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MOLECULAR IDENTIFICATION OF MAIZE (*ZEA MAYS* L.) GENOTYPES USING START CODON TARGETED (SCoT) MARKERS POLYMORPHISM

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SUMMARY

The genetic diversity estimation in 10 maize (*Zea mays* L.) genotypes (Somar, Fajer1, Al maha, Baghdad-3, Al hajen nhren, DKC 6777, ZP.glorya, PIOWEE R, KWS, and Syngenta) grown in Iraq was this latest study's aim. It used DNA markers based on the polymerase chain reaction (PCR). The current study showed the results of molecular detection using the start codon targeted (SCoT) markers to assess the variation of 10 genotypes studied through the existence of single, multi-shapes, and unique packages. Even some primers showed a unique imprint of the genetic structures of maize plants. The synthesis comprised a genetic relationship tree of various genetic structures, constructed to estimate the genetic diversity between maize plants' different genetic structures. They appeared in varying number and size of multiplier pieces and the efficiency of prefixes used to produce different DNA packages in the maize genome. This research employed DNA markers based on PCR to examine the genetic makeup of these maize cultivars. The study utilized various DNA markers, including those developed by SCoT and others, to examine the genetic variation among the studied maize genotypes. The presented study identified the presence of single, polymorphic, and unique genetic markers, thus, providing a unique fingerprint for specific maize genotypes.

Keywords: Maize (*Zea mays* L.), DNA markers, maize cultivars, polymorphic, markers polymorphism (SCoT)

Key findings: The quantity and size of multiplier pieces as well as the efficiency of prefixes used to create different DNA packages in the maize genome determined the genetic variations estimation among the genotypes of maize (*Zea mays* L.).

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INTRODUCTION

Maize (*Zea mays* L.), globally an important cereal crop, is a key staple of human nourishment and an essential resource in producing animal feeds. Its supreme importance in the realm of agriculture and food security axis for its rich genetic diversity is a characteristic drawing keen consideration of researchers worldwide (Abdul Mohsin and Farhood, 2023). The genetic composition and diversity of maize are not only subjects of academic intrigue but hold the key to advancing the breeding programs, vital for meeting growing demands of the ever-expanding global population (Zimmer, 2023).

The start codon targeted (SCoT) markers are a powerful tool for molecular identification of maize genotypes. These codons are sequences of few nucleotides used in genes to determine the start point of translation. These codons are crucial because they help determine the correct reading frame of the gene, thus, determining which protein will be produced (Chňapek *et al.,* 2023). Several studies have shown that SCoT markers can serve to characterize maize genotypes with high accuracy. Al-Tamimi (2020) used SCoT markers to genotype 10 synthetic local and exotic maize genotypes. Chňapek *et al.* (2023) also used SCoT markers to genotype 40 maize species from Eastern European countries and Russia. However, both studies resulted in discovering SCoT markers are competent to distinguish the different maize populations with high accuracy.

The use of SCoT markers for maize genotyping has several advantages. First, SCoT markers are relatively easy to develop and use. Second, the SCoT markers are highly polymorphic, which means these markers can help distinguish numerous maize genotypes, and third, they are relatively inexpensive to use (Al-Musawi and Al-Abedy, 2020).

Over the years, many scientific investigations have explored the particulars of maize genetics and detection of a vast reservoir of genetic variation within maize populations. These studies have provided profound insights into vital parameters, such as yield potential, drought tolerance, resistance to pests and diseases, and nutritional content. In this introduction, an exploration of maize's genetic diversity, guided by the findings of distinguished scientific sources, each contributed a valuable perspective to this vital field of study.

Khan *et al*.'s (2014) study stands to understand the maize genetics. Their comprehensive research illuminated the molecular characterization and genetic relationship among diverse maize genotypes, sorting out the genetic diversity that supports the adaptability of this crucial cereal crop. The work of Ashkani *et al*. (2015) marked a paradigm shift in maize genetics. This research guided for the utilization of polymerase chain reaction (PCR)-based DNA markers for assessing genetic diversity within maize populations—a revolutionary approach that has since become primary to genetic studies in maize.

The historical journey about tracing maize's lineage to the Andean region of South America in this context has significantly enriched our understanding of maize genetics, shedding light on the crop's ancestral roots (Zhang *et al*., 2016). Kumar *et al*. (2009) explored the practical applications of genetic diversity studies within maize breeding programs, highlighting the transformative potential of genetic research in exploring maize crop on a global level. As for Pejic *et al*. (2003), they investigated the complex genetic relationship among different maize populations, laying the foundation for more targeted and effective breeding strategies. Therefore, the current study aimed to estimate genetic diversity in 10 genetic patterns of maize.

MATERIALS AND METHODS

Genetic material

In 10 maize (*Zea mays* L.) genotypes, seeds of five cultivars, i.e., Somar, Fajer1, Al maha, Baghdad-3, and Al hajen nhren, came from the Research and Development Department, Ministry of Agriculture, Iraq. The other five cultivars' seeds, viz., DKC 6777, ZP.glorya,

PIOWEE R, KWS, and Syngenta were a provision from a commercial seed supplier. The DNA sequencing using Scott primers prepared from Macrogen Korea helped determine the genetic composition of each variety.

DNA extraction and purification

DNA extraction from the maize seeds used a modified CTAB method. Briefly, the process comprised 50 mg of ground maize samples incubated with 600 µL of CTAB extraction buffer at 65 °C for 45 min. After centrifugation, the supernatant's transfer to a new tube continued its extraction with chloroform: isoamyl alcohol (24:1). DNA's precipitation with isopropanol followed, and then washed with 70% ethanol. The purified DNA's re-suspension in TE buffer reached quantification using a Nanodrop spectrophotometer (Minas *et al.,* 2011).

Thermoplastic device PCR

PCR amplification of the DNA proceeded using a thermoplastic device with integrated microfluidic channels. The PCR reaction mixture contained 10 ng of DNA template, 1 µL of Taq polymerase, 0.5 µL of each forward and reverse primer, 1 µL of dNTPs, and 4 µL of reaction buffer. The PCR program consisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min. A final extension step was at 72 °C for 5 min. PCR products' analysis employed gel electrophoresis.

Electrical relay device and dyes

The PCR products' analysis used an electrical relay device and dyes. The device contained a microfluidic chip with integrated electrodes and a detection chamber. Mixing PCR products with SYBR Green I dye continued loading these onto the chip. Applying electricity to the electrodes drove the PCR products through the microfluidic channels into the detection chamber. Fluorescence signals' detection used a CCD camera, and then analyzed using the software.

Prefixes, volumetric guide

All experimental procedures proceeded as per standard protocols and guidelines. The use of prefixes and units was according to the International System of Units (SI). Volumes' measurement used calibrated micropipettes and tubes. All statistical analyses had the study using the GraphPad Prism software (Table 1).

Data analysis

The data obtained from the ultraviolet device underwent analysis to calculate the number of alleles, genetic diversity, polymorphism content, and main allele frequency. These are the data required in the genetic variation study of groups because with more alleles, the

Table 1. SCoT Primers sequences (5′–3′).

SCoT primers	Primer sequence (5′–3′)	SCoT primers	Primer sequence (5′–3′)
SCoT 29	5′-CCATGGCTACCACCGGCC-3′	SCoT 54	5′-ACAATGGCTACCACCAGC-3′
SCoT 36	5′-GCAACAATGGCTACCACC-3′	SCoT 9	5′-AACAATGGCTACCAGCA-3′
SCoT 6	5′-CAACAATGGCTACCACGC-3′	SCoT 40	5′-CAATGGCTACCACTACAG-3′
SCoT 44	5′-CAATGGCTACCATTAGCC-3′	SCoT 60	5′-ACAATGGCTACCACCACA-3′
SCoT 30	5′-CCATGGCTACCACCGGCG-3′	SCoT 63	5′-ACCATGGCTACCACGGGC-3′
SCoT 12	5′-ACGACATGGCGACCAACG -3′	SCoT 8	5′-CAACAATGGCTACCACGT-3′
SCoT 23	5′-CACCATGGCTACCACCAG-3′		

Table 2. SCoT primer amplification product analysis.

Markers	Molecular size of packages	Major Allele Frequency	Allele No.	Gene Diversity	PIC
SCoT 8	1200-450bp	0.7000	6.0000	0.4900	0.4701
SCoT 9	800-250bp	0.6750	9.0000	0.5263	0.5095
SCoT 12	1200-300bp	0.6000	15.0000	0.6275	0.6184
SCoT 23	1200-300bp	0.6000	13.0000	0.6213	0.6075
SCoT 29	1200350bp	0.6500	10.0000	0.5613	0.5473
SCoT 30	1100-350bp	0.4000	15.0000	0.8025	0.7894
SCoT 53	1700-250bp	0.4750	13.0000	0.7400	0.7236
SCoT 60	1800-300bp	0.2500	15.0000	0.8788	0.8687
SCoT 63	1600-200bp	0.1000	21.0000	0.9363	0.9326
Mean		0.4944	13.0000	0.6871	0.6741

Table 3. Genetic relationships among maize genotypes.

Genotypes	Somar	Fajer 1	Al maha	3 Baghdad	Al hajen nhren	DKC 6777	ZP.glorya	POIWEER	KWS	Syngenta
Somar	1									
Fajer 1	0.823529	1								
Al maha	0.777778	0.842105	1							
3 Baghdad	0.777778	0.75	0.714286	1						
Al hajen nhren	0.705882	0.777778	0.833333	0.571429	1					
DKC 6777	0.611111	0.684211	0.65	0.65	0.764706	1				
ZP.glorya	0.631579	0.7	0.666667	0.75	0.52381	0.6	1			
POIWEER	0.526316	0.6	0.571429	0.571429	0.578947	0.666667	0.684211	1		
KWS	0.631579	0.7	0.666667	0.666667	0.684211	0.777778	0.7	0.684211	1	
Syngenta	0.529412	0.526316	0.428571	0.578947	0.5	0.588235	0.526316	0.588235	0.611111	1

greater the genetic diversity using programs. Gene set Analyzer (Version 2.0), Gen Mapper (Ver 3.7), and the resulting outcomes' assessment used the Power Marker, V.3. A resulting value aided to know the distinctive information given by the spatial locus by calculating the frequency of alleles of this locus in the studied samples (Table 2). The genetic relationships among calculated samples applied the PAST program (Ver. 1.19) (Table 3). The genetic tree construction adopted the Neighbor-Joining method to obtain the tree of genetic relationships (phylogenetics) using the PAST program (Hammer and Harper, 2001). Afterward, engaging another program helped to extract the genetic dimension and clarify the tree, which is 1.6 Tree view version, shown in Figure 8.

RESULTS AND DISCUSSION

For extricating the genetic details of two genetically distinct maize cultivars A and B, and their molecular differentiation, the study employed a comprehensive set of materials and methods. These methodologies included the possession and classification of the maize genetic compositions, DNA extraction and purification, the utilization of a thermoplastic device for PCR amplification, and the subsequent analysis of PCR products, using an electrical relay device and dyes. These thorough procedures adhered to established protocols and standards, employing precise measurements and statistical analyses. The zenith of these efforts has yielded the exhaustive results forming the basis for the

following discussion. In this section, the outcomes of each experimental step's investigation examined the genetic composition of maize cultivars. Furthermore, exploring the implications of these results will continue, considering their significance in the context of maize genetics and potential applications.

The outcomes of these carefully executed procedures had the study untangle the genetic information of maize cultivars A and B. The broader implication of these findings is a consideration, probing how these findings can contribute to understanding genetic diversity within maize populations and how such knowledge can persuade future advancements in maize breeding and its agrotechnology. The results and their discussion will also shed light on these crucial characteristics that provide an inclusive viewpoint on the molecular characterization of maize genotypes through the integration of cutting-edge technologies and precise scientific methodologies (Tables 2 and 3).

DNA replication based on SCoT's indices

The primer SCoT 63

For the SCoT 63 primer, the results about the bands' molecular size revealed the bands ranged between 1600-200 bp. The frequency of the major allele was 0.1000, the number of alleles was 21,000, the genetic diversity was 0.9363, and the polymorphism content was 0.9326 (Figure 1).

The primer 8 and SCoT 9

The results for the SCoT 8 primer appeared about the molecular size, and the bands ranged between 1200-450 bp. The frequency of the major allele was 0.7000, the number of alleles was 6.000, and the genetic diversity was 0.4900. Meanwhile, the polymorphism content was 0.4701. The results of the SCoT 9 primer also materialized. The molecular size of the bands ranged between 800-250 bp. The frequency of the main allele was 0.6750, the number of alleles was 9,000, the genetic diversity was 0.5263, and the polymorphism content was 0.5095 (Figure 2).

The primer SCoT 29 and SCoT 30

The outcomes for primer SCoT 29 appeared, with the bands ranging between 1200-350 bp. The frequency of the major allele was 0.6500, the number of alleles was 10,000, the genetic diversity was 0.5613, and the polymorphism content was 0.5473 (Figure 3). The results for the SCoT 30 primer also showed. The bands ranged between 1100-350 bp. The frequency of the major allele was 0.4000, the number of alleles was 15000, the genetic diversity was 0.8025, while the polymorphism content was 0.7894.

The primer SCoT 12 and SCoT 23

The SCoT 12 primer results revealed the molecular size, and the bands ranged from 1200-300 bp, the major allele frequency was 0.6000, the number of alleles was 15.000, genetic diversity at 0.6275, and polymorphism content of 0.6184. The results of the primer SCoT 23 showed the molecular size range, with the bands ranging between 1200-300 bp, the frequency of the major allele at 0.6000, the number of alleles was 13.000, the genetic diversity was 0.6213, and the polymorphism content of 0.6075 (Figure 4).

The primer SCoTs 44, 40, 6, and 36

For these four SCoT primers, there was no match with the maize genotypes (Figures 5 and 6).

The primer SCoT 53 and SCoT 60

The results for the SCoT 53 primer on the molecular size appeared, and the bands ranged between 1700-250 bp. The frequency of the major allele was 0.4750, the number of alleles was 13,000, the genetic diversity was 0.7400, and the polymorphism content was 0.7236. The results for the SCoT 60 primer also occurred, and the bands ranged between 1800-300 bp. The frequency of the major allele was 0.2500, the number of alleles was 15,000, the genetic diversity was 0.8788, and the polymorphism content was 0.8687 (Figure 7).

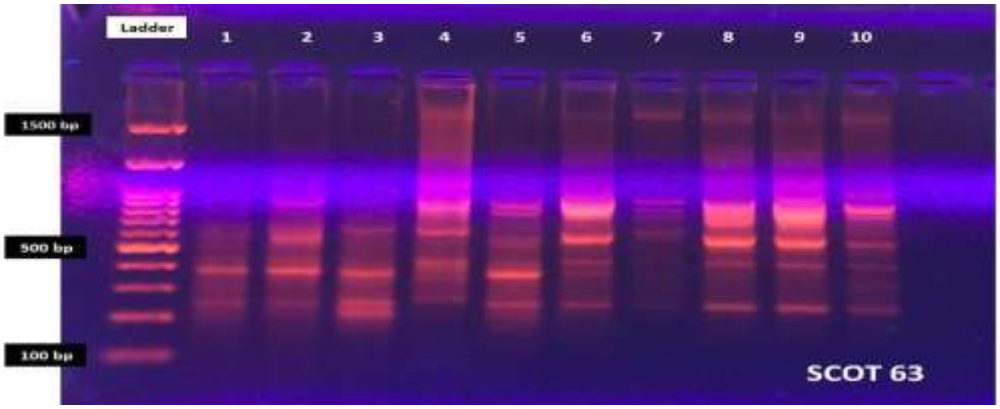

Figure 1. Representing the products of the replication of the SCoT 63 primer staged on a 1.5% agarose gel for 2 h with the standard volumetric guide (M) at a current of 30 amps and a voltage of 70, according to the following order of genotypes: 1. Somar, 2. Fajer1, 3. Al maha, 4. Baghdad 3, 5. Al hajen nhren, 6. DKC 6777, 7. ZP.glorya, 8. POIWEER, 9. KWS, 10. Syngenta (maize).

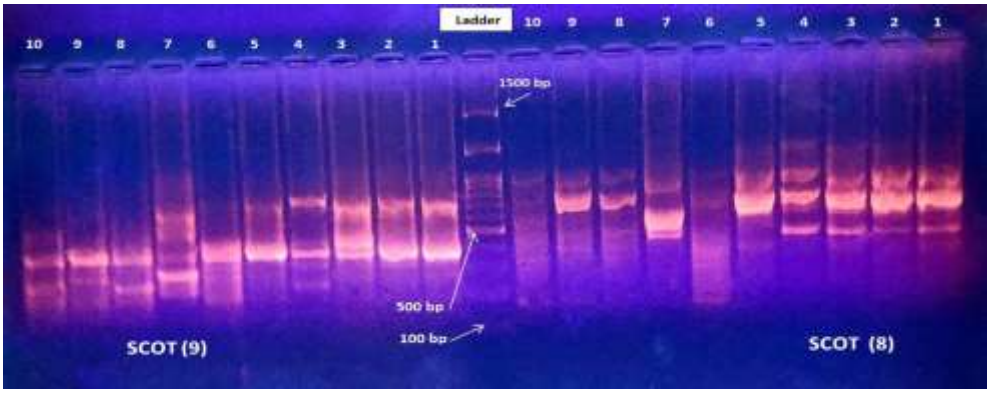

Figure 2. Representing the products of the replication of the primer SCoT 8 and SCoT 9 staged on agarose gel at a concentration of 1.5% for 2 h with the standard volume index (M) at a current of 30 amps and a voltage of 70, according to the following arrangement of genetic structures of maize: 1.

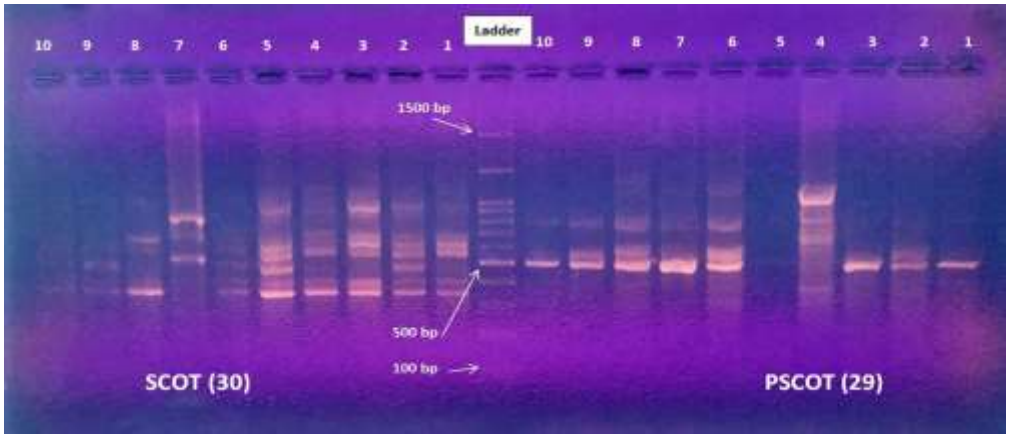

Figure 3. Representing the products of the replication of the primer SCoT 29 and SCoT 30 staged on agarose gel at a concentration of 1.5% for 2 h with the standard volume index (M) at a current of 30 amps and a voltage of 70, according to the following arrangement of the genetic structures of maize.

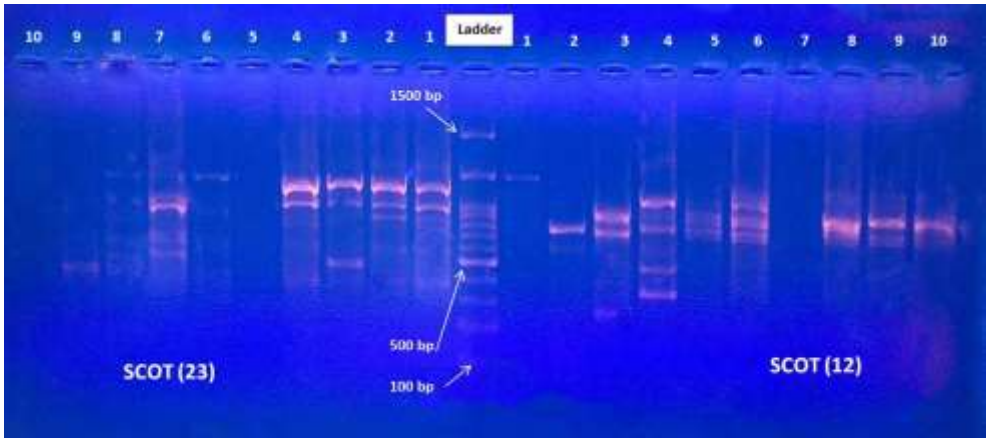

Figure 4. The products of multiplying the starter SCoT 12 and SCoT 23 represent the stage on an agarose gel at a concentration of 1.5% for 2 h with a standard volumetric index (M) at a current of 30 amperes and a voltage of 70, according to the following arrangement of the genotypes of an atom.

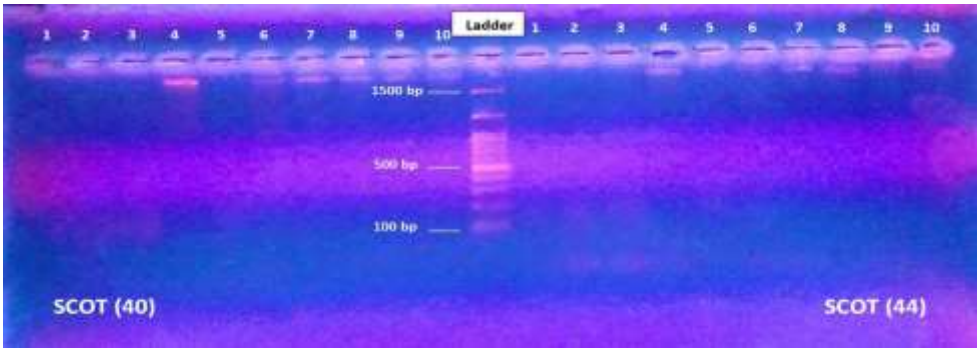

Figure 5. Representing the products of the replication of the primer SCoT 44 and SCoT 40 staged on an agarose gel at a concentration of 1.5% for 2 h with the standard volumetric guide (M) at a current of 30 amps and a voltage of 70, according to the following arrangement for the genetic structures of maize.

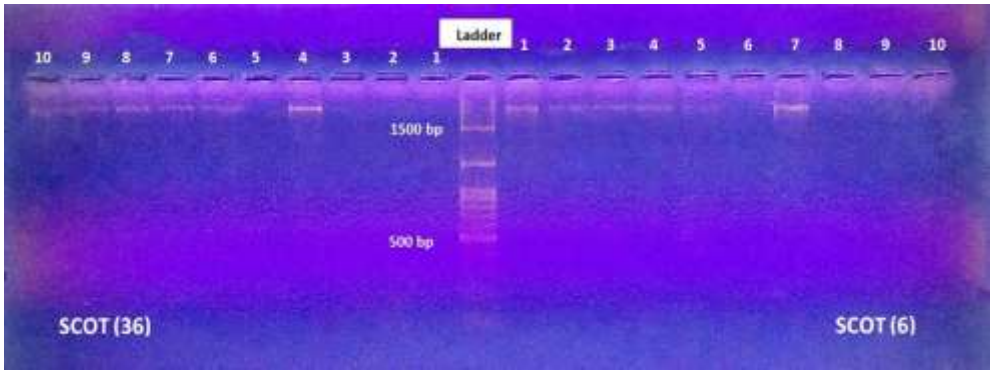

Figure 6. Representing the products of the replication of the primer SCoT 6 and SCoT 36 staged on an agarose gel at a concentration of 1.5% for 2 h with the standard volume index (M) at a current of 30 amps and a voltage of 70, according to the following arrangement of the genetic structures of maize.

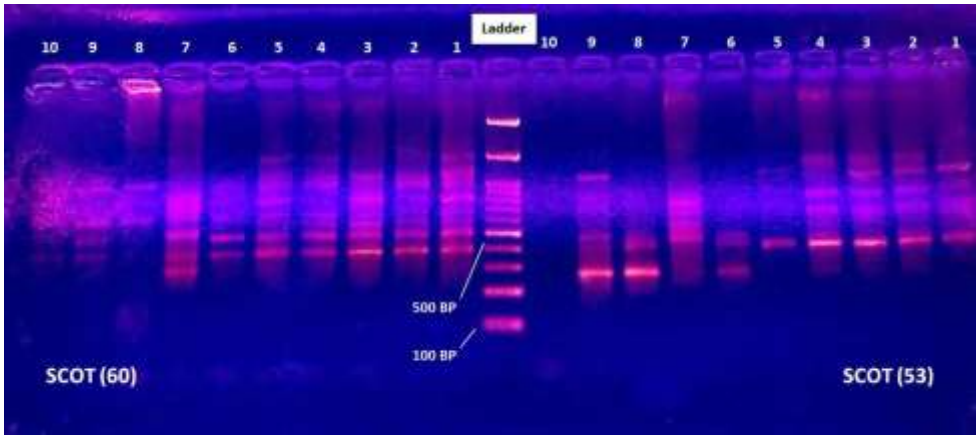

Figure 7. Representing the products of the replication of the primer SCoT 53 and SCoT 60 staged on an agarose gel at a concentration of 1.5% for 2 h with the standard volumetric guide (M) at a current of 30 amps and a voltage of 70, according to the following arrangement for the genetic structures of maize.

Isolation and assessment of genomic DNA

Genomic DNA's successful isolation from the leaves of the genetically synthesized maize plants used a well-established method provided by specialized prefabricated companies, including Geneaid Biotech Ltd and Promega. Following the isolation, a critical step involved assessing the efficiency of the process by estimating DNA concentration and purity. The concentration of the isolated DNA fell within the range of 153–315 ng/mg, with a purity of 1.8. Determining these values employed a UV-Vis Spectrophotometer at wavelengths of 260 and 280 nm. The high purity of the DNA could refer to the efficiency of the extraction method, which is particularly suitable for plant DNA isolation due to its speed and simplicity. The chemicals used in the process effectively removed unwanted cellular components while preserving the extracted DNA.

Electrophoresis results indicated the DNA fragments predominantly appeared towards the top of the gel, indicating good quality and higher molecular sizes. Further scrutiny of the DNA quality was by visualizing the samples using the ethidium bromide dye.

Genetic relationship analysis

The genetic relationships among the maize compositions received analysis using the Jaccard technology. The results revealed varying degrees of genetic similarity, as shown in Table 2. In Table 3, the highest similarity was evident between specific compositions, such as orb and dawn 1, with a similarity ratio of 0.842105. In contrast, the composition between Al maha and Syngenta exhibited lower similarity, with a ratio of 0.428571.

The analysis further detailed the genetic dimension and relationship among the studied maize compositions. The SCoT labels were instrumental in estimating genetic variation and proximity among the 10 maize compositions. Despite the innate nature of species, the genetic variation had notable manifestations between the compositions, particularly between orb and dawn 1, indicating a high degree of similarity. On the contrary, the lowest genetic dimension was apparent in cases where the genetic material matched completely, signifying that these compositions belong to the same genetic group and share a common genetic ancestry.

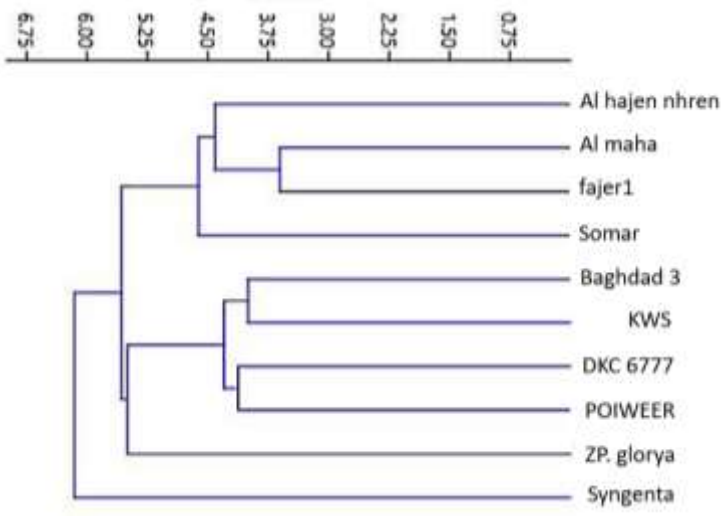

Figure 8. The Diagram (Dendrogram) represents the genetic relationship tree between the studied maize genotypes using the Jaccard scale and according to the following arrangement of the maize genotypes: 1. Somar, 2. Fajer1, 3. Al Maha, 4. Baghdad 3, 5. Al hajen nhren, 6. DKC 6777, 7. ZP.glorya, 8. POIWEER, 9. KWS, and 10. Syngenta.

The findings further suggested that common phenotypic traits, such as physiological maturity, plant height, and location, contributed to an increased genetic similarity among the studied compositions. Additionally, the genetic differences identified between the compositions used SSR markers, which can serve for analytical purposes, revealing information about their origin and geographical location.

Genetic relationship tree

Figure 8 shows cumulative analysis results presented in the form of a dendrogram tree (genetic relationship tree), highlighting two main genetic groups. The first group consisted of a single composition, Syngenta, while the second group encompassed the remaining compositions. Notably, some compositions within the same group shared common origins, such as dawn-1, Maha, Sumar, and Baghdad-3, which were the local compositions.

The clustering of compositions not solely depended on their origins, indicating that those differences in phenotypic qualities and certain genes related to resistance to environmental stresses contributed to their grouping. Additionally, some compositions may fall under different labels due to commercial considerations and their involvement in hybridization and breeding programs.

Understanding the genetic dimensions of plant compositions through genetic analyses at the DNA level is crucial for developing desired traits and creating genetically compatible combinations. These insights can aid plant breeders in enhancing qualities like disease resistance and environmental adaptability by selecting the suitable parental combinations. It also allows for introducing specific gene-controlled traits without significantly altering the species' genetic makeup, preserving desirable qualities (Mukhlif *et al*., 2023; Sobirova *et al*., 2023).

In the presented study, the assessment of genetic polymorphism among the maize genotypes using 11 SCoT primers, exposed exciting insights into the diversity within the germplasm. Notably, we observed varying degrees of polymorphism across the tested maize genotypes, with the highest level of polymorphism reaching an impressive 81% by utilizing the primer SCoT 29. The results revealed the efficacy of SCoT 29 in capturing genetic variations within an individual population.

The varied performance of primers in exposing polymorphism could be due to their uneven abilities to recognize distinct regions within the genome. Generally, primers that can recognize many annealing sites are considerably more valuable for genetic polymorphism studies. This is because a higher recognition of annealing sites leads to producing more amplified fragments, consequently increasing the likelihood of detecting polymorphism among the individuals within a population. In this regard, SCoT primers offer an optimistic advantage compared with other marker types like the Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) primers (Souframanien and Reddy, 2012; Luo *et al*., 2014; Meteab *et al.*, 2015). The study also highlighted the superior polymorphism-revealing capacity of primers recognizing multiple annealing sites.

CONCLUSIONS

Utilizing SCoT primers provided valuable insights into the genetic polymorphism found within the maize genotypes. Despite some limitations, such as the relatively lower polymorphism compared with prior studies, the promising results contribute to understanding genetic diversity in maize germplasm. These outcomes are crucial for breeding programs aiming to harness specific traits and enhance maize genetic resources for further improvement. Future research, including a broader selection of genotypes, may divulge additional layers of genetic diversity within the maize germplasm.

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