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Research article

# Genetic relationships among maize inbred lines as revealed by start codon targeted (SCoT) analysis

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**Key words:** Maize; Molecular markers; SCoT analysis.

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**Abstract**

Maize (*Zea mays* L.), is a cereal crop with a remarkable potential for production, it is the third most important grain crop after wheat and rice. Molecular analysis is frequently used by maize breeders for selecting promising inbred lines to develop hybrid combination. In the present study 8 yellow maize inbred lines (Gm730,Gm 731, Gm 739, Gm 743, Gm 744, Gm 745, Gm 746 and Gm 749) developed by Maize Research Program, Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Egypt were analyzed using 10 Start codon targeted (SCoT) markers. These primers produced total 136 fragments across 8 maize inbred lines, of which 74 (54.4%) were polymorphic with an average of 7.4 polymorphic fragments per primer and number of amplified fragments ranged from 4 (SCoT- 07) to 13 (SCoT- 08). The genetic similarity between pairs of inbred lines ranged from 78 to 91. The highest genetic similarity was between inbred lines Gm 745 and Gm746.While the lowest genetic similarity was between inbred lines Gm 730 and Gm 749 and between inbred line Gm 731 and both of inbred lines Gm 739 and Gm 749. The dendrogram of eight maize inbred lines based on SCoT markers using UPGMA comprised two main clusters; the first cluster grouped two inbred lines; Gm 730 and Gm 731 which derived from the same genetic source. The second cluster divided into two sub clusters; one sub- cluster contains one inbred line (Gm 739). While the other sub-cluster grouped the other five inbred lines in two groups. In general, Cluster analysis based on SCoT data grouped inbred lines largely consistent with their pedigree. This present study showed effectiveness of employing SCoT markers in analysis of maize genotypes, and it would be a promising marker for further studies in population genetics, genetic diversity and genotypes improvement.

## Introduction

Knowledge about germplasm diversity and of genetic relationships among elite breeding materials has a significant impact on the improvement of crop plants [1]. Therefore, analysis of genetic relationship in crop species is an important component of crop improvement programs, as it serves to provide information about genetic diversity, and is a platform stratified sampling of breeding populations [2].

Maize (*Zea mays* L.), is a cereal crop with a remarkable potential for production, it is the third most important grain crop after wheat and rice [3]. In maize breeding programs the development and selection of inbred lines are rather costly and time-consuming tasks and large number of hybrids produced so, extensive yield trials are required to evaluate hybrid performance [4].

Different methodologies have been used to characterize genetic diversity in the maize germplasm including morphological characters, pedigree analysis, heterosis and the detection of variation at the DNA level using markers [5].

Molecular characterization is frequently used by maize breeders as an alternative method for selecting more promising genotypes and reducing the cost and time needed to develop hybrid combinations [6]. A number of molecular markers have been employed for genetic diversity evaluation in maize for instance, Random amplified polymorphic DNA (RAPD)[7], Amplified Fragment Length Polymorphism (AFLP) [8], inter-simple sequence repeat (ISSR) [9-10] and simple sequence repeats (SSR) [11].

A novel method was developed for generating plant DNA marker system based on conserved regions flanking the ATG regions of the start codon in plant genes. It is called as SCoT (Start Codon Targeted) marker. SCoT was first developed by [12]. This marker system requires no prior knowledge about the sequence under study. It is reproducible, reliable, efficient and easy to use. It has been used to evaluate genetic relationships in plants. It is

useful for plant breeding, accessing genetic relationships and QTL mapping [13].

The usefulness of SCoT markers in diversity analysis and diagnostic fingerprinting has been successfully demonstrated by many authors in many crops, such as potato [14], tomato [15], citrus [16], mango [17] and grabs [18]. The effectiveness of employing SCoT markers in analysis of maize was studied by [19-20] their results indicated that SCoT markers would be useful for further studies in population genetics, conservation genetics and genotypes improvement.

In this present study, eight yellow maize inbred lines were assayed by SCoT markers to achieve the following objectives; (i) assess the genetic relationships among the eight maize inbred lines at the molecular level by SCoT markers, (ii) evaluate the usefulness of SCoT marker for analysis of genetic diversity of maize.

## Materials and methods

### Plant material and DNA isolation

Eight ($S_6$) yellow maize inbred lines developed by Maize Research Program, Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Egypt. Their names and sources were presented in Table 1. These inbred lines were assayed by using SCoT marker. Genomic DNA was isolated from the 14 days leaves with DNAeasy Plant Mini Kit (Qiagen, Santa Clarita, CA) (Catno. 69104). Laboratory experiments were performed at Agricultural Genetic Engineering Research Institute (AGRI), Agricultural Research center, Egypt.

**Table 1. inbred lines used in this study.**

| No | Inbred line | Origin of inbred line |
|---|---|---|
| 1 | Gm 730 | Comp-# 45- Egypt |
| 2 | Gm 731 | Comp-# 45- Egypt |
| 3 | Gm 739 | Gm yellow population-Egypt |
| 4 | Gm 743 | Gm yellow population- Egypt |
| 5 | Gm 744 | Comp-# 21- Egypt |
| 6 | Gm 745 | Comp-# 21- Egypt |
| 7 | Gm 746 | CIMMYT- pop 31 |
| 8 | Gm 749 | CIMMYT- pop 41 |

Gm: Gemmieza, Com: Composed, Pop: Population.

### SCoT amplification

A ten SCoT primers employed in the present study (Table 2) were designed by [12] based on the consensus sequences of translation initiation codon region in higher plants with ATG codon at positions +1, +2, +3; 'G' at position + 4; and 'A,' 'C,' and 'C' at positions + 7, + 8, and + 9, respectively. The PCR reaction was performed as mention by [21] in a total volume of 25 µl containing 1X reaction buffer, 1.5Mm MgCl2, 0.2 mM dNTPs, 0.4 µM of a single primer; 50 ng genomic DNA and 2U of Taq DNA polymerase.

The PCR amplification conditions were carried out as follows: an initial denaturation step at 94$^{O}$C for 3 min. followed by 36 cycles of 94$^{O}$C for 50 s. 50$^{O}$C for 1 min. and72$^{O}$C for 2 min; the final extension at 72$^{O}$C was held for 5min. A total of 5 µl PCR products were separated on 1.5% agarose gel in 1 X TBE buffer using electrophoresis technique. The agarose gel was visualized UV light after staining with ethidium bromide.

### The PCR scoring Data analysis

PCR fragments on gels were recorded as presence (1) or absence (0) for all samples and final data sets included both polymorphic and monomorphic bands. Only patterns that are existed in both sample replicate were chosen for analysis. Then a binary statistic matrix was constructed. Dice's similarity matrix coefficients were calculated between accessions using the unweighted pair group method with arithmetic averages (UPGMA) and this matrix was used to construct a phylogentic dendrogram using the online dendrogram construction utility Dendro UPGMA [22].

## Results and Discussion

### Polymorphism analysis detected by SCoT markers

In this study, ten SCoT primers which used for analysis of eight yellow maize inbred lines produced amplification products and all resulted in polymorphic fingerprint patterns except for, SCoT-9 (Table 2). Ten primers produced 136 DNA fragments (Figure 1) with an average of 13.6 bands per primer. Out of the total of 136 amplified fragments, 74 were polymorphic, with an average of 7.4 polymorphic bands per primer. This represented a level of polymorphism of 54.4% from these ten primers. Primer SCoT-08, was the most polymorphic bands, where 13 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (4) was detected by primer SCoT-07. The polymorphism percentage ranged from 40% (SCoT-07) to 75% (SCoT-19). The different SCoT primers revealed different numbers of unique positive and /or negative markers across the studied inbred lines. The total number of markers was 14 unique positive markers and 5 unique negative markers. In this regard, [19] assessed the genetic diversity of 40 genotypes of maize using 20 Start Codon Targeted markers. These primers, produced 114 bands across 40 maize genotypes, of which 86 (76.43%) were polymorphic with an average of 4.30 polymorphic fragments per primer. Similar range of polymorphism in maize was scored by (5) SCoT primers [20]. However [23] observed High levels of polymorphism were; 98.70% (ISSR) and 100% (SCoT) in durum wheat, which indicated that these markers are useful tools for detection of genetic variation. Similarly in potato and peanut, SCoT markers were effective for fingerprinting, detecting DNA polymorphism, and

studying genetic relationships among closely related genotypes [14, 24].

### Genetic relationship among inbred lines

The data showed that the genetic similarity between pairs of inbred lines ranged from 78 to 91 (Table 3). The highest genetic similarity (91) was between inbred lines Gm 745 (derived from Comp-21 Egypt) and Gm746 which derived from (CIMMYT- pop 31). Then followed by similarity degree 89 between inbred lines Gm 744 and Gm 745 where both of them derived from (comp- #21 Egypt) where have the same genetic back ground, and these results completely agreement with pedigree data (Table 1). Similarity degree 88 between inbred lines Gm 743 (drived from Gm yellow pop-Egypt) and Gm 745. While the lowest genetic similarity (78) between inbred lines Gm 730 (derived from comp- #45 Egypt) and Gm 749 (derived from CIMMYT- pop 41) and between inbred line Gm 731 (derived from comp- #45 Egypt) and both of inbred lines Gm 739 (drived from Gm yellow pop-Egypt) and Gm 749, where there are differences in their genetic back ground. In general, the range of genetic similarity from 78 to 91 among studied inbred lines may be due to low number of genotypes under study. In this respect [25] pointed that in general, a higher number of investigated accessions and more varied genetic background result in a higher expected polymorphic rate. Similar results were obtained for SCoT marker in other crops. [26] Studied genetic relationship among 107 sugarcane accessions and found genetic similarity ranged from 0.375 to 0.881. In addition [18] in grapes and [23] in durum wheat.

### Cluster analysis based on SCoT marker

The dendrogram of eight maize inbred lines based on SCoT markers using UPGMA and similarity matrix computed according to Dice coefficient (Figure 2). The dendrogram comprised two main clusters; the first cluster grouped two inbred lines; Gm 730 and Gm 731 which derived from the same genetic source (Comp-# 45-Egypt). The second cluster divided into two sub clusters; one sub- cluster contains one inbred line (Gm 739). While the other sub-cluster grouped five inbred lines in two groups as follows; the inbred line Gm 749 in a singular cluster, the other four inbred lines grouped in another cluster and distributed into two sub-clusters; inbred line Gm 743 in singular sub- cluster. However, the other sub-cluster divided in two groups as follows, inbred line Gm 744 in a single group and the two inbred lines (Gm 745 and Gm 746) clustered in one group.

**Table 2. Ten SCoT primer sequences used in this study, the total bands (TB), polymorphic bands (PB), percentage of polymorphic bands (%PB) positive unique marker (PUM) and negative unique marker (NUM).**

| Primer | Sequence | TB | PB | %PB | PUM | NUM |
|---|---|---|---|---|---|---|
| SCoT-03 | ACGACATGGCGACCCACA | 20 | 9 | 45 | 950bp | 350bp |
| SCoT-05 | CAATGGCTACCACTAGCG | 14 | 8 | 57 | 210-290-890 | 800 |
| SCoT-06 | CAATGGCTACCACTACAG | 11 | 8 | 73 | 270 | 620 |
| SCoT-07 | ACAATGGCTACCACTGAC | 10 | 4 | 40 | - | 440-490 |
| SCoT-08 | ACAATGGCTACCACTGAG | 20 | 13 | 65 | 460-610 | - |
| SCoT-09 | ACAATGGCTACCACTGCC | 9 | - | 0 | - | - |
| SCoT-10 | ACAATGGCTACCACCAGC | 14 | 9 | 64 | 130-720-850 | - |
| SCoT-17 | CCATGGCTACCACTACCC | 15 | 7 | 47 | 1050 | - |
| SCoT-18 | CCATGGCTACCACTAGCA | 15 | 10 | 67 | 630-700 | - |
| SCoT-19 | CCATGGCTACCACCGGCG | 8 | 6 | 75 | 220 | - |
| Total | | 136 | 74 | | 14 | 5 |
| **Average** | | 13.6 | 7.4 | 54.4 | | |

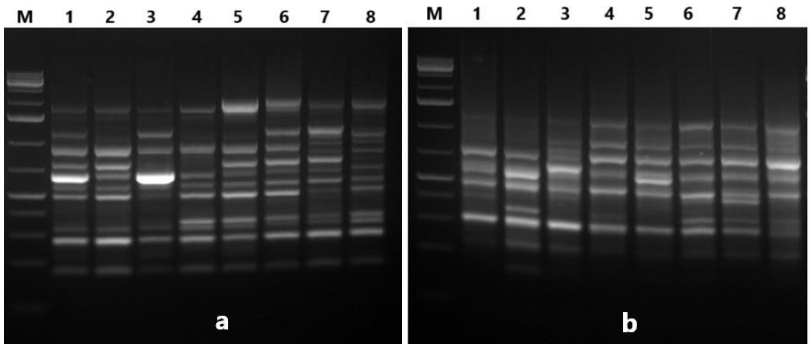


**Figure 1. SCoT profiles of the eight maize inbred lines using primers (SCoT-17) and (SCoT-10). 1: Gm 730, 2: Gm 731, 3: Gm 739, 4: Gm 734, 5: Sd 744, 6: Gm 745, 7: Gm 746 and 8: Gm 749. M: DNA molecular weight marker (1000bp ladder).**

**Table 3. Genetic similarity (GS) as revealed by SCoT data.**

| Lines | Gm 730 | Gm 731 | Gm 739 | Gm 743 | Gm 744 | Gm 745 | Gm 746 | Gm 749 |
|---|---|---|---|---|---|---|---|---|
| Gm 730 | 100 | | | | | | | |
| Gm 731 | 88 | 100 | | | | | | |
| Gm 739 | 80 | 78 | 100 | | | | | |
| Gm 743 | 80 | 84 | 86 | 100 | | | | |
| Gm 744 | 83 | 84 | 81 | 86 | 100 | | | |
| Gm 745 | 82 | 80 | 85 | 88 | 89 | 100 | | |
| Gm 746 | 80 | 79 | 83 | 86 | 86 | 91 | 100 | |
| Gm 749 | 78 | 78 | 80 | 86 | 85 | 85 | 87 | 100 |

It might be due to there is a degree of similarity in their genetic background. These results confirmed the results of similarity matrix (Table 2). In general, cluster analysis based on SCoT data grouped inbred lines largely consistent with their pedigree. Similar results indicated that, SCoT markers have been proved to be useful in genetic diversity studies because of their high reproducibility and great power for the detection of polymorphism [26, 27]. [20] Confirmed that polymorphism revealed by SCoT technique was so abundant and could be used for molecular genetics study of the maize accessions, providing high-valued information for the management of germplasm, improvement of the current breeding strategies, and conservation of the genetic resources of maize

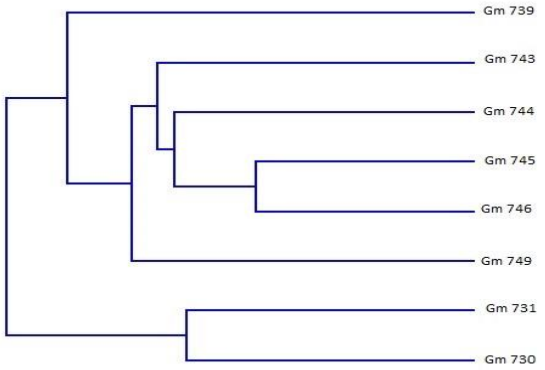


**Figure 2. Dendrogram for the eight maize inbred lines constructed from SCoTs data using UPGMA and similarity matrix computed according to Dice coefficient.**

## Conclusion

The present research indicated that, the SCoT marker analysis was effective for evaluating the genetic relationships among maize genotypes. It would be a promising marker for further studies in population genetics, genetic diversity and genotypes improvement.

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