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START CODON TARGETED (SCOT) POLYMORPHISM REVEALS GENETIC DIVERSITY IN EUROPEAN OLD MAIZE (*ZEA MAYS* L.) GENOTYPES

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ABSTRACT

Maize (*Zea mays* L.) is one of the world's most important crop plants following wheat and rice, which provides staple food to large number of human population in the world. It is cultivated in a wider range of environments than wheat and rice because of its greater adaptability. 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. In the present investigation 40 genotypes of maize from Czechoslovakia, Hungary, Poland, Union of Soviet Socialist Republics, Slovakia and Yugoslavia were analysed using 20 Start codon targeted (SCoT) markers. These primers produced total 114 fragments across 40 maize genotypes, of which 86 (76.43%) were polymorphic with an average of 4.30 polymorphic fragments per primer and number of amplified fragments ranged from 2 (SCoT 45) to 8 (SCoT 28 and SCoT 63). The polymorphic information content (PIC) value ranged from 0.374 (ScoT 45) to 0.846 (SCoT 28) with an average of 0.739. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. The hierarchical cluster analysis showed that the maize genotypes were divided into two main clusters. Unique maize genotype (cluster 1), Zuta Brzica, originating from Yugoslavia separated from others. Cluster 2 was divided into two main clusters (2a and 2b). Subcluster 2a contained one Yugoslavian genotype Juhoslavanska and subcluster 2b was divided in two subclusters 2ba and 2bb. The present study shows effectiveness of employing SCoT markers in analysis of maize, and would be useful for further studies in population genetics, conservation genetics and genotypes improvement.

Keywords: Dendrogram; Maize; Molecular markers; SCoT analysis

INTRODUCTION

Maize (*Zea mays* L.) is one of the world's most important crop plants following wheat and rice, which provides staple food to large number of human population in the world **(Ahmad et al., 2011; Iqbal, et al., 2015).** Determining genetic diversity can be based on agronomic, morphological, biochemical, and molecular types of information, among others **(Goncalves et al., 2009)**. 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 **(Garcia et al., 2004)**. In recent years, a number of molecular markers have been employed for genetic diversity evaluation, genetic mapping, and quantitative trait locus analysis. These types of molecular techniques included random amplified polymorphic dna (RAPD) **(Štefúnová et al., 2015)**, amplified fragment length polymorphism (AFLP) **(Molin et al., 2013)**, inter-simple sequence repeat (ISSR) **(Idris et al., 2012; Žiarovská et al., 2013)** and simple sequence repeats (SSR) **(Shehata et al., 2009)**.

Recently, a simple novel DNA marker technique namely start codon targeted (SCoT) polymorphism, was developed by **Collard and Mackill (2009)**. Primers for SCoT marker analysis were designed from the conserved region surrounding the translation initiation codon, ATG **(Joshi et al., 1997; Sawant et al., 1999)**. Single 18-mer oligonucleotides were used as both forward and reverse primer for PCR, and the annealing temperature was set at 50 °C. The amplicons were resolved using standard agarose gel electrophoresis. Suitability of SCoT markers for the construction of genetic maps, fingerprinting and phylogenetic studies has been proved by many authors in many crops, such as tomato **(Shahlaei et al., 2014)**, citrus **(Mahjbi et al., 2015)**, date palm **(Al-qurainy et al., 2015)**, castor (**Kallamadi et al., 2015)** and mango **(Gajera et al., 2014)**.

The goals of this study were to examine the effectiveness of scot markers for analysis of genetic diversity of maize and to study genetic relationships among 40 maize accessions originating from various geographic regions of europe.

MATERIAL AND METHODOLOGY

Plant material: Forty genotypes of old maize lines originating from six different geographical areas (Table 1) (CZE - Czechoslovakia, HUN - Hungary, POL - Poland, SUN – Union of Soviet Socialist Republics, SK – Slovakia, YUG - Yugoslavia) of Europe were obtained from the Gene Bank Praha-Ruzyně (Czech Republic) and from the Gene Bank in Piešťany (Slovakia). Genomic DNA was isolated from the 14 days leaves with GeneJET Plant Genomic DNA Purification Mini Kit.

Table 1 List of 40 analyzed genotypes of maize.

Genotypes	Country of origin	Year of registration
1. Feheres Sarga Filleres	Hungary	1965
2. Mindszentpusztai Feher	Hungary	1964
3. Zakarpatskaja	Union of Soviet Socialist Republics	1964
4. Przebedowska Burskynowa	Poland	1964
5. Krasnodarskaja	Union of Soviet Socialist Republics	1964
6. Mesterhazy Sarga Simaszemu	Hungary	1964
7. Slovenska biela perlova	Czechoslovakia	1964
8. Zuta Brzica	Yugoslavia	1975
9. Zloty Zar	Poland	1964
10. Slovenska Florentinka	Czechoslovakia	1964
11. Juhoslavska	Yugoslavia	1964
12. Kostycevskaja	Union of Soviet Socialist Republics	1964
13. Mindszentpusztai Sarga Lofogu	Hungary	1964
14. Stodnova	Czechoslovakia	1964
15. Slovenska žltá	Slovak Republic	1964
16. Slovenska krajová velkozrná	Slovak Republic	1964
17. Partizanka	Union of Soviet Socialist Republics	1964
18. Voroneskaja	Union of Soviet Socialist Republics	1964
19. Kocovska Skora	Slovak Republic	1964
20. Milada	Czechoslovakia	1964
21. Moldavskaja	Union of Soviet Socialist Republics	1964
22. Bučiansky Konský Zub	Slovak Republic	1964
23. Hodoninský konský zub žltý	Czechoslovakia	1964
24. M Silokukurica	Hungary	1964
25. Valticka	Czechoslovakia	1964
26. Przebedowska Biala	Poland	1964
27. Toschevska	Slovak Republic	1964
28. Šamorinsky konský zub	Hungary	1964
29. Wielkopolanka	Poland	1964
30. Czechnicka	Poland	1964
31. Manalta	Czechoslovakia	1964
32. Zlota gorecka	Poland	1964
33. Celchovicka ADQ	Czechoslovakia	1964
34. Belaja mestnaja	Union of Soviet Socialist Republics	1964
35. Bučanská žltá	Slovak Republic	1964
36. Iregszemeseil 2 hetes	Hungary	1964
37. Dnepropetrovskaja	Union of Soviet Socialist Republics	1964
38. Bezuncukskaja	Union of Soviet Socialist Republics	1964
39. Mikulická	Czechoslovakia	1964
40. Aranyozon sarga lofogu	Hungary	1964

SCoT amplification: A total of 20 SCoT primers developed by **Collard and Mackill (2009)** were selected for the present study (Table 2). Each 15-μL amplification reaction consisted of 1.5 μL (100 ng) template DNA, 7.5 μL Master Mix (Genei, Bangalore, India), 1.5 μL 10 pmol primer, and 4.5 μL distilled water. Amplification was performed in a programmed thermocycler (Biometra, Germany) using 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; a final extension at 72 °C for 5 min. Amplified products were separated in 1.5% agarose in 1 × TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system UVP PhotoDoc-t® camera system. A dendrogram was constructed based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA). For the assessment of the polymorphism between genotypes maize and usability SCoT markers in their differentiation we used polymorphic information content (PIC) **(Weber, 1990)**.

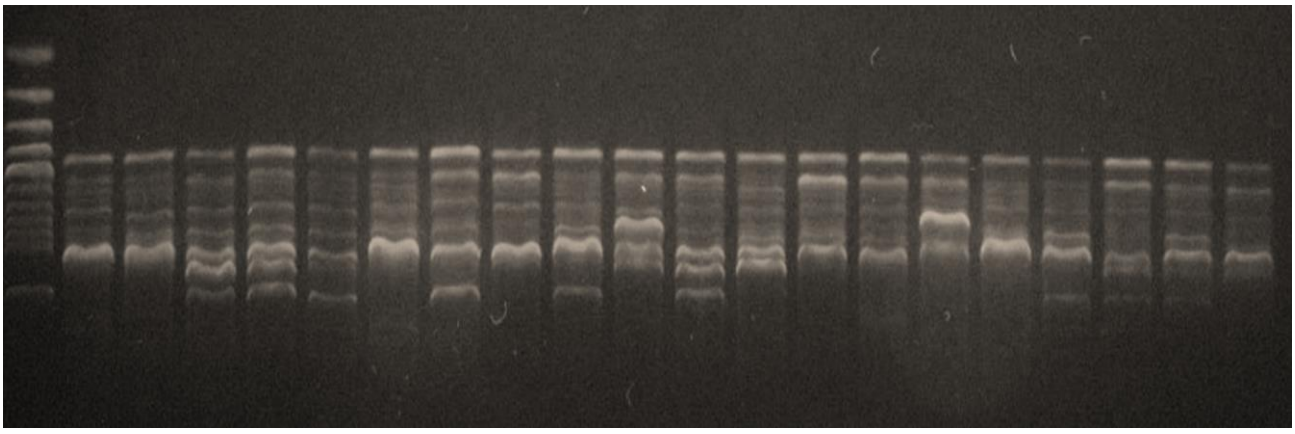
RESULTS AND DISCUSSION

In this work, all 20 SCoT primers used for analysis of 40 European old maize genotypes produced amplification products and all resulted in polymorphic fingerprint patterns. Twenty primers produced 114 DNA fragments (Figure 1) with an average of 5.7 bands per primer (Table 2). Out of the total of 114 amplified fragments, 86 (76.43 %) were polymorphic, with an average of 4.30 polymorphic bands per primer. From these twenty primers, primers SCoT 28 and SCoT 63, respectively, were the most polymorphic, where 8 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (2) was detected by primer SCoT 45. To determine the level of polymorphism in the analysed group of maize genotypes, polymorphic information content (PIC) was calculated (Table 2).

Table 2 Statistical characteristics of the SCoT markers used in maize.

SCoT Primers	Primer sequence (5´ – 3´)	TNoB	NoPB	PoPB	PIC
SCoT 6	CAACAATGGCTACCACGC	5	4	80.00	0.729
SCoT 8	CAACAATGGCTACCACGT	4	4	100.00	0.652
SCoT 9	CAACAATGGCTACCAGCA	6	4	66.66	0.780
SCoT 12	ACGACATGGCGACCAACG	7	5	71.43	0.715
SCoT 23	CACCATGGCTACCACCAG	7	5	71.43	0.816
SCoT 26	ACCATGGCTACCACCGTC	5	4	80.00	0.714
SCoT 28	CCATGGCTACCACCGCCA	8	5	62.50	0.846
SCoT 29	CCATGGCTACCACCGGCC	6	4	66.66	0.810
SCoT 30	CCATGGCTACCACCGGCG	7	6	85.71	0.825
SCoT 36	GCAACAATGGCTACCACC	7	7	100.00	0.812
SCoT 40	CAATGGCTACCACTACAG	6	5	83.33	0.731
SCoT 44	CAATGGCTACCATTAGCC	4	2	50.00	0.710
SCoT 45	ACAATGGCTACCACTGAC	2	2	100.00	0.374
SCoT 54	ACAATGGCTACCACCAGC	5	3	60.00	0.717
SCoT 59	ACAATGGCTACCACCATC	6	3	50.00	0.794
SCoT 60	ACAATGGCTACCACCACA	6	3	50.00	0.790
SCoT 61	CAACAATGGCTACCACCG	6	5	83.33	0.808
SCoT 62	ACCATGGCTACCACGGAG	4	4	100.00	0.618
SCoT 63	ACCATGGCTACCACGGGC	8	7	87.50	0.832
SCoT 65	ACCATGGCTACCACGGCA	5	4	80.00	0.697
Average		**5.70**	**4.30**	**76.43**	**0.739**
Total		**114**	**86**	**-**	**-**

Note: TNoB – Total number of bands, NoPB – Number of polymorphic bands, PoPB – Percentage of polymorphic bands (%), PIC- Polymorphic information content.



M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Figure 1 PCR amplification products of 20 genotypes of maize produced with SCoT 54 primer. Lane M is 1-kb DNA ladder and lanes 1-20 are maize genotypes.

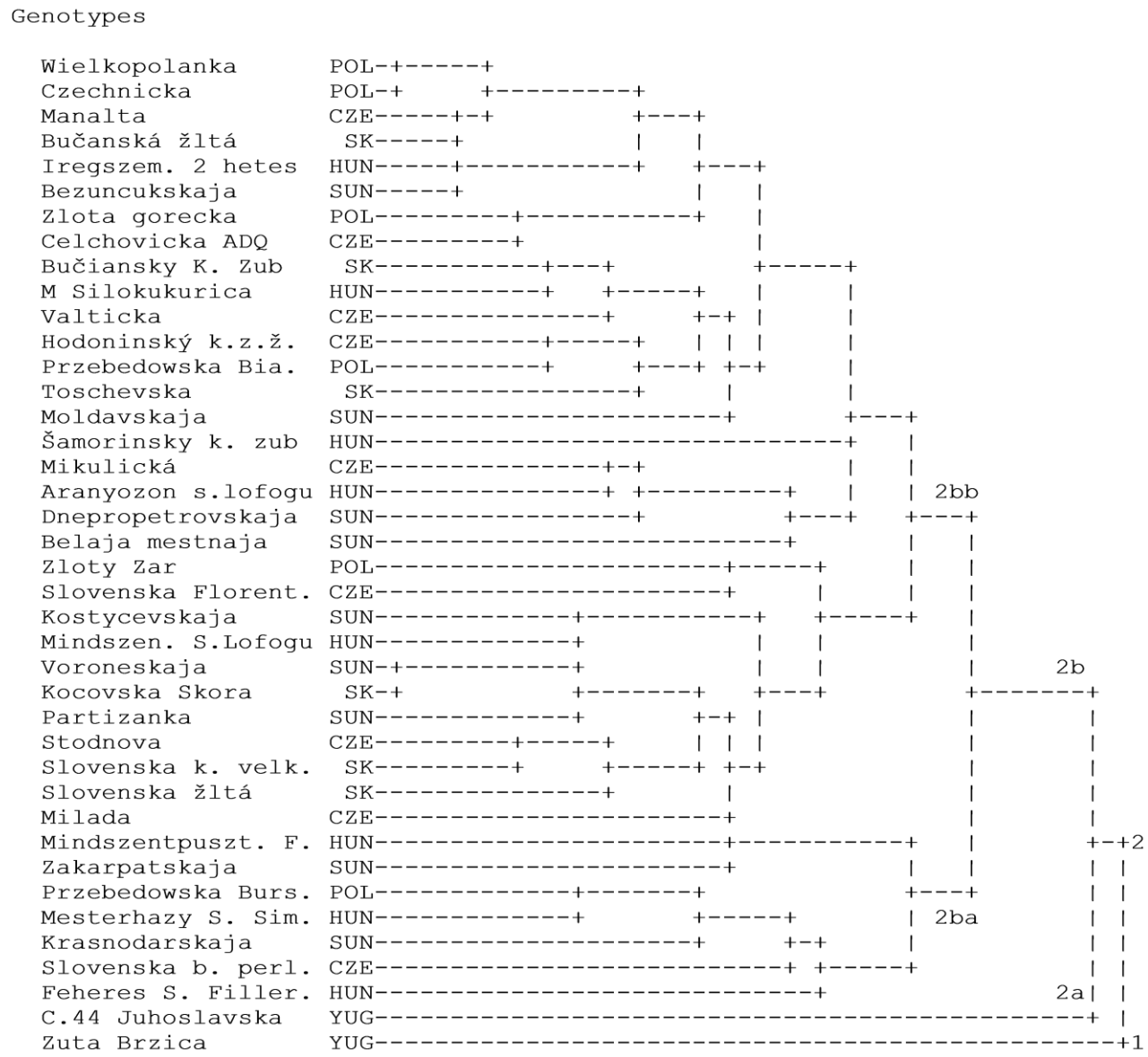


Figure 2 Dendrogram of 40 maize genotypes prepared based on 20 SCoT markers. CZE – Czechoslovakia, HUN – Hungary, POL – Poland, SUN – Union of Soviet Socialist Republics, SK – Slovakia, YUG – Yugoslavia.

The polymorphic information content (PIC) value ranged from 0.374 (SCoT 45) to 0.846 (SCoT 28) with an average of 0.739. The dendrogram of 40 maize genotypes based on SCoT markers using UGMA algorithm was constructed (Figure 2). The hierarchical cluster analysis divided maize genotypes into two main clusters. Unique maize genotype Zuta Brzica, originated from Yugoslavia (cluster 1), separated from others. Cluster 2 containing 39 genotypes was divided into two main subclusters (2a and 2b). Subcluster 2a contained one Yugoslavian genotype Juhoslavanska and subcluster 2b was divided in two subclusters 2ba and 2bb. In the subcluster 2ba were grouped 7 genotypes from Hungary (42.87%), Poland (14.29%), Czechoslovakia (14.29%) and Union of Soviet Socialist Republics (28.58%). Subcluster 2bb of 31 genotypes included genotypes of Polish origin (16.15%), Union of Soviet Socialist Republics origin (22.61%), Slovakia origin (19.38%), Czechoslovak origin (25.84%) and Hungarian origin (16.15%). Two genotypes of 2bb subcluster (Czechnicka and Wielkopolanka) from Poland and two genotypes (Voroneskaja and Kocovska Skora) from Union of Soviet Socialist Republics and Slovakia, respectively, were genetically the closest. We can assume that they have close genetic background.

Level of polymorphism in analysed maize genotypes was determined by calculated polymorphic information content (PIC) (Table 2). The PIC values ranged from 0.374 (SCoT 45) to 0.846 (SCoT 28) with an average of 0.739. Similar values of PIC were detected by other authors **(Luo et al., 2012; Arya et al., 2014; Gajera et al., 2014; Que et al., 2014; Gao et al., 2014; Fang-Yong et al., 2014; Jiang et al., 2014; Huang et al., 2014; Satya et al., 2015)** and these values presented a high level of polymorphism of genotypes detected by SCoT markers**. Huang et al., (2014)** assessed the genetic diversity of six Hemarthria cultivars using seven SCoT primers, which together amplified 105 bands with an average of 15 bands per sample. Start codon-targeted markers were utilized by **Gajera et al., (2014)**

who used 19 SCoT markers for characterization and genetic comparison among 20 mango cultivars. These primers produced total 117 loci across 20 cultivars, of which 96 (79.57 %) were polymorphic. In the study **Que et al., (2014)**, used 20 start codon targeted (SCoT) marker primers to assess the genetic diversity among 107 sugarcane accessions within a local sugarcane germplasm collection. These primers amplified 176 DNA fragments, of which 163 were polymorphic (92.85%). The aim of **Gao et al., (2014)** was to estimate the genetic diversity across 43 varieties of Lycoris. Of 57 SCoT primers screened, 23 SCoT primers were identified to be high polymorphism. **Fang-Yong et al., (2014)** assessed the genetic diversity of 31 germplasm resources of Myrica rubra from Zhejiang Province, the major gathering site and the largest producer of M. rubra in China using start codon-targeted polymorphism (SCoT) markers. Authors used 38 primers to perform PCR amplification of 31 genotypes, from which 298 reproducible bands were obtained, including 251 polymorphic bands (84.23%). **Satya et al., (2015)** used 24 start codon targeted (SCoT) markers to assess genetic diversity and population structure of indigenous, introduced and domesticated ramie (Boehmeria nivea L. Gaudich.). **Jiang et al., (2014)** used start codon-targeted (SCoT) markers to analyze the diversity and genetic relationships among 95 orchardgrass accessions. In total, 273 polymorphic bands were detected with an average of 11.4 bands per primer. In the study **Zhang et al., (2015)** used SCoT markers to study the genetic diversity and relationships among 53 Elymus sibiricus accessions.

Studies of genetic diversity across individuals of plant have been realized by different PCR-based DNA marker methods: random amplified polymorphic DNA (RAPD) **(Molin et al., 2013; Balážová et al., 2016; Kuťka Hlozáková et al., 2016)**, simple sequence repeat (SSR) **(Terra et al., 2011; Molin et al., 2013; Gálová et al., 2015; Balážová et al., 2016)**, amplified fragment length polymorphism (AFLP) **(Molin et al., 2013),** inter-simple sequence repeat (ISSR) **(Žiarovská et al., 2013; Molin et al., 2013)**. These methods are technically simple, fairly cheap and generate a relatively large number of markers per sample. **Molin et al., (2013)** pointed that in general, a higher number of investigated accessions and more varied genetic background result in a higher expected polymorphic rate. Start codon targeted polymorphism (SCoT) is a simple and novel marker system first described by **Collard and Mackill (2009)**, which is based on the short conserved region flanking the ATG translation start codon in plant genes. The higher primer lengths and subsequently higher annealing temperatures ensure higher reproducibility of SCoT markers, compared to RAPD markers **(Rajesh et al., 2015)**. **Gorji et al., (2011)** presented that SCoTs markers were more informative and effective, followed by ISSRs and AFLP marker system in in fingerprinting of potato varieties.

CONCLUSION

The present work is the first report on genetic variability of maize using SCoT markers. In summary, SCoT marker analysis was successfully developed to evaluate the genetic relationships among the genus maize accessions originated from various regions. The hierarchical cluster analysis showed that the maize genotypes were divided into 2 main clusters. One maize genotype Zuta Brzica, origin from Yugoslavia (cluster 1), was separated from others. Cluster 2 was divided into two main clusters (2a and 2b). Four genotypes of 2bb subcluster (Czechnicka and Wielkopolanka) from Poland and two genotypes (Voroneskaja and Kocovska Skora) from Union of Soviet Socialist Republics and Slovakia, respectively, were genetically the closest. 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 species.

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