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GENETIC RELATIONSHIP OF SOYBEAN (*GLYCINE MAX* L.) GENOTYPES USING SCoT MARKERS

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ABSTRACT

In the present investigation 28 genotypes of soybean were analysed using 37 start codon targeted (SCoT) markers and 37 primers produced 260 DNA fragments with an average of 7.03 bands per primer. From these 37 primers, primers SCoT 33 and SCoT 65 wos the most polymorphic, where 10 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (2) was detected by primers SCoT 8, SCoT 14, SCoT 19, SCoT 31 and SCoT 59. Of the 260 amplified bands, 200 (77.27 %) were polymorphic, with an average of 5.41 polymorphic bands per primer. To determine the level of polymorphism in the analysed group of soybean genotypes, polymorphic information content (PIC) was calculated. The polymorphic information content (PIC) value ranged from 0.512 (ScoT 66) to 0.968 (SCoT 12) with an average of 0.777. The dendrogram of genetic relationships among 28 soybean genotypes based on 37 SCoT markers was constructed. The hierarchical cluster analysis showed that the soybean geno-types were divided into 4 main clusters. The markers used in this study created a number of polymorphic bands among the different cultivars that can be utilized as molecular markers for their differentiation. The obtained data indicated that SCoT technique could be used efficiently for identification and differentiation of the soybean genotypes.

Keywords: Molecular markers, SCoT analysis, Polymorphism, Dendrogram, PIC, Soybean

INTRODUCTION

Soybean (*Glycine max* L.) has an economic importance between leguminous plants. It is considered as a source of seed proteins and oil. Recent investigations found that genetic diversity of elite soybean germplasm is limited **(Shi *et al.*, 2018)**. Along with the increase in the number of cultivars having minimal differences and the possibilities of phenotypic variability, examination of cultivars by DNA markers is timely **(Tasma *et al.*, 2011)**. Soybean contains high levels of proteins, and eight essential amino acids such as lysine, arginine, and leucine. It also has high levels of fatty acids and appreciable levels of vitamins and minerals. Soybeans contain a huge amount of omega-6 fatty acid, linolenic acid, and isoflavones. Soybean contains about 30% of soluble and insoluble carbohydrates **(Rotundo and Westgate, 2009)**. Molecular markers play an important role in assessing the phylogenetic relationships between and within different species and cultivars. Deoxyribonucleic acid (DNA)- based markers were commonly used in studies of genetic diversity, comparative biology, morphological characters, environmental conditions, conservation, and phylogenic phenomena between plant species and cultivars **(Haq *et al.*, 2014)**.
Studies of soybean based on SCoT polymorphism are used to determine the relationship between genotypes, the purity of commercial cultivars and to assess the genetic diversity **(Sun *et al.*, 2018)**. Start codon targeted (SCoT) polymorphism was considered as a novel molecular marker. This marker targets on short start codon ATG within plant genes and has been developed by **(Collard and Mackill, 2009)**. It was characterized by higher polymorphism and better marker resolvability than other DNA marker techniques like random amplified polymorphic DNAs (RAPD) and ISSR (inter simple sequence repeat), therefore earning its popularity for its superiority. SCoT markers have salutary properties such as easy to use, cheaper, faster, and including nonradioactive materials than other molecular markers. SCoTs are more directly used in constructing marker assisted breeding programs than RAPDs, ISSRs, and SSRs (microsatellite markers). SCoT marker is concerned with the conserved start codon in plant genes or flanking short region of the ATG translation initiation **(Collard and Mackill, 2009)**. SCoT markers have been used in many crop plant species such as cowpea **(Igwe *et al.*, 2017)**, plantago **(Rahimi *et al.*, 2018)**, castor **(Vivodík *et al.*, 2018)**, maize **(Sadek and Ibrahim, 2018; Al-Tamimi, 2020)**, flax **(Ahmed *et al.*, 2018)**, triticale **(El-Safty and Attia, 2019)**, wheat **(Alsamman *et al.*, 2020)**, *Elaeagnus angustifolia* L. **(Sevindik *et al.*, 2022)**, *Eriobotrya japonica* **(Sevindik & Delibay, 2022)**. Molecular markers has proven to be quite efficient in detecting genetic variations and used for diversity assessment and for identifying germplasm in a number of species **(Žiarovská *et al.*, 2014; Petrovičová *et al.*, 2015; Vyhnánek *et al.*, 2015; Ražná *et al.*, 2016; Žiarovská *et al.*, 2017; Štiasna *et al.*, 2019; Prysiazhniuk *et al.*, 2019; Aslan-Parviz *et al.*, 2020; Balaska *et al.*, 2021; Beranová *et al.*, 2022; Žiarovská *et al.*, 2022; Golian *et al.*, 2022; Žiarovská and Urbanová, 2022; Sevindik *et al.*, 2023a; Sevindik *et al.*, 2023b).**
The goal of this research is to evaluate the 37 SCoT markers effectiveness to determine the phylogenetic relationships among 28 soybean (*Glycine max* L.) genotypes.

MATERIAL AND METHODS

Plant material and DNA extraction

Soybean genotypes (28) (Table 1) were obtained from the Gene Bank in Piešťany, the Slovak Republic. Genomic DNA was isolated from the 14 days leaves with GeneJET™ (Thermo Scientific, USA). according to the manufacturer's instructions. Soybean genotypes were grown in a growth chamber on humus soil. Concentrations of isolated DNA were estimated using UV-VIS spectrophotometer and the final concentration of DNA was adjusted to 50 ng/µl. All the DNA samples were stored at − 20 °C.

SCoT amplification and statistical analysis

37 SCoT primers developed by **(Collard and Mackill, 2009)** were used to analyze 37 soybean genotypes. The PCR reaction mixture had a volume of 15 µl and consisted of the following components and volumes: 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. DNA replication 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. PCR amplification results were separated on 1.00 % agarose gels and visualized with ethidium bromide stain. In the obtained 1.00% agarose gels, PCR fragments were evaluated as present (1) or absent (0) and a dendrogram was constructed from the obtained binary matrix using the UPGMA algorithm. For the assessment of the polymorphism between genotypes soybean and usability SCoT markers in their differentiation we used polymorphic information content (PIC) **(Weber, 1990)**.

Table 1 List of analyzed 28 soybean genotypes

Number	Genotype	Code designation	Country of origin
1.	Anko	SVK001 L06 00013	unknown
2.	Baron	SVK001 L06 00015	Canada
3.	Polanka	SVK001 L06 00036	Czech Republic
4.	Sluna	SVK001 L06 00037	Czech Republic
5.	Jihomoravska Zluta	SVK001 L06 00047	Czech Republic
6.	Chmelarova Brnenska	SVK001 L06 00050	Czech Republic
7.	Dacota	SVK001 L06 00054	Czech Republic
8.	Canton	SVK001 L06 00098	USA
9.	Fred	SVK001 L06 00120	France
10.	Ishigo Wase	SVK001 L06 00148	Japan
11.	Kador	SVK001 L06 00158	France
12.	Lokus	SVK001 L06 00284	unknown
13.	Chabarovskaja	SVK001 L06 00287	Union of Soviet Socialist Republics
14.	Anoka	SVK001 L06 00323	USA
15.	Holt	SVK001 L06 00453	USA
16.	Comet	SVK001 L06 00485	unknown
17.	Korada	SVK001 L06 00513	Canada
18.	Zora	SVK001 L06 00520	Slovak Republic
19.	Armor	SVK001 L06 00525	France
20.	Recor	SVK001 L06 00527	France
21.	Maverick	SVK001 L06 00586	USA
22.	Cesar	SVK001 L06 00597	Canada
23.	Gaillard	SVK001 L06 00598	Canada
24.	Mario	SVK001 L06 00599	Canada
25.	Ugo	SVK001 L06 00601	Canada
26.	Bristol	SVK001 L06 00612	Canada
27.	Cardiff	SVK001 L06 00613	Canada
28.	Primus	SVK001 L06 00617	Canada

RESULTS AND DISCUSSION

In this work, 37 primers were screened for PCR amplification of DNA and SCoT analysis in 28 soybean genotypes. Table 2 shows sequences of these primers, total number of amplified fragments (TNoB) from 28 soybean genotypes, the number of polymorphic bands (NoPB) and the polymorphic information content (PIC) for each primer. 37 primers produced 260 DNA fragments (Table 2, Figure 2) with an average of 7.03 bands per primer. From these 37 primers, primers SCoT 33 and SCoT 65 were the most polymorphic, where 10 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (2) was detected by primers SCoT 8, SCoT 14, SCoT 19, SCoT 31 and SCoT 59. Of the 260 amplified bands, 200 (77.27 %) were polymorphic, with an average of 5.41 polymorphic bands per primer. To determine the level of polymorphism in the analysed group of soybean genotypes, polymorphic information content (PIC) was calculated (Table 2). The polymorphic information content (PIC) value ranged from 0.512 (ScoT 66) to 0.968 (SCoT 12) with an average of 0.777.

The dendrogram of genetic relationships among 28 soybean genotypes based on 37 SCoT markers was constructed (Figure 1). The hierarchical cluster analysis showed that the soybean genotypes were divided into 4 main clusters. Cluster 1 contained 2 genotypes - Anko and Comet, which have an unknown genetic origin. Cluster 2 contained 3 genotypes - Maverick, Holt and Anoka, which are of USA origin. Cluster 3 contained only one genotype - Chabarovskaja, originating from the Union of Soviet Socialist Republics, and separated from all analyzed soybean genotypes. Cluster 4 is the largest of all 4 clusters and is divided into subclusters 4A and 4B. Subcluster 4A contains only one genotype - Kador, which originates from France. Subcluster 4B is divided into further subclusters - 4BA, 4BB and 4BC. Subcluster 4BA contains 7 genotypes originating from Canada (Baron, Primus, Cesar, Ugo, Bristol, Cardiff and Mario). Subcluster 4BB contains 2 genotypes from Canada (Gaillard and Korada), 3 genotypes from France (Fred, Armor and Recor) and one genotype of unknown origin - Lokus. Subcluster 4BC is the most diverse and contains one soybean genotype from Japan (Ishigo Wase), contains all 5 soybean genotypes from the Czech Republic (Polanka, Chmelarova Brnenska, Dacota, Sluna and Jihomoravska Zluta) and one genotype each from Slovakia (Zora) and the USA (Canton) (Figure 1). Based on the obtained dendrogram, we can say that with the help of 37 SCoT markers, we managed to distinguish soybean genotypes based on their genetic origin. Thus, we confirmed the usability of SCoT markers for DNA analyzes of soybeans.

Table 2 List of used 37 SCoT primers **(Collard and Mackill, 2009)** and the statistical characteristics of the 37 SCoT markers used in soybean

SCoT Primers	Primer sequence (5´-3´)	TNoB	NoPB	PoPB	PIC
ScoT 2	CAACAATGGCTACCACCC	10	8	80.00	0.789
ScoT 3	CAACAATGGCTACCACCG	9	6	66.67	0.722
SCoT 6	CAACAATGGCTACCACGC	8	7	87.50	0.851
SCoT 8	CAACAATGGCTACCACGT	3	2	66.67	0.742
SCoT 9	CAACAATGGCTACCAGCA	7	5	71.43	0.888
SCoT 11	AAGCAATGGCTACCACCA	5	4	80.00	0.911
SCoT 12	ACGACATGGCGACCAACG	9	9	100.00	0.968
SCoT 13	ACGACATGGCGACCATCG	10	8	80.00	0.744
SCoT 14	ACGACATGGCGACCACGC	3	2	66.67	0.668
SCoT 15	ACGACATGGCGACCGCGA	9	7	77.78	0.811
SCoT 16	ACCATGGCTACCACCGAC	8	5	62.50	0.769
SCoT 17	ACCATGGCTACCACCGAG	4	3	75.00	0.730
SCoT 18	ACCATGGCTACCACCGCC	9	6	66.67	0.555
SCoT 19	ACCATGGCTACCACCGGC	3	2	66.67	0.896
SCoT 20	ACCATGGCTACCACCGCG	6	5	83.33	0.711
SCoT 21	ACGACATGGCGACCCACA	7	7	100.00	0.922
SCoT 22	AACCATGGCTACCACCAC	8	7	87.50	0.780
SCoT 23	CACCATGGCTACCACCAG	9	6	66.67	0.630
SCoT 26	ACCATGGCTACCACCGTC	8	5	62.50	0.755
SCoT 28	CCATGGCTACCACCGCCA	6	4	66.67	0.744
SCoT 29	CCATGGCTACCACCGGCC	10	5	50.00	0.629
SCoT 30	CCATGGCTACCACCGGCG	10	8	80.00	0.896
SCoT 31	CCATGGCTACCACCGCCT	3	2	66.67	0.744
SCoT 33	CCATGGCTACCACCGCAG	10	10	100.00	0.956
SCoT 34	ACCATGGCTACCACCGCA	4	3	75.00	0.869
SCoT 36	GCAACAATGGCTACCACC	6	5	83.33	0.731
SCoT 40	CAATGGCTACCACTACAG	6	5	83.33	0.800
SCoT 44	CAATGGCTACCATTAGCC	5	4	80.00	0.709
SCoT 45	ACAATGGCTACCACTGAC	8	6	75.00	0.666
SCoT 54	ACAATGGCTACCACCAGC	7	6	85.71	0.900
SCoT 59	ACAATGGCTACCACCATC	3	2	66.67	0.709
SCoT 60	ACAATGGCTACCACCACA	9	6	66.67	0.767
SCoT 61	CAACAATGGCTACCACCG	4	3	75.00	0.799
SCoT 62	ACCATGGCTACCACGGAG	7	5	71.43	0.787
SCoT 63	ACCATGGCTACCACGGGC	8	6	75.00	0.748
SCoT 65	ACCATGGCTACCACGGCA	10	10	100.00	0.923
SCoT 66	ACCATGGCTACCAGCGAG	9	6	66.67	0.512
Average		**7.03**	**5.41**	**77.27**	**0.777**
Total		**260**	**200**	-	-

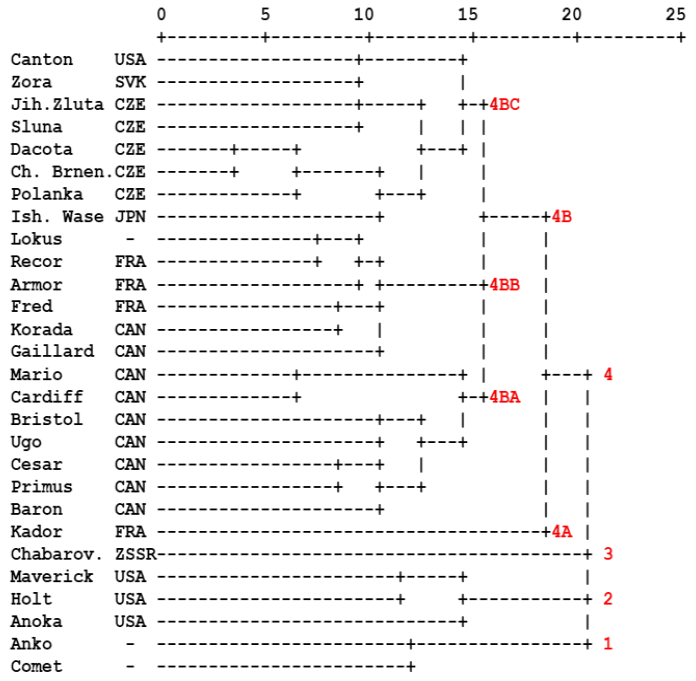

Figure 1 Dendrogram of 28 soybean genotypes prepared based on 37 SCoT markers.

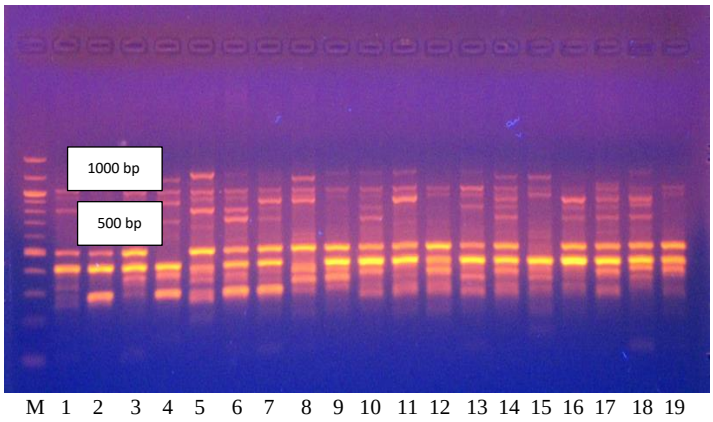

Figure 2 SCoT profiles of bands in soybean genotypes (SCoT 30). M- Quick-Load ® 2-Log DNA ladder and 1-19 are analyzed genotypes of soybean (Table 1).

Rayan and Osman (2019) using 11 SCoT primers to analysis of soybean genotypes. Using 11 SCoT primers, they detected together 106 fragments, from them 52 polymorphic and 54 monomorphic fragments with the overall degree of polymorphism 49.11%. Dendrogram obtained using techniques SDS-PAGE and SCoT markers revealed two main clusters; the first cluster contained 2 genotypes of soybean (Giza111, Giza21), while the second cluster contained 4 genotypes of soybean (Giza82, Giza35, Giza22, Giza83). The second cluster was further subdivided into 2 subclusters; the first subcluster contained 2 genotypes of soybean (Giza82, Giza35) and the second subcluster contained 2 genotypes of soybean (Giza22, Giza83). The aim of the study by **Jadhav *et al.* (2018)** was to determine the relatedness and polymorphism of 30 analyzed soybean genotypes using 20 SCoT primers. Using 20 SCoT primers, they detected an intermediate degree of polymorphism among the analyzed soybean genotypes (58.9%). The Polymorphism information content (PIC) of 20 SCoT primers ranged from 0.27 to 0.70 with an average value of 0.41. The dendrogram was constructed based on DNA SCoT profiling and soybean genotypes were grouped into 3 clusters. **Nosair *et al.* (2016)** analyzed genetic variability of 9 species of nutritional and medicinal important Fabaceae: faba bean (*Vicia faba*), fenugreek (*Trigonella foenum graecum*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), soybean (*Glycine max*), lupine (*Lupines termis*), cowpea (*Vigna unguiculata*), bean (*Phaseollus vulgaris*), pea (*Pisum sativum*) using 10 start codon targeted (SCoT) markers. Together, in 9 analyzed legume species, they detected 183 DNA fragments with a high degree of polymorphism (93.99%) between the analyzed legume genotypes. Dendogram analysis also reveals genetic variation among the nine species. The present study discloses the SCoT markers system as an effective technique for genetic diversity estimation of various leguminosae species. Such results boost utilization of the SCoT markers system in evolutionary studies, conservation and plant breeding. **Gao *et al.* (2016)** were applied SCoT markers to detect the genetic diversity among 159 cultivated soybeans released during 1923-2005 in China from Huang-Huai-Hai and southern region. Twenty seven primers were selected from 80 SCoT primers, 130 DNA bands were produced by 27 primers including 110 polymorphic bands, with an average of 84.62%. Nei's range of gene diversity ranged from 0.24 to 0.49, and the average was 0.37. The average value of locus polymorphism information contents 0.27. Dendrograms generated from coefficient of genetic distance between species on the basis of SCoT markers showed that 99 cultivated soybeans were mainly from Huang-Huai-Hai in Cluster 1, 60 cultivated soybeans of Group II were mainly from Nanfang. From 1923, the genetic diversity of cultivated soybean accessions is increasing, reached the highest in 1971-1990 and unchanged in the next two periods. This showed that the genetic diversity of cultivated soybean increased from 1970s. The results indicated that SCoT markers were feasible and effective to analyze the genetic diversity of cultivated soybean and provided an important reference for broadening the genetic base of soybean cultivars.

CONCLUSION

In summary, SCoT marker analysis was successfully developed to evaluate the genetic relationships among the genus soybean accessions originated from various area. The hierarchical cluster analysis showed that the soybean genotypes were divided into 4 main clusters. Polymorphism revealed by SCoT technique was so abundant and could be used for molecular genetics study of the soybean accessions, providing highvalued information for the management of germplasm, improvement of the current breeding strategies, and conservation of the genetic resources of soybean species.

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