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GENETIC DIVERSITY ANALYSIS OF MAIZE (*ZEA MAYS* L.) USING SCoT MARKERS

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ABSTRACT

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 20 genotypes of maize from Czechoslovakia, Hungary, Poland, Union of Soviet Socialist Republics, Slovakia and Yugoslavia were analysed using 5 Start codon targeted (SCoT) markers. These primers produced total 29 fragments across 20 maize genotypes, of which 22 (77.90 %) were polymorphic with an average of 4.40 polymorphic fragments per primer and number of amplified fragments ranged from 4 (SCoT 8) to 7 (SCoT 12 and SCoT 23). The polymorphic information content (PIC) value ranged from 0.652 (ScoT 8) to 0.816 (SCoT 23) with an average of 0.738. The dendrogram of 20 maize genotypes based on SCoT markers using UGMA algorithm was constructed. The hierarchical cluster analysis divided maize genotypes into two main clusters. Unique 2 maize genotype Slovenska žltá and Slovenska krajová velkozrná, originated from Slovak Republic, separated from others. Cluster 2 containing 18 genotypes was divided into two main subclusters. Subcluster 2a contained two Poland genotypes Przebedowska Burskynowa and Zloty Zar, two genotypes of Union of Soviet Socialist Republics- Partizanka and Krasnodarskaja and one Czechoslovakian genotypes Milada. In subcluster 2b were grouped 13 maize genotypes. 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; PIC

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) (**Petrovičová *et al*., 2015**; **Štefúnová *et al*., 2015; Vivodík *et al*., 2015; Žiarovská *et al*., 2016**), 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; Lancíková *et al*., 2015; Balážová *et al*., 2016; Vivodík *et al.*, 2016**).
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 20 maize accessions originating from various geographic regions of Europe.

MATERIAL AND METHODS

Plant material

Twenty 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). Maize genotypes were grown in a growth chamber on humus soil. Genomic DNA was isolated from the 14 days leaves with GeneJET Plant Genomic DNA Purification Mini Kit.

SCoT amplification

A total of 5 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)**.

Table 1 List of 20 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

RESULTS AND DISCUSSION

In this work, all 5 SCoT primers used for analysis of 20 European old maize genotypes produced amplification products and all resulted in polymorphic fingerprint patterns. Five primers produced 29 DNA fragments (Figure 1) with an average of 5.80 bands per primer (Table 2). Out of the total of 29 amplified fragments, 22 (77.90 %) were polymorphic, with an average of 4.40 polymorphic bands per primer. From these five primers, primers SCoT 12 and SCoT 23, respectively, were the most polymorphic, where 7 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (4) was detected by primer SCoT 8. To determine the level of polymorphism in the analysed group of maize genotypes, polymorphic information content (PIC) was calculated (Table 2). The polymorphic information content (PIC) value ranged from 0.652 (SCoT 8) to 0.816 (SCoT 23) with an average of 0.738. The dendrogram of 20 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 2 maize genotype Slovenska žltá and Slovenska krajová velkozrná, originated from Slovak Republic (cluster 1), separated from others. Cluster 2 containing 18 genotypes was divided into two main subclusters (2a and 2b). Subcluster 2a contained two Poland genotypes Przebedowska Burskynowa and Zloty Zar, two genotypes of Union of Soviet Socialist Republics- Partizanka and Krasnodarskaja and one Czechoslovakian genotypes Milada. In subcluster 2b were grouped 4 genotypes from Hungary (30.77%), 3 genotypes from Czechoslovakia (23.08%), 3 genotypes from Union of Soviet Socialist Republics (23.08%), 2 genotypes from Yugoslavia (15.38%) and 1 genotypes from Slovak Republic (7.70%).

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
Average		**5.80**	**4.40**	**77.90**	**0.738**
Total		**29**	**22**	-	-

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

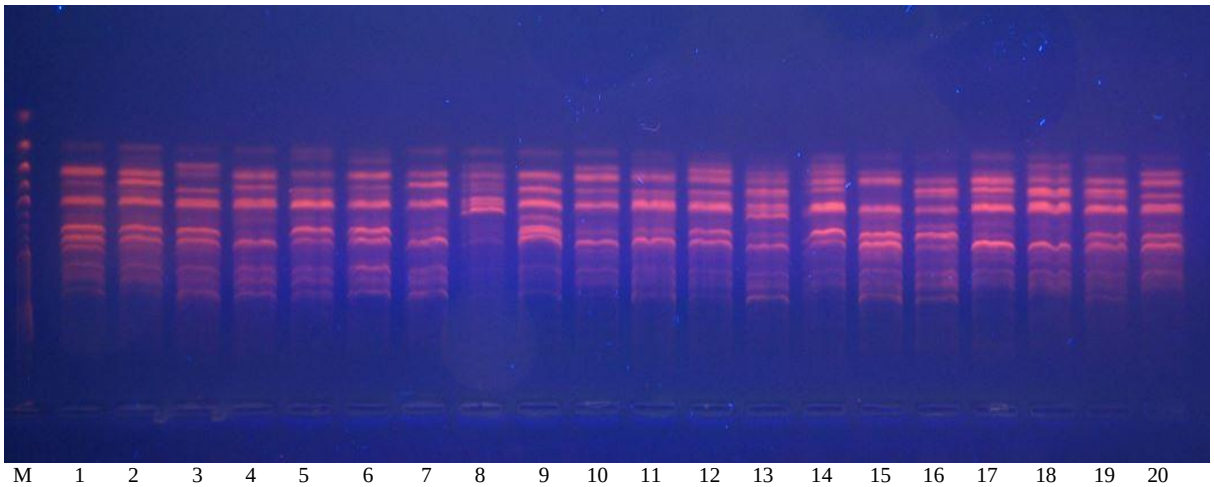

Figure 1 PCR amplification products of 20 genotypes of maize produced with SCoT marker SCoT 12. Lanes 1 - 20 are maize genotypes (Table 1) and M- 100 bp DNA ladder

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. **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.

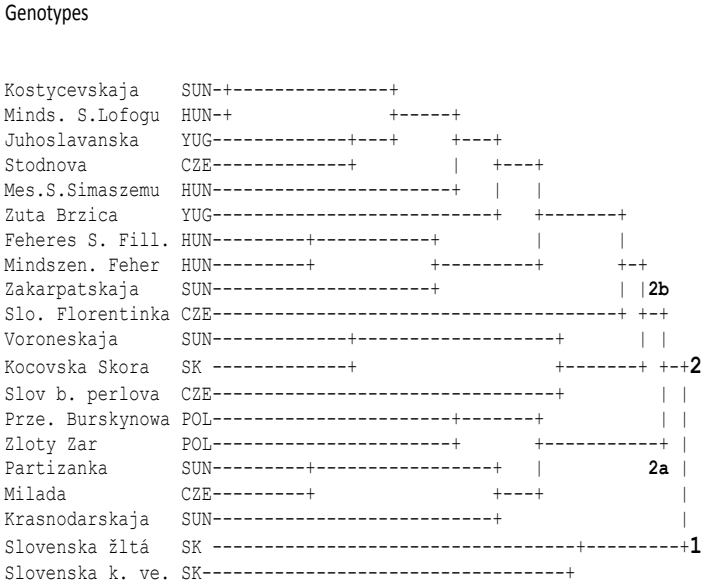

Figure 2 Dendrogram of 20 maize genotypes prepared based on 5 SCoT markers.

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. Unique 2 maize genotype Slovenska žltá and Slovenska krajová velkozrná, originated from Slovak Republic (cluster 1), separated from others. Cluster 2 containing 18 genotypes was divided into two main subclusters (2a and 2b). Subcluster 2a contained two Poland genotypes Przebedowska Burskynowa and Zloty Zar, two genotypes of Union of Soviet Socialist Republics- Partizanka and Krasnodarskaja and one Czechoslovakian genotypes Milada. In subcluster 2b were grouped 13 maize genotypes. 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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