73	Cylindrical	23	Large

Table 3. SCoT primer amplification product analysis.

Primers	Molecular size (bp)	Main bands	Amplified bands	Monomorphic bands	Polymorphic bands	Unique bands	Polymorphism (%)	Primer efficiency	Discriminatory value (%)	Genotype with unique fingerprint
SCoT-29	1039-213	11	49	1	9	1	81	0.183	16	1,8,9
SCoT-36	724-223	7	51	4	1	2	14.2	0.019	1.78	3,6,10
SCoT-6	528-214	8	58	3	4	1	50	0.068	7.14	10
SCoT-44	1670-102	22	117	3	10	9	45	0.085	17.85	1,2,5,6,7
SCoT-30	661-166	10	64	4	4	2	20	0.062	7.14	1,9,10
SCoT-12	1182-162	19	66	2	10	7	52	0.151	17.85	1,5,6,7,8,9,10
SCoT-54	771-102	7	51	3	4	0	57	0.078	7.14	1,2,8
SCoT-9	767-146	9	75	4	5	0	55	0.066	8.9	3,7
SCoT-40	948-208	9	52	2	4	3	44	0.076	7.14	6
SCoT-60	392-222	4	28	1	3	0	75	0.107	5.3	0
SCoT-63	551-269	4	16	1	2	1	50	0.125	3.57	4

Table 4. Genetic relationships among maize genotypes.

Genotypes	SNH-8605	IPA-5012	Sarah	Buhooth-106	IPA-5011	Biotech Bag	DKC-5783	3078	89-May-70	Pio-3751
SNH-8605	0									
IPA-5012	0.21998	0								
Sara	0.32275	0.3119	0							
Buhooth-106	0.27649	0.26373	0.23618	0						
IPA-5011	0.27619	0.28839	0.167	0.20412	0					
Biotech Bag	0.29982	0.23511	0.39037	0.42459	0.37231	0				
DKC-5783	0.31056	0.24934	0.38188	0.43263	0.36294	0.22036	0			
3078	0.4166	0.40784	0.35308	0.35332	0.26342	0.47111	0.50668	0		
89-May-70	0.23618	0.30093	0.27709	0.18738	0.22074	0.44864	0.44052	0.36294	0	
Pio-3751	0.31216	0.34367	0.30093	0.25034	0.27619	0.46374	0.45607	0.43263	0.26405	0

samples was used to amplify the PCR products relative to 11 SCoT primers. In the present study, all the 11 SCoT primers were used for the analysis of 10 local and exotic maize genotypes produced amplification products and resulted in varying polymorphic banding patterns. The amplified fragments from all maize samples based on 11 SCoT primers were illustrated (Figures 1, 2, 3, 4, and 5). The fragments amplified from the maize genotypes with primers SCoT-29, SCoT-36, and SCoT-6 are given in Figure 2. A few bands with these primers can be seen as variable.

Furthermore, primers SCoT-44, SCoT-30, and SCoT-30 produced fragments with different patterns (Figure 3). Among these primers, SCoT-44 and SCoT-12 showed considerable variation in banding pattern. Variation in amplified fragments could also be seen with primers SCoT-54 and SCoT-9 (Figure 4). Primers SCoT-40, SCoT-60, and SCoT-63 failed to produce variable bands in the tested maize genotypes. Among all the tested primers, SCoT-29, SCoT-44, and SCoT-12 produced highly diverse fragments as revealed in the abovementioned figures. The patterns of the amplified PCR products of the studied primers were used for calculation and data analysis (Table 3). The 11 primers produced a total of 627 fragments across 10 maize genotypes. Of these total fragments, 56 were found to be polymorphic with an average of 5.09 polymorphic fragments per primer, and the number of amplified fragments ranged from 16 (SCoT-63) to 117 (SCoT-44).

Maximum polymorphism (81%) was observed for primer SCoT-29. Among all the tested primers, SCoT-44 primer produced both the highest

and lowest molecular sized amplified products, i.e., 1670 and 102 bp, respectively. Primer SCoT-44 produced the highest values for the main, amplified, and unique bands, i.e., 22, 117, and 9, respectively. Monomorphic bands reached four in primers SCoT-36, SCoT-30, and SCoT-9. Primers SCoT-44 and SCoT-12 produced the highest number of polymorphic bands, and each produced 10 bands. The highest polymorphism and primer efficiency reached 81 and 0.183, respectively, in primer SCoT29. Primers SCoT-12 and SCoT-44 produced the highest value for discrimination (17.85). Primer SCoT-12 was found to be successful in giving a unique fingerprint for seven genotypes: SNH-8605, IPA-5011, Biotech Bag, DKC-5783, 3078, 89-May-70, and Pio-3751. The lowest value for main bands was 4 in primers SCoT-60 and SCoT-63, whereas the lowest value for monomorphic bands produced by primers SCoT-29, SCoT-60, and SCoT-63 was 1. Primer SCoT63 produced the lowest number of amplified bands that reached 16 bands. Primer SCoT-36 produced the lowest values for polymorphic bands, polymorphism, primer efficiency, and discrimination value that reached one band, 14.2, 0.019, and 1.78, respectively. Primers SCoT-54, SCoT-9, and SCoT-60 gave no unique bands. Primer SCoT-60 failed to give any unique fingerprint for all studied genotypes (Table 3).

Genetic distances among the studied maize genotypes were recorded (Table 4). Variable genetic distances were observed between all of the genotypes. Local genotypes showed the lowest distance, whereas exotic genotypes revealed the highest genetic distances. The lowest genetic distance, i.e., 0.167, was observed

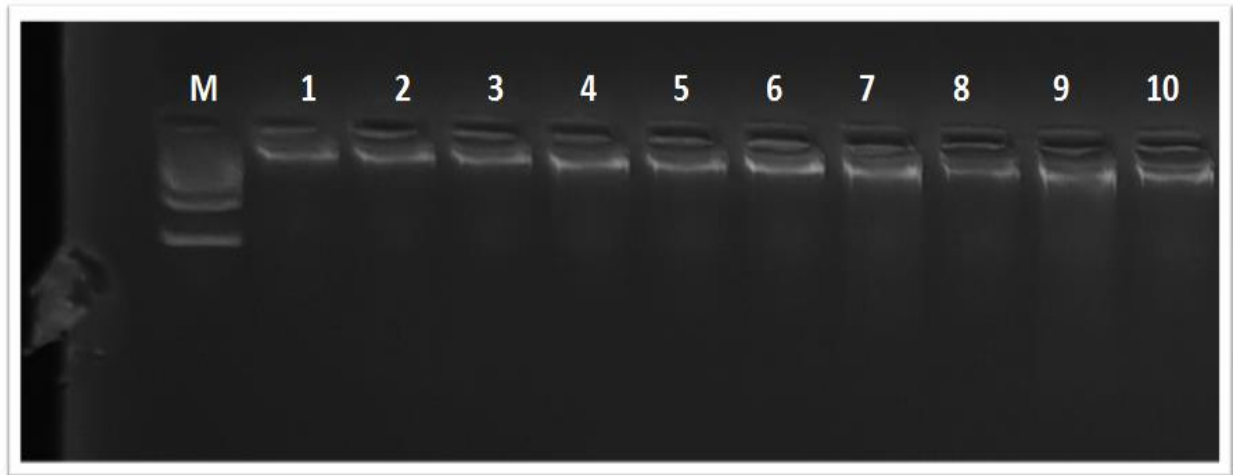


Figure 1. Agarose gel electrophoresis of genomic DNA using 2% agarose on 0.9% agarose for half an hour at 90 V for 1) SNH-8605, 2) IPA-5012, 3) Sarah, 4) Buhooth-106, 5) IPA-5011, 6) Biotech Bag, 7) DKC-5783, 8) 3078, 9) 89-May-70, and 10) Pio-3751 maize genotypes. M: DNA ladder.

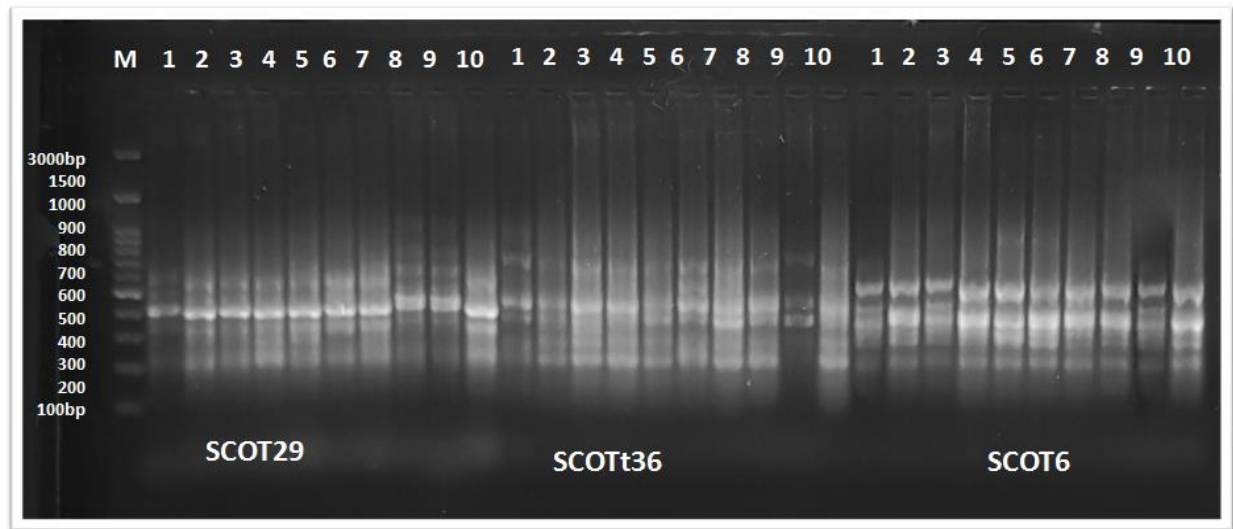


Figure 2. PCR amplification products of 10 maize genotypes produced with SCoT markers SCoT-29, SCoT-36, and SCoT-6. Lanes 1–10: maize genotypes. M: 100 bp plus ladder.

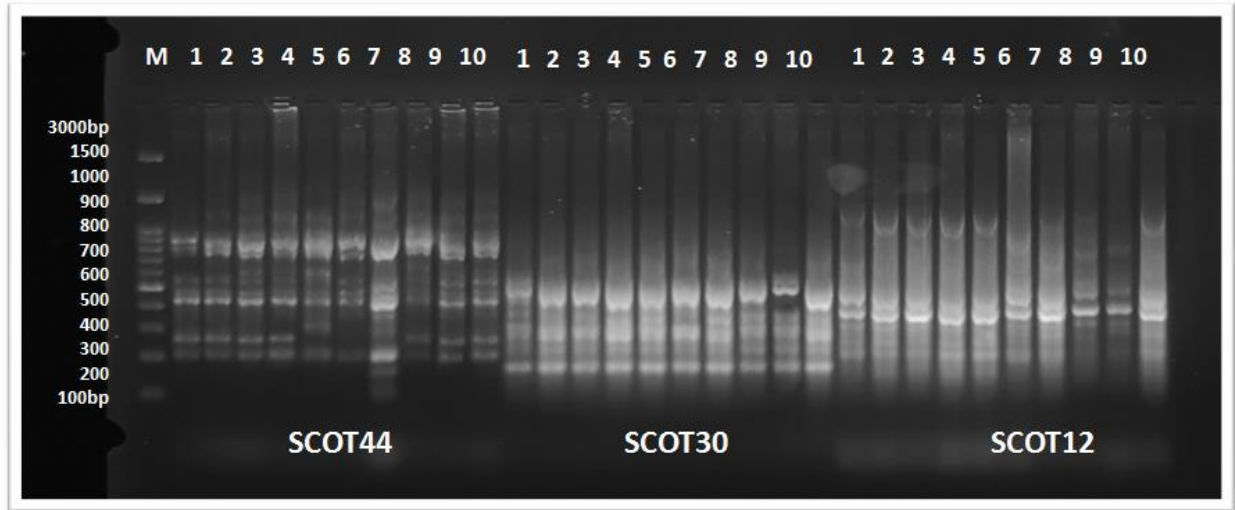


Figure 3. PCR amplification products of 10 maize genotypes produced with SCoT markers SCoT-44, SCoT-30, and SCoT-12. Lanes 1–10: maize genotypes. M: 100 bp plus ladder.

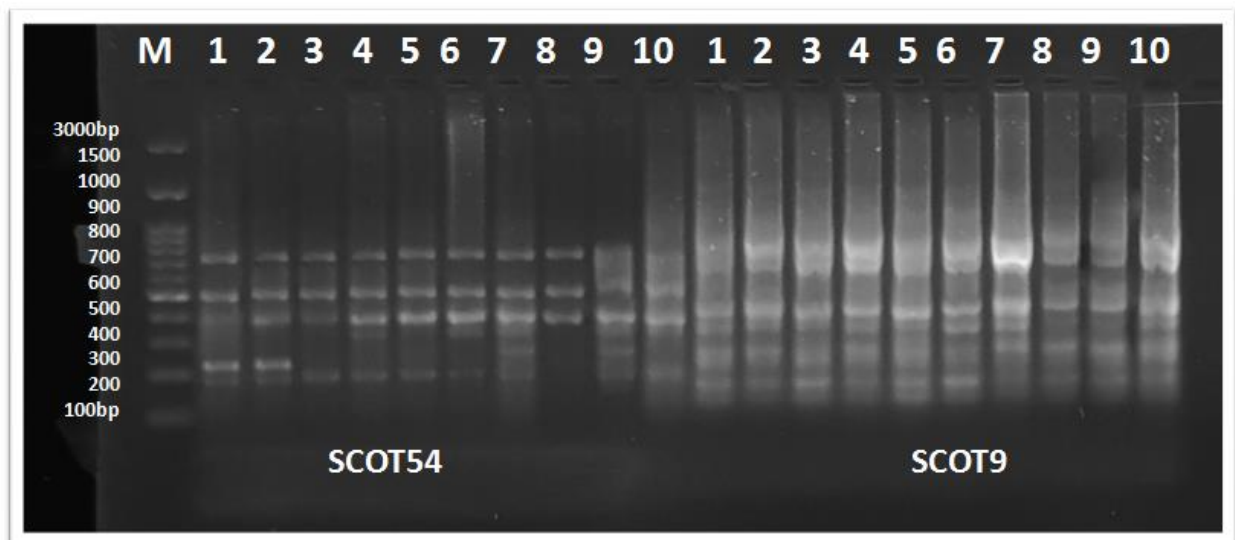


Figure 4. PCR amplification products of 10 maize genotypes produced with SCoT markers SCoT-54 and SCoT-9. Lanes 1–10: maize genotypes. M: 100 bp plus ladder.

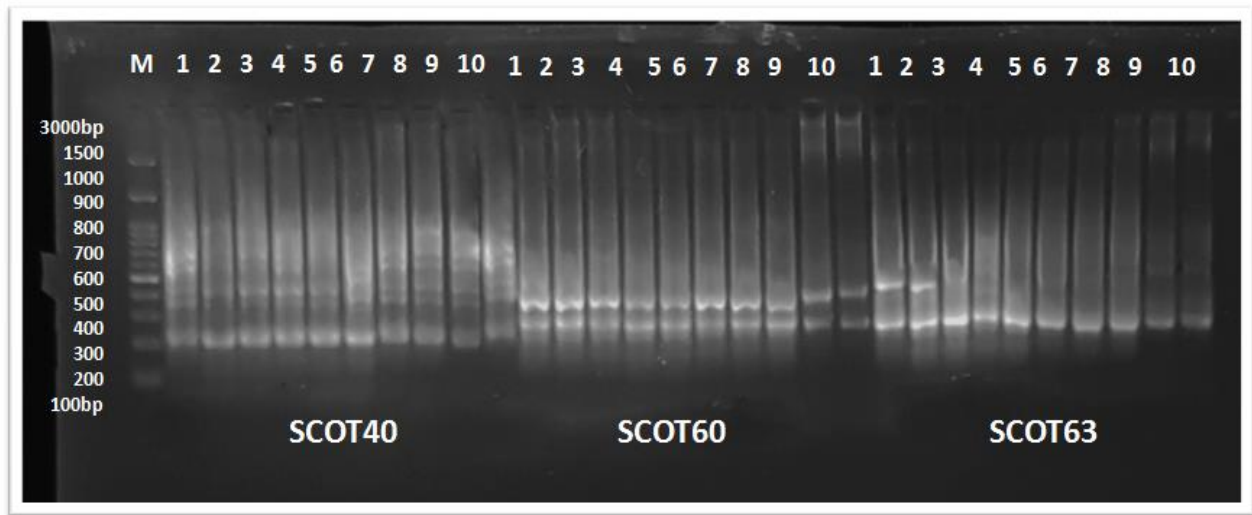


Figure 5. PCR amplification products of 10 maize genotypes produced with SCoT markers SCoT-40, SCoT-60, and SCoT-63. Lanes 1–10: maize genotypes. M: 100 bp plus ladder.

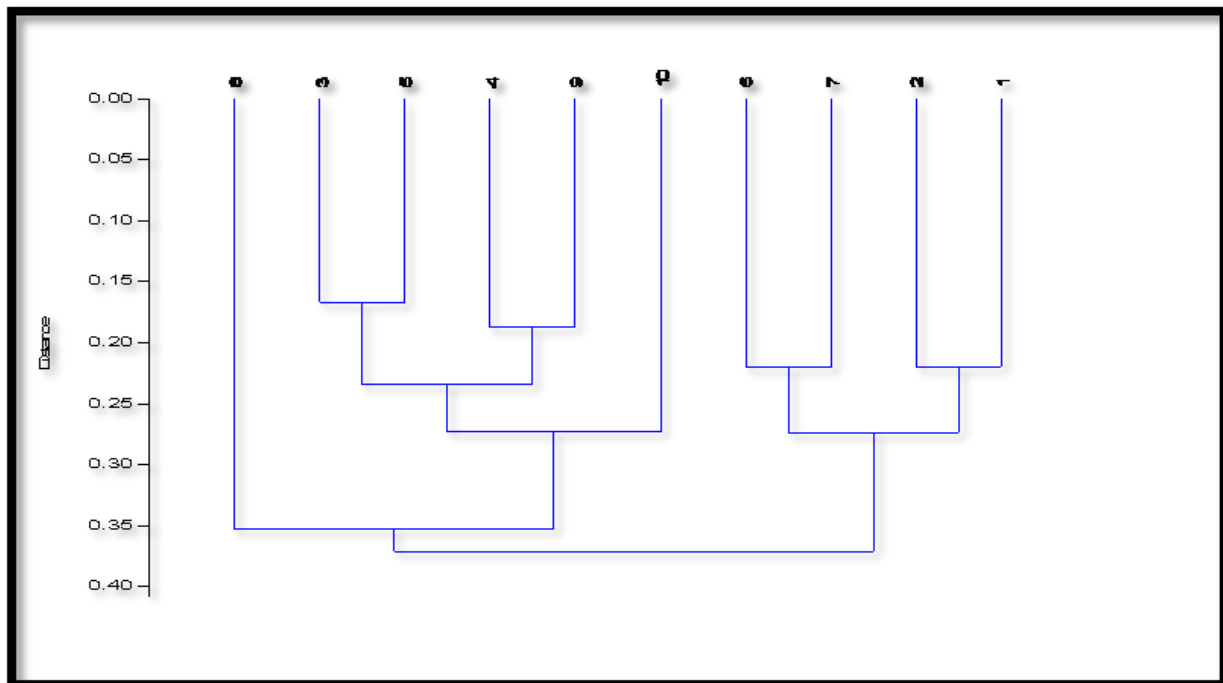


Figure 6. UPGMA dendrogram of the phylogenetic relationship among 10 maize genotypes by 11 SCoT primers.

between Sarah (local) and IPA-5011 (local), whereas the highest value of 0.5066 was noted between DKC-5783 (USA) and 3078 (Russia). Nearly the same genetic distance was also observed between the maize genotypes SNH-8605 and Buhoth-106 and SNH-8605 and IPA-5011. Phylogenetic relationship revealed that the 10 maize genotypes were grouped in two main clusters (Figure 6). The first small cluster included only 3078 (Russia), whereas the other large cluster included SNH-8605 (Turkey), IPA-5012 (local), Sarah (local), Buhoth-106 (local), IPA-5011 (local), Biotech Bag (USA), DKC-5783 (USA), 89-May-70 (USA), and Pio-3751 (USA). The large main cluster was further divided into two sub-clusters. The first included SNH-8605 (Turkey), IPA-5012 (local), Biotech Bag (USA), and DKC-5783 (USA). The other subcluster included Sarah (local), Buhoth-106 (local), IPA-5011 (local), 89-May-70 (USA), and Pio-3751 (USA).

DISCUSSION

In the present study, the 11 SCoT primers revealed varying degrees of polymorphism among the tested maize genotypes. The highest polymorphism, i.e., 81%, was achieved for primer SCoT-29. This showed the ability of this primer to reveal genetic variation in an individual germplasm. Primers differ in their ability to recognize different regions (annealing sites) in the genome. Generally, the primers that recognize more annealing sites are considered more useful for genetic polymorphism studies than primers that recognize a low number of annealing sites because the

recognition of more annealing sites will result in the production of a high number of amplified fragments and thus high chances of polymorphism among individuals of a population. Given these characteristics, SCoT primers provide comparatively more chances of revealing polymorphism in individuals as compared with RAPD and ISSR primers (Williams *et al.*, 1990; Xiong *et al.*, 2009; Collard and Mackill, 2009). DNA polymorphism at the molecular level is the product of several causes, including point mutations (base deletion or insertion) at the primer annealing site in the genome that consequently prevents primer amplification (Williams *et al.*, 1990; Hurtado and Rodriguez, 1999). In the present study, nearly all the tested primers revealed polymorphic bands in all maize genotypes.

In the current study, low polymorphism reached 49% per primer as compared with 76.54% per primer reported in a previous study (Vivodik *et al.* 2016). This low polymorphism might be due to the close pedigrees and geographical origins of the studied genotypes (Al-Badeiry, 2013). The amplification products generated by these markers are always associated with functional genes and their traits. Therefore, the presence of unique bands indicates the determination of a particular agricultural trait (Fadoul *et al.*, 2013). In the present study, the maize genotypes revealed 56 polymorphic bands out of 110 main bands. This polymorphism might be due to the inherent variation in the morphological traits and origins of the selected maize germplasm. Maize germplasm belonging to different origins were reported to show variations in morphological traits (Badeiry, 2013).

The variation in the molecular sizes of amplified products was found to be related to an insertion or deletion between primer annealing sites (Hurtado and Rodriguiz, 1999). In the present study, a low number of amplicons was produced by SCoT markers, i.e., 62.7 per primer, in comparison with RAPD markers that produced 100 amplicon per primer (Al-Badeiry, 2013). The reason is that the SCoT primers amplify only functional gene sequences. Therefore, a low number of bands per primer is expected. On the contrary, RAPD markers are independent of coding regions, and therefore more bands are expected (Vivodik *et al.*, 2016). The presence of monomorphic bands related to all the studied genotypes belonging to one particular species, thus they share several genome sequences, which are called constant or conserved sequences, that appear as monomorphic bands (Al-Judy, 2004; Al-Badeiry, 2013). The stability of these conserved sequences is important to study plant response to different stress factors. The presence of these common sequences among the number of genotypes only without other genotypes may refer to certain characters between these genotypes, such as resistance to unsuitable environmental factors and disease (Al-Tamimi, 2014). This study established a high number of unique bands, which reached nine for primer SCoT-44; these unique sequences indicated that each genotype possessed one or more particular sequences (novel sequence) that were not found in other genotypes. Such unique bands have been successfully used as genetic markers for the identification and differentiation of these genotypes (Vishwanath *et al.*,

2010; Al-Badeiry, 2013; Al-Tamimi, 2014).

Variation among SCoT markers in revealing genetic variations is based on SCoT primer design. For example, the change in a single nucleotide within the last three nucleotides at the 3' end can affect banding patterns. In the current study, SCoT-29 and SCoT-30 differed by a single nucleotide; SCoT-54 and SCoT-60 differed by two nucleotide; and SCoT-6 and SCoT-9 differed in the last four nucleotides at the 3' end. This nucleotide variation resulted in the production of different electrophoretic profiles and consequently their polymorphism as proven in a previous study (Vivodik *et al.*, 2016). Other primers were intensively different and thus produced intensively different DNA profiles. The higher discriminatory value (17.85) for primers SCoT-44 and SCoT-12 was concerned with high polymorphic bands that reached 10 bands. The discriminatory power of a particular primer can be increased by increasing the number of identified genotypes (Arif *et al.*, 2010; Al-Badeiry, 2013; Al-Tamimi, 2014). An efficient primer is unnecessary to produce a high number of amplified bands, but its ability to show differences between studied genotypes is important (Newton and Graham, 1997). This was clearly shown in primers SCoT-44 and SCoT-29 because these two primers produced the highest number of unique fingerprints for the studied genotypes. Our results are in complete agreement with those of previously reported studies (Vishwanath *et al.*, 2010; Al-Badeiry, 2013; Al-Tamimi, 2014). The variation in genetic distance in relation to a genotype's geographical origin and pedigree was established by

Sadek and Ibrahim (2018). The results of the present study are supported by previous studies that established the efficiency in using SCOT markers in studying genetic variation among maize germplasm (Vivodik *et al.*, 2016; Vivodik *et al.*, 2017; Sadek and Ibrahim, 2018) and other crops including *Elymus sibiricus* (Zhang *et al.*, 2015), sweet potato (Nair *et al.*, 2016), durum wheat (Etminan *et al.*, 2016), and *Mentha* (Khan *et al.*, 2017).

CONCLUSION

SCoT markers can efficiently reveal polymorphism and fingerprinting in Iraqi and exotic maize genotypes. Thus, our study established that these markers can be used in genetic diversity studies. Given that these markers are associated with coding regions in the genomes, they may be used for QTL mapping and targeted fingerprinting in the future, in addition to their importance in choosing appropriate gene pools for genetic improvement, especially for industrially important crops. High genetic distances between genotypes, especially between DKC-5783 (USA) and 3078 (Russia), may be successfully used for hybridization programs for heterosis.

REFERENCES

- Adu GB, Awuku FJ, Amegbor IK, Haruna A, Manigben KA, Aboyadana PA (2019). Genetic characterization and population structure of maize populations using SSR markers. *Ann. Agric. Sci.* 64: 47-54.
- AhmadS, Khan S, Ghaffar M, Ahmad F (2011). Genetic diversity analysis for yield and other parameters in maize (*Zea mays* L.) genotypes. *Asian J. Agric. Sci.* 3: 385-388.
- AL-Badeiry NAM (2013). Molecular and cytological studies on some *Zea mays* varieties in Iraq. PhD Dissertation, Department of Biology, Faculty of Science, University of Kufa, Iraq.
- Ali S, Khan NU, Gul R, Naz I, Goher R, Ali N, Khan SA, Hussain I, Saeed M, Saeed M (2018). Genetic analysis for earliness and yield traits in maize. *Pak. J. Bot.*50: 1395-1405.
- Ali S, Khan NU, Gul S, Goher R, Naz I, Khan SA, Ali N, Saeed M, Hussain I, Khan SM, Ali I (2019). Heterotic effects for yield related attributes in F₁ populations of maize. *Pak. J. Bot.* 51: 1675-1686.
- Ali S, Khan NU, Khalil IH, Iqbal M, GuL S, Ahmed S, Ali N, Sajjad M, Afridi K, Ali I, Khan SM (2017). Environment effects for earliness and grain yield traits in F₁ diallel populations of maize (*Zea mays* L.). *J. Sci. Food Agric.*97: 4408-4418.
- Al-Judy NJ (2004). Detecting of DNA fingerprints and genetic relationship analysis in local and improved rice (*Oryza sativa* L.) varieties in Iraq using RAPD markers. Ph.D Dissertation, Department of Biology, Faculty of Science, Baghdad University, Iraq.
- Al-Niemi SN, Rehani SI, Hassn FA (2019). Genetic variability and morphological diversity of open pollinated maize (*Zea mays* L.) varieties under the effect of potassium fertilization. *Int. J. Agric. Edu. Ext.* 5: 197-202.
- Al-Nuaimi JJJ, Al-Myali AAH, Hussein HT (2019). Environmental study of genetic behavior of oil and protein ratio with correlation yield of *Zea mays* L. *Ekoloji* 28: 141-151.
- Al-Qurainy F, Salim K, Mohammad N, Mohamed T (2015). SCoT markers for the assessment of genetic diversity in Saudi Arabian date palm cultivars. *Pak. J. Bot.* 47: 637-643.
- Al-Tamimi AJT (2014). Genetic diversity of some tomato genotypes using RAPD and SSR markers in Iraq. Ph.D Dissertation, Department of Biology, Faculty of Science, University of Kufa, Iraq.

- Arif IA, Bakir MA, Khan HA, Al-Farhan AH, Al-Homaidan AA, Bahkali AH, Al-Sadoon, M, Shobrak M (2010). Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. *Genet. Mol. Res.* 9: 2191-2198.
- Baghizadeha A, Dehghan E (2018). Efficacy of SCoT and ISSR markers in assessment of genetic diversity in some Iranian pistachio (*Pistacia vera* L.) cultivars. *Pistachio and Health J.* 1: 37-43.
- Bauer I, Drinic-Mladenovic S, Drinic G (2007). Assessing temporal changes in genetic diversity of maize hybrids using RAPD markers. *Cer. Res. Comm.* 35: 1563 -1571.
- Collard BCY, Mackill DJ (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.
- Dhaka BL, Meena BS, Suwalka RL (2010). Popularization of improved maize production technology through frontline demonstrations in South-Eastern Rajasthan. *J. Agric. Sci.* 1: 39-42.
- Eo I, Jo O (2017). Weed infestation, growth and yield of maize (*Zea mays* L.) as influenced by periods of weed interference. *Adv. Crop Sci. Tech.* 5: 267.
- Etminan A, Alireza P, Reza M, Amin A, Afsaneh N, Zahra M, Zahra M (2016). Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes. *Biotechnol. Biotechnol. Equip.* 30: 1075-108.
- Fadoul, HE, El-Siddig MA, El Hussein AA (2013). Assessment of genetic diversity among Sudanese wheat cultivars using RAPD markers. *Int. J. Curr. Sci.* 6: 51-57.
- Gorji AM, Poczai P, Polgar Z, Taller J (2011). Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. *Am. J. Potato Res.* 88: 226–237.
- Graham J, McNicol RJ (1995). An examination of the ability of RAPD markers to determine the relationships within and between *Rubus spp.* *Theor. Appl. Genet.* 90: 1128-1132.
- Hammer O, Harper DAT, Ryan PD (2001). PAST: Palaeontological statistics software package for education and data analysis. *Paleontologia Eletronica* 4: 1-9.
- Hunter PR, Gaston MA (1988). Numerical index of discriminatory ability of simpson's index of diversity. *J. Clin. Mic.* 26: 2465-2466.
- Hurtado A, Rodriguiz F (1999). Accessory DNA in the genomes of representatives of the *Escherichia coli* reference collection. *J. Bacteriol.* 181: 2548-2554.
- Iqbal J, Shinwari ZK, Rabbani MA, Khan SA (2015). Genetic divergence in maize (*Zea mays* L.) germplasm using quantitative and qualitative traits. *Pak. J. Bot.* 47: 227-238.
- Khan PN, Saudan SP, Sunita SD (2017). Development of species specific SCoT markers and analysis of genetic diversity among *Mentha* genotypes. *Int. J. Innov. Sci. Eng. Technol.* 4: 244-264.
- Kumar LS (1999). DNA markers in plant improvement: an overview. *Biotechnol. Adv.* 17: 1430-1482.
- Kumar P, Gupta VK, Misra AK, Pandey BK (2009). Potential of molecular markers in plant biotechnology. *Plant Omics J.* 2: 141-162.
- Luo C, He XH, Hu Y, Yu HX, Ou SJ, Fang ZB (2014). Oligo-dT anchored cDNA-SCoT: a novel differential display method for analyzing differential gene expression in response to several stress treatments in mango (*Mangifera indica* L.). *Gene* 548: 182-189.
- Miro K, Tibor N, Edit K, Ferenc M (2017). Discrimination of grape varieties by Start Codon Targeted genotyping using partially degenerate primers. *Acta Biol. Szegediensis* 61: 77-83.
- Mulpuri S, Muddanuru T, Francis G (2013). Start codon targeted (SCoT) polymorphism in toxic and non-toxic accessions of *Jatropha curcas* L. and

- development of a codominant SCAR marker. *Plant Sci.* 207: 117-127.
- Nair AGH, Vidya P, Mohan C (2016). Analysis of genetic variability in sweet potato accessions using start codon targeted (SCoT) polymorphism. *Int. J. Biotechnol. Biochem.* 12: 111-121.
- Nerling D, Coelho CMM, Brümmer A (2018). Biochemical profiling and its role in physiological quality of maize seeds. *J. Seed Sci.* 40: 7-15.
- Newton CR, Graham A (1997). Polymerase Chain Reaction. 2nd ed. Bios. Scientific Publishers Ltd., Oxford, UK.
- Poehlman JM (1977). Breeding Field Crops. 2nd. The AVI Publish. Co. Inc. Westport, CT, USA.
- Ranum P, Pena-Rosas JP, Garcia-Casal MN (2014). Global maize production, utilization, and consumption. *Ann. New York Acad. Sci.* 1312 : 105-112.
- Sadek MSE, Ibrahim SD (2018). Genetic relationships among maize inbred lines as revealed by start codon targeted (SCoT) analysis. *J. Innov. Pharm. Biol. Sci.* 5: 103-107.
- Sharma R, Maloo SR, Jain D (2018). Assessment of molecular diversity in diverse Maize genotypes using RAPD and ISSR markers. *Int. J. Curr. Microbiol. Appl. Sci.* 6: 2812-2821.
- Shrestha J (2019). Morphological variation in maize inbred lines. *Int. J. Environ.* 3: 98-107.
- Sprague GF, Fuccillo DA, Perelman LS, Stelly M (1988). Corn and Corn Improvement: 3rd Ed. Amer. Society of Agron. Madison, Wisconsin, USA.
- Sultana R (2019). Molecular and morphological characterization of maize genotypes for opaque2 gene and yield contributing traits. *Big Data in Agric. (BDA)*. 1: 7-14.
- Vishwanath K, Prasanna KPR, Pallvi HM, Rajendra P, Ramegowda S, Devaraju PJ (2010). Identification of tomato (*Lycopersicon esculentum*) varieties through total soluble seed proteins. *Res. J. Agric. Sci.* 2: 8-12.
- Vivodik M, Balazova Z, Galova Z, Petrovicova L (2017). Genetic diversity analysis of maize (*Zea Mays* L.) using SCoT markers. *J. Microbiol. Biotechnol. Food Sci.* 6: 1170-1173.
- Vivodik M, Zdenka G, Zelmira B, Lenka P (2016). Start Codon Targeted (SCoT) polymorphism reveals genetic diversity in European old maize (*Zea mays* L.) genotypes. *Potravinarstvo Scien. J. Food Ind.* 10: 563-569.
- Wattoo FM, Saleem M, Ahsan M, Sajjad M, Ali W (2009). Genetic analysis for yield potential and quality traits in maize (*Zea mays* L.). *Am. Eur-Asian J. Agric. Environ. Sci.* 6(6): 723-729.
- Williams JG, Kubelik AR, Livak KJ, Rafaski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.
- Xanthopoulou A, Ganopoulos I, Kalivas A, Nianiou-Obeidat I, Ralli P, Moysiadis T, Tsaftaris A, Madesis P (2015). Comparative analysis of genetic diversity in Greek Genebank collection of summer squash (*Cucurbita pepo*) landraces using start codon targeted (SCoT) polymorphism and ISSR markers. *Aust. J. Crop Sci.* 9: 14-21.
- Xiong FQ, Tang RH, Chen ZL, Pan LH, Zhuang WJ (2009). SCoT: A novel gene targeted marker technique based on the translation start codon. *Mol. Plant Breed.* 7: 635-638.
- Zhang J, Xie W, Wang Y, Zhao X (2015). Potential of start codon targeted (SCoT) markers to estimate genetic diversity and relationships among Chinese *Elymus sibiricus* accessions. *Molecules* 20: 5987-6001.