JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

GENETIC DIVERSITY ANALYSIS OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) GENOTYPES USING SCOT POLYMORPHISM

*Zuzana Hromadová**, *Lucia Mikolášová, Želmíra Balážová, Martin Vivodík, Milan Chňapek, Zdenka Gálová*

Address(es): Ing. Zuzana Hromadová
Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

*Corresponding author: xhromadovaz@uniag.sk

https://doi.org/10.55251/jmbfs.5919

ARTICLE INFO

Received 2. 3. 2022
Revised 6. 6. 2022
Accepted 15. 6. 2022
Published 1. 8. 2022

Regular article

OPEN ACCESS

ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is legume crop of worldwide importance and due to optimal content of proteins and other essential compounds it has a potential as a functional food. Genetic diversity studies are significant from the point of obtaining information important for breeding process. The goal of present work was to analyze genetic diversity among 34 genotypes of common bean from different countries using 5 SCoT (Start Codon Targeted) markers. Altogether 82 DNA fragments were amplified, out of which 66 fragments were polymorphic with an average of 11 polymorphic fragments per primer. The highest number of polymorphic fragments was detected by marker SCoT 59 (15). The percentage of polymorphism ranged from 57.17% (SCoT 2) to 78.57% (SCoT 19) with an average of 67.3%. PIC values varied from 0.719 (SCoT 19) to 0.886 (SCoT 3) and average PIC value was 0.802. The DI values varied between 0.722 (SCoT 19) and 0.888 (SCoT3), with an average of 0.804. The dendrogram of 34 genotypes of common bean, constructed based on hierarchical cluster analysis separated genotypes into two clusters (I and II). Cluster I was formed by two subclusters. Thirty-three genotypes were included in cluster I and genotype Albena (Slovak Republic), which significantly differed from other genotypes in seed size was included in cluster II. Obtained results support the effectiveness of SCoT markers in the analysis of common bean useful for genotypes differentiating and assessment of genetic diversity in the set of common bean germplasm.

Keywords: common bean, dendrogram, genetic variability, gene specific markers

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is diploid plant (2n = 22) from *Fabaceae* family, with relatively small genome of 587 Mbp **(Capella-Gutiérrez *et al*., 2017)**. Common bean is often referred to as one of the most significant grain legumes, with great importance in human nourishment **(Marzooghian *et al*., 2013)**. Optimal proportions of proteins, complex carbohydrates and dietary fibre, also vitamins (A, C, and folate) and minerals (e.g. Ca, Fe, Cu, Cu, K, and Zn) contribute to nutritional value of this legume **(Assefa *et al*., 2019)**. Good nutritional properties of common bean, as well as its relatively easy production, tasty flavour, and many alternatives of food preparation determine its growing popularity. Especially for the people living in developing countries common bean provides good source of protein, calories and nutrients **(Myers and Kmiecik, 2017)**. It is also considered to be one of the main crops in terms of ensuring food security for nations, who are at the risk of malnutrition. This is the reason why many international breeding programs focus their attention on genetic improvements of common bean. Throughout recent decades, new successful varieties of common bean have been obtained. However, current situation regarding the climate change, as well as the lack of acceptance of adequate cultivating technologies, have affected the common bean production in negative terms. That is why new methods and approaches in breeding strategies should be accepted **(Jiménez, 2019)**. Genetic variability analysis allows breeders to gain information about germplasm and estimate its genetic potential. Information about genetic diversity ensure effective usage of germplasm resources and suitable breeding method with the aim of improving crop species **(Aljumaili *et al*., 2018)**.

Over the last years, several different molecular marker techniques have been developed and employed in breeding process of many important crops **(Rasmussen, 2020; Bohar *et al*., 2020)**. DNA markers are considered to be valuable tool for the indirect selection of important genes and their use in plant breeding **(Jiang, 2015)**. Through the years, a large number of marker systems were developed and implemented in the estimation of genetic diversity among plant genotypes **(Shekhawat *et al*., 2018; Zhang *et al*., 2015)**, population structure analysis **(Chen *et al*., 2020)**, genetic mapping **(Gujaria-Verma *et al*., 2016)** and the construction of genetic linkage maps **(Zheng *et al*., 2019)**.

Information regarding the genetic, as well as phenotypic diversity of common bean were obtained using the morphological characteristics, allozymes, seed protein - phaseolin, biochemical-nutritional characteristics, and DNA markers **(Chávez-Servia *et al*., 2016)**. With the comparison to previously used techniques, such as morphological markers, higher informative DNA markers are nowadays used for the genetic diversity studies and for the crop evaluation **(Gill-Langarica *et al*., 2011)**.

For the studies of common bean collections different DNA marker systems, such as Random Amplified Polymorphic DNA (RAPD; **Asifa *et al*., 2015)**, Amplified Fragment Length Polymorphism (AFLP; **Gill-Langarica *et al*., 2011)**, ISSR (Inter Simple Sequence Repeat; **Cabral *et al*., 2018)**, microsatellite markers **(Vidak *et al*., 2017)**, have been used. Among the various types of markers, a rather recent gene-targeted marker system called Start Codon Targeted (SCoT) Polymorphism, for the first time used by **Collard and Mackill (2009)**, is getting more attention. The principle of this DNA marker technique is in utilization of primers designed based on the conserved parts, which flanks the ATG (initiation) codon of plant genes. In the polymerase chain reaction (PCR) single 18-mer primers are used as forward and reverse primer at the annealing temperature of 50°C. SCoT markers are dominant markers suitable for quantitative trait loci mapping, bulk segregation and genetic analysis **(Collard and Mackill, 2009)**.

The aim of present study was to evaluate genetic diversity among the set of 34 common bean genotypes originating from different countries using 5 SCoT markers, and to testify usefulness of these markers in terms of differentiation and characterization of common bean genotypes. The information gathered here may be valuable for the future use in genomic mapping studies leading to obtain new common bean cultivars with improved traits and management of genetic resources.

MATERIAL AND METHODS

Plant material

In the present study, thirty-four common bean genotypes (*Phaseolus vulgaris*, L.) were used. Seeds of seventeen genotypes were obtained from the Gene Bank of Research Institute of Plant Production (RIPP) Piešťany in Slovak Republic (Table 1) and seventeen seeds of genotypes were provided by the Gene Bank of Research Institute of Plant Production (RIPP) Prague-Ruzyně in Czech Republic (Table 2). Genomic DNA was extracted from fresh leaves of fourteen-days old plant tissue and purified with the GeneJET Plant Genomic DNA Purification Mini Kit (Thermo Scientific™) following the manufacturer´s instructions. Concentrations of isolated DNA were estimated using UV-VIS spectrophotometer.

Table 1 List of analyzed common bean genotypes provided by Gene Bank of RIPP Piešťany (Slovak Republic)

Number	Genotype	Code designation	Country of origin
1.	Albena	SVK001 L05 01325	Slovak Republic
2.	Alicante	SVK001 L05 01130	USA
3.	Amanda	SVK001 L05 01032	Netherlands
4.	Atlanta	SVK001 L05 01036	unknown
5.	Belinda	SVK001 L05 01040	unknown
6.	Cabernet	SVK001 L05 01131	Netherlands
7.	Canada	SVK001 L05 01045	Canada
8.	Fullcrop	SVK001 L05 01065	USA
9.	Goliat	SVK001 L05 00455	Poland
10.	Marika	SVK001 L05 00932	Czech Republic
11.	Meteorit	SVK001 L05 01150	unknown
12.	Michael	SVK001 L05 01154	France
13.	Olga	SVK001 L05 00508	Germany
14.	Pesak	SVK001 L05 01166	Bulgaria
15.	Sancrop	SVK001 L05 01174	unknown
16.	Wawero	SVK001 L05 01188	unknown
17.	Zlaty Roh	SVK001 L05 01164	Slovak Republic

Table 2 List of analyzed common bean genotypes provided by Gene Bank of RIPP Prague-Ruzyně (Czech Republic)

Number	Genotype	Code designation	Country of origin
1.	Amethyst	09L0505134	Netherlands
2.	Amulet	09L0505139	France
3.	Augustynka	05L0500062	Poland
4.	Avans	05L0500271	Romania
5.	Enso	09L0505322	Sweden
6.	Favorit	09L0505350	Netherlands
7.	Fruca Simpla	09L0505384	Italy
8.	Gangtok bila	05L0500332	Ukraine
9.	Golden Dream	09L0505417	Denmark
10.	Grasa de Transilvania	09L0505437	Romania
11.	Herold	09L0505472	United Kingdom
12.	Kaboon	09L0500256	Hungary
13.	Katja	09L0500078	Sweden
14.	Kharkovskaya	05L0500151	Ukraine
15.	Mona	05L0500006	Czechoslovakia
16.	Nordstern	09L0500233	Germany
17.	Start	05L0500054	Hungary

SCoT markers assay

Altogether 5 SCoT primers (Table 3), designed according to **Collard and Mackill (2009)** and **Luo *et al.* (2010)** were used for analyses. The volume of amplification reaction was 15 µL, consisting of 7.5 µL of 2 × Master Mix (GoTaq® G2 Green Master Mix, USA), 4.5 µL of nuclease-free water, 1.5 µL of 10 pmol primer and 1.5 µL of template DNA (100 ng). The amplifications were carried out in the thermocycler (Biometra; Germany) with the following steps and conditions of amplification: an initial denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute, extension at 72°C for 2 minutes; and final extension at 72°C for 5 minutes. The amplification products were separated on 1.5% agarose in 1×TBE (Tris-borate-EDTA) buffer, with the addition of ethidium bromide as intercalating agent. Electrophoretic separation of amplified fragments was performed at constant voltage of 50V, while using 0.5×TBE buffer. The gels documentation was carried out with camera system PhotoDoc-It® (Ultra-Violet Products Ltd., United Kingdom). The size of DNA products was determined by comparison of obtained fragments with the DNA length marker Quick-Load® 2-Log DNA ladder (New England Biolabs Inc.).

Table 3 List of applied SCoT markers

SCoT marker	Primer sequence (5´→3´)
SCoT2[a]	CAACAATGGCTACCACCC
SCoT3[a]	CAACAATGGCTACCACCG
SCoT19[a]	ACCATGGCTACCACCGGC
SCoT34[a]	ACCATGGCTACCACCGCA
SCoT59[b]	ACAATGGCTACCACCATC

Legend: [a] - designed by **Collard and Mackill (2009)**, [b] - designed by **Luo *et al.* (2010)**

Statistical analysis

Qualitative (detection of DNA fragments) and quantitative (the size of amplified fragments – number of base pairs) evaluation of amplified DNA profiles was performed. Binary matrix was constructed based on the scoring of (1) for presence or (0) for absence of SCoT fragments and the data were used for the estimation of polymorphism level. Using the UPGMA algorithm (Unweighted Pair Group Method with Arithmetic Mean) the hierarchical cluster analysis was employed to construct the dendrogram with the statistic software SPPS Professional Statistics, version 17. For the assessment of the polymorphism of the common bean genotypes polymorphic information content (PIC) **(Weber, 1990)**, diversity index (DI) **(Weir, 1990)**, and probability of identity (PI) **(Paetkau *et al.*, 1995)** were calculated.

RESULTS AND DISCUSSION

In the present study, genetic diversity among 34 common bean genotypes was analyzed using Start Codon Targeted polymorphism. All 5 SCoT primers used for the analysis produced amplification products, whereas regarding the individual primers, different levels of polymorphism were detected. The number of DNA fragments ranged from 12 (SCoT 34) to 22 (SCoT 3) (Figure 1) and the amplicon size varied from 190 to 5100 bp. A total of 82 DNA fragments with an average of 16.4 fragments per primer using SCoT markers were generated. Altogether, 66 (80.49%) of produced fragments were polymorphic, with an average of 11 polymorphic fragments per primer. Primer SCoT 59 amplified the highest number (15) of polymorphic fragments. On the other hand, the lowest number of amplified polymorphic fragments (8) was detected using primers SCoT 2 and SCoT 34. The percentage of polymorphic fragments ranged from 57.17% (SCoT 2) to 78.57% (SCoT 19) with an average polymorphism of 67.3%. Three different coefficients: polymorphic information content (PIC), diversity index (DI), and probability of identity (PI) were determined to evaluate the level of polymorphism in the group of analyzed common bean genotypes (Table 4). PIC values varied from 0.719 (SCoT 19) to 0.886 (SCoT 3), with an average of 0.802 and DI value ranged from 0.722 (SCoT 19) to 0.888 (SCoT3), with an average of 0.804. PI value ranged from 0.002 (SCoT 3) to 0.035 (SCoT 19), with an average of 0.013.

Table 4 Features of 5 SCoT markers used for the genetic diversity study of common bean genotypes

SCoT primer	TNF	NPF	PPF (%)	PIC	DI	PI
SCoT2	14	8	57.17	0.779	0.780	0.012
SCoT3	22	13	59.09	0.886	0.888	0.002
SCoT19	14	11	78.57	0.719	0.722	0.035
SCoT34	12	8	66.67	0.768	0.772	0.014
SCoT59	20	15	75.00	0.857	0.859	0.004
Average	16.40	11	67.30	0.802	0.804	0.013
Total	82	66	-	-	-	-

Legend: TNF - Total Number of Fragments, NPF - Number of Polymorphic Fragments, PPF - Percentage of Polymorphic Fragments, PIC - Polymorphic Information Content, DI - Diversity Index, PI - Probability of Identity

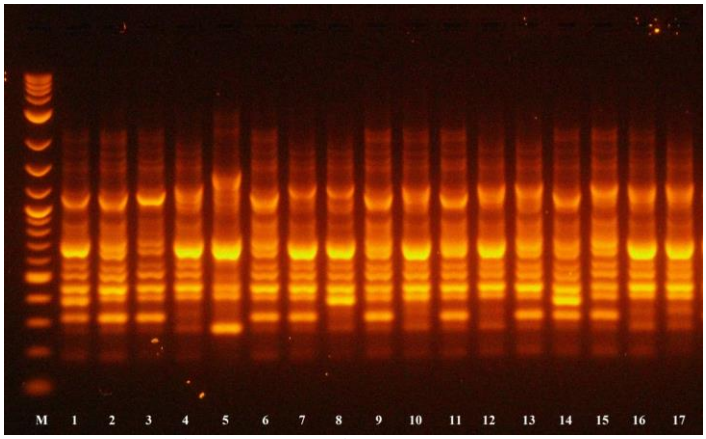

Figure 1 PCR amplification products of 17 common bean genotypes generated by SCoT 3 marker. M - Quick-Load ® 2-Log DNA ladder; 1-17 represent common bean genotypes provided by RIPP Prague-Ruzyně (Table 2)

SCoT markers were previously used for genetic diversity analysis of many agricultural crop species such as buckwheat **(Balážová *et al*., 2018)**, tomato **(EL-Mansy *et al*., 2021)**, garlic **(El-Fiki and Adly, 2020)**, durum wheat **(Etminan *et al*., 2016)**, maize **(Sadek and Ibrahim, 2018; Vivodík *et al*., 2017)**, rye **(Petrovičová *et al*., 2017)**, wheat **(Pour-Aboughadareh *et al*., 2017)**; as well as several important legume species including chickpea **(Hajibarat *et al*., 2015; Ahmad and Talebi, 2017)**, cowpea **(Igwe *et al*., 2017; Hussein and Osman, 2020)**, field pea **(Osman and Ali, 2020)**, pigeon pea **(Singh *et al*., 2018)**, mungbean (**Jena *et al*., 2017),** soybean **(Rayan and Osman, 2019)** and common bean **(Yeken *et al*., 2020).**

Due to the limited amount of information regarding the use of SCoT markers in common bean molecular variability analyzes, we focused on comparison of the results with those of genetic diversity studies performed on legumes. As reported by **Wang *et al.* (2017),** legume family altogether includes 650 genera and more than 18860 of species. Members of this family are characterized by great variability in genome size (e.g. ~400 Mbp in *Medicago truncatula* Desr. and 1150 Mbp in *Glycine max* L.), and the number of chromosomes varying from 6 to 20. Thus, we mainly chose *Fabaceae* species which are, in terms of size of the genome and ploidy, similar to common bean.

Lower average PIC values were obtained by many authors for example **Yeken *et al*. (2022), Hajibarat *et al*. (2015), Ahmad and Talebi (2017), Igwe *et al*. (2017), Rayan and Osman (2019), Osman and Ali (2021),** who studied different legume crops using SCoT markers.

Compared to our results **Yeken *et al*. (2022)** obtained higher an average percentage of polymorphism (87.51%). It was the first and the only study focusing on the evaluation of genetic diversity among common bean genotypes using SCoT markers. They analyzed genetic variability among 87 accessions of common bean (*Phaseolus vulgaris* L.) using 8 SCoT markers, which produced 118 evaluable DNA fragments. Out of total number (118) of amplified fragments 105 fragments were polymorphic (88.98 %). Even if they detected higher average number of polymorphic fragments (13.13) compared to our results (11) the average PIC value was lower (0.34). **Hajibarat *et al*. (2015)** used 9 SCoT markers to analyze 48 genotypes of chickpea (*Cicer arietinum* L.). Nine SCoT markers produced 145 fragments of which 133 (91.72 %) were polymorphic. The PIC values ranged from 0.43 to 0.47 with an average of 0.45. **Ahmad and Talebi (2017)** analyzed genetic diversity in set of 36 chickpea (*Cicer arietinum* L.) genotypes using 14 SCoT primers. They achieved in total 135 amplified fragments of which 100 fragments were polymorphic. The average percentage of polymorphism was similar to our results (72.4%), but average PIC value was lower compared to our results (0.36). **Rayan and Osman (2019)** used SCoT technique to evaluate its effectiveness for determination of the phylogenetic relationships among six Egyptian soybean (*Glycine max* L.) cultivars using 11 primers, which produced a total number of 106 fragments of which 106 were polymorphic. They obtained also lower average PIC values (0.44) compared to our results. **Osman and Ali (2021)** used three different molecular marker systems for the assessment of genetic relations among six field pea (*Pisum sativum* L.) subspecies. Out of the three used markers (RAPD, ISSR, SCoT), the SCoT markers were proven as the most informative but an average value of PIC was low only 0.228. Totally 105 fragments were produced using 8 SCoT primers of which 79 fragments were polymorphic with an average percentage of polymorphism 75.24%.

Singh *et al*. (2018) detected similar average PIC value (0.7345) compared to our results. They applied 15 SCoT primers in molecular diversity analysis of sixteen pigeon pea (*Cajanus cajan* L.) germplasm lines. Out of 15 used primers 8 SCoT primers amplified PCR products. Similar average PIC value (0.81) also obtained **Jena *et al*. (2017)** in the study of genetic variability among 38 mungbean (*Vigna radiata* (L.) R. Wilczek) genotypes using 15 SCoT markers. They amplified 230 evaluable fragments of which 222 were polymorphic with the higher percentage of polymorphism (96.52%) compared to our results. **Igwe *et al*. (2017)** assessed the genetic diversity of eighteen cowpea (*Vigna unguiculata* L. Walp.) genotypes using five SCoT primers. They detected values of PIC ranged from 0.6364 to 0.9210 with an average of 0.80 which were also comparable to our results.

Higher PIC observed **Chai et al. (2017)** who applied 5 SCoT primers for genetic diversity analysis among 240 individuals of four common vetch (*Vicia sativa* L.) accessions. Calculated PIC values varied from 0.9339 (SCoT 28) to 0.9520 (SCoT 36) with an average PIC value of 0.9434.

The genetic variability of common bean was also studied by the application of other types of molecular markers. **Pipan and Meglič (2019)** used 33 SSR markers to specify and explain the genetic relations and variability of 782 common bean accessions originated from 12 areas of southern Europe. They observed comparable average PIC value of SSR markers (0.800), representing high level of polymorphism.

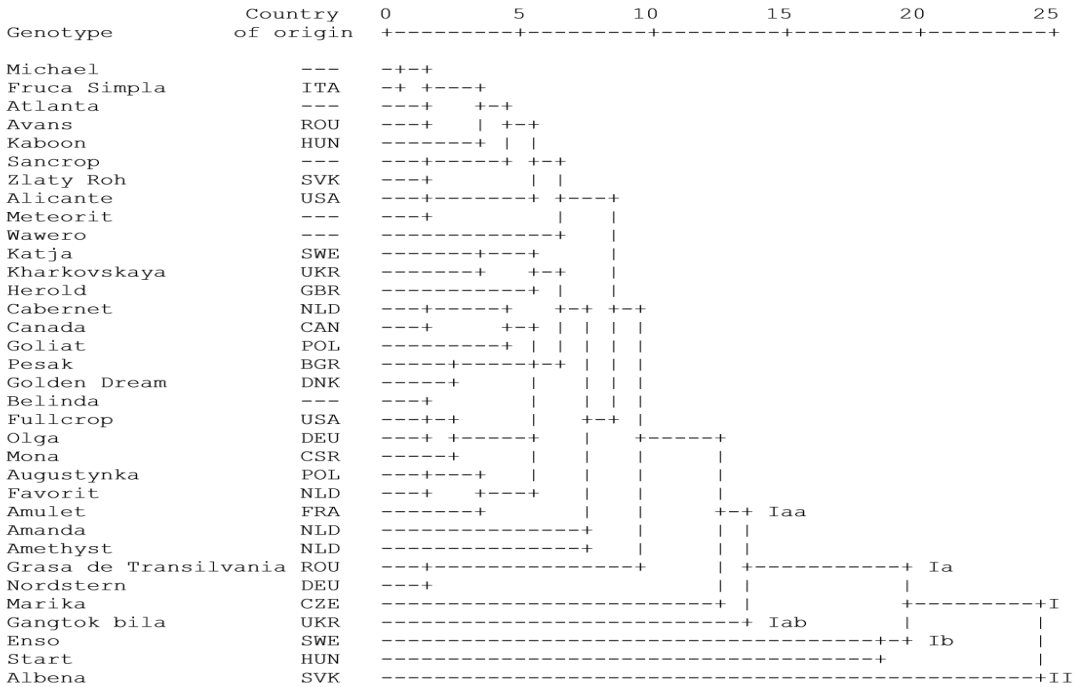

Figure 2 Cluster analysis of 34 common bean genotypes generated based on polymorphism of 5 SCoT markers

Comparable levels of polymorphism were observed by **Hamouda *et al*. (2020)**, who evaluated five populations of common bean using 6 ISSR markers. The primers amplified 117 bands, out of which 85 bands were polymorphic with the average percentage polymorphism 72.65%.

Asifa *et al*. (2015) characterized the genetic diversity of 45 common bean genotypes by using 19 RAPD markers. Out of the 253 observed fragments, 236 (94.22%) were polymorphic. The highest number of polymorphic bands (20) was produced by primer OPB-07. They obtained the average PIC value of 0.54. **Šustar-Vozlič *et al*. (2006)** assessed the structure of genetic diversity among 139 bean genotypes from Slovenia using 10 AFLP markers. Altogether 10 AFLP markers generated a total of 424 evaluable fragments of which 303 fragments (71%) were polymorphic. An average percentage of polymorphism was 71%. The results prove the high level of genetic variability among genetic resources of common bean, even within a rather small region like Slovenia.

For the effective visualization of genetic relationships in the set of analyzed plant genotypes a dendrogram based on hierarchical cluster analysis using UPGMA algorithm was constructed. Thirty-four common bean genotypes (Figure 2) were divided into two main clusters (I and II). Thirty-three genotypes were included in the cluster I. Genotype Albena originated from Slovak Republic, which differed morphologically in considerably larger seed size, separated from other genotypes in cluster II. Cluster I was further subdivided into two subclusters (Ia and Ib). Subcluster Ib included two genotypes Start and Enso from Hungary and Sweden, respectively. Subclaster Ia, involving thirty-one genotypes, was further divided into two subclusters (Iaa and Iab). Subcluster Iab separated genotype Gangtok bila from Ukraine. Subcluster Iaa consisting of thirty genotypes was further subdivided into two clusters, whereas genotype Marika from Czech Republic was separated from remaining genotypes in one subclaster. Genotype Fruca Simpla from Italy and genotype Michael of unknown origin included in the subcluster Iaa were considered as genetically the most similar. Despite of the application of relatively small number of SCoT markers it was possible to effectively distinguish and cluster genetic resources.

Similarly, many authors were able to differentiate genotypes of legumes using SCoT markers. **Yeken *et al*. (2022)** according to cluster analysis (UPGMA) and genetic structure based on SCoT data, were able to separate common bean accessions into Andean (PopA) and Mesoamerican (PopB) gene pools. Moreover, accessions were mostly placed in the same groups/subgroups according to their geographical origin. **Rayan and Osman (2019)** demonstrated that the dendrogram prepared based on UPGMA algorithm gave two main clusters of Egyptian soybean genotypes and the SCoT technique could be used efficiently for identification and differentiation of the selected Egyptian soybean genotypes. **Ahmad and Talebi (2017)** analyzed genetic diversity of 36 chickpea genotypes using 14 SCoT markers. They found that there was no strong relationship between morphological and molecular diversity pattern. The rate of diversity for morphological characters and SCoT-PCR based markers was different. They anticipate that the source of detected diversity is different.

CONCLUSION

The present work proved the utilization of SCoT markers in genetic diversity evaluation of selected common bean (*Phaseolus vulgaris* L.) genotypes. In total five SCoT markers were successfully applied in the analysis of genetic relationships among 34 common bean genotypes of different origin. Dendrogram divided genotypes into main two clusters (I and II). Cluster I contained 33 genotypes further subdivided into two subclusters. Cluster II separated genotype Albena (Slovak Republic), which is significantly different in the seed size. The cluster analysis did not separate analyzed genotypes according to the area of their origin, which may be assumably caused by the extensive genetic variability of common bean, associated with relatively parallel introduction of bean genetic resources belonging to two main gene pools to certain areas and their migration, as well as their mutual crossing. Also, as we assume, using of higher number of markers may contribute to more efficient differentiation of genotypes in the future. The level of polymorphism revealed by SCoT markers was abundant enough and thus could be efficiently applied for the genetic variability study of analyzed genotypes. Knowledge regarding the genetic variability of studied common bean genotypes may provide information important for the improvement of existing common bean cultivars in the breeding process, as well as the preservation and maintenance of common bean germplasm resources.

Acknowledgments: This publication was supported by the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund (20%); VEGA project No. 1/0291/21 (50%), KEGA project No. 026SPU-4/2021 (15%) and KEGA project No. 027SPU-4/2021 (15%).

REFERENCES

Ahmad, K., & Talebi, R. (2017). Genetic Diversity Assessment in Chickpea Using Morphological Characters and Start Codon Targeted (SCoT) Molecular Markers. *Annual Research & Review in Biology*, *15*(1), 1-8. https://doi.org/10.9734/arrb/2017/34203

Aljumaili, S. J., Rafii, M. Y., Latif, M. A., Sakimin, S. Z., Arolu, I. W., & Miah, G. (2018). Genetic Diversity of Aromatic Rice Germplasm Revealed By SSR Markers. *BioMed Research International*, *2018*, 11. https://doi.org/10.1155/2018/7658032

Asifa, B., Bhat, M. A., Ahmad, M., & Saleem, N. (2015). Examination of genetic diversity in common bean (*Phaseolus vulgaris* L.) using random amplified polymorphic DNA (RAPD) markers. *African Journal of Biotechnology*, *14*(6), 451-458. https://doi.org/10.5897/AJB2014.14281

Assefa, T., Mahama, A. A., Brown, A. V., Cannon, E. K. S., Rubyogo, J. C., Rao, I. M., Blair, M. W., & Cannon, S. B. (2019). A review of breeding objectives, genomic resources, and marker-assisted methods in common bean (*Phaseolus vulgaris* L.). *Molecular Breeding*, *39*(20), 1-23. https://doi.org/10.1007/s11032-018-0920-0

Balážová, Ž., Gálová, Z., Vivodík, M., Chňapek, M., & Hornyák Gregáňová, R. (2018). Molecular analysis of buckwheat using gene specific markers. *Potravinarstvo*, *12*(1), 546-552. https://doi.org/10.5219/954

Bohar, R., Chitkineni, A., & Varshney, R. K. (2020). Genetic molecular markers to accelerate genetic gains in crops. *BioTechniques*, *69*(3), 158-160. https://doi.org/10.2144/btn-2020-0066

Cabral, P. D. S., de Souza, L. C., da Costa, G. F., Silva, F. H. L., & Soares, T. C. B. (2018). Investigation of the genetic diversity of common bean (*Phaseolus vulgaris*.) cultivars using molecular markers. *Genetics and Molecular Research*, *17*(4), 1-11. http://dx.doi.org/10.4238/gmr18106

Capella-Gutiérrez, S., Vlasova, A., & Gabaldón, T. (2017). Phylogenomics: The Evolution of Common Bean as Seen from the Perspective of All of Its Genes. In: Pérez de la Vega M., Santalla M., Marsolais F. (eds). *The Common Bean Genome*. Compendium of Plant Genomes. Springer, Cham. 263–287. https://doi.org/10.1007/978-3-319-63526-2_12

Chai, X., Dong, R., Liu, W., Wang, Y., & Liu, Z. (2017). Optimizing Sample Size to Assess the Genetic Diversity in Common Vetch (*Vicia sativa* L.) Populations Using Start Codon Targeted (SCoT) Markers. *Molecules*, *22*(4), 567-577. https://doi.org/10.3390/molecules22040567

Chávez-Servia, J. L., Heredia-García, E., Mayek-Pérez, N., Aquino-Bolaños, E. N., Hernández-Delgado, S., Carrillo-Rodríguez, J. C., Gill-Langarica, H. R., & Vera-Guzmán, A. M. (2016). Diversity of Common Bean (*Phaseolus vulgaris* L.) Landraces and the Nutritional Value of their Grains. In: Goyal, A. K. *Grain Legumes*. IntechOpen. 1-33. https://doi.org/10.5772/63439

Chen, C., Chu, Y., Ding, C., Su, X., & Huang, Q. (2020). Genetic diversity and population structure of black cottonwood (*Populus deltoides*) revealed using simple sequence repeat markers. *BMC Genet*, *21*(2), 1-12. https://doi.org/10.1186/s12863-019-0805-1

Collard, B. C. Y., & Mackill, D. J. (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Report*, *27*, 86-93. https://doi.org/10.1007/s11105-008-0060-5

El-Fiki, A., & Adly, M. (2020). Morphological, molecular, and organosulphur compounds characterization in irradiated garlic (*Allium sativum*) by GC–MS and SCoT markers. *Journal of Radiation Research and Applied Sciences*, *13*(1), 61–70. https://doi.org/10.1080/16878507.2019.1697079

EL-Mansy, A. B., Abd El-Moneim, D., ALshamrani, S. M., Alsafhi, F. A., Abdein, M. A., & Ibrahim, A. A. (2021). Genetic Diversity Analysis of Tomato (*Solanum lycopersicum* L.) with Morphological, Cytological, and Molecular Markers under Heat Stress. *Horticulturae*, *7*(4), 65-83. https://doi.org/10.3390/horticulturae7040065

Etminan, A., Pour-Aboughadareh, A., Mohammadi, R., Ahmadi-Rad, A., Noori, A., Mahdavian, Z., & Moradi, Z. (2016). Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes. *Biotechnology & Biotechnological Equipment*, *30*(6), 1075–1081. https://doi.org/10.1080/13102818.2016.1228478

Gill-Langarica, H. R., Muruaga-Martínez, J. S., Vargas-Vázquez, M. L. P., Rosales-Serna, R., & Mayek-Pérez, N. (2011). Genetic diversity analysis of common beans based on molecular markers. *Genetics and Molecular Biology*, *34*(4), 595–605. https://doi.org/10.1590/S1415-47572011005000056

Gujaria-Verma, N., Ramsay, L., Sharpe, A. G., Sanderson, L.-A., Debouck, D. G., Tar'an, B., & Bett, K. E. (2016). Gene-based SNP discovery in tepary bean (*Phaseolus acutifolius*) and common bean (*P. vulgaris*) for diversity analysis and comparative mapping. *BMC Genomics*, *17*(239), 1-16. https://doi.org/10.1186/s12864-016-2499-3

Hajibarat, Z., Saidi, A., Hajibarat, Z., & Talebi, R. (2015). Characterization of genetic diversity in chickpea using SSR markers, Start Codon Targeted Polymorphism (SCoT) and Conserved DNA-Derived Polymorphism (CDDP). *Physiology and Molecular Biology of Plants*, *21*(3), 365–373. https://doi.org/10.1007/s12298-015-0306-2

Hamouda, M., Haider, A., Elshanshory, A., Elbehairy, E. (2020). Genetic variation among common beans cultivars (*Phaseolus vulgaris* L.) as revealed by morphological, protein and molecular markers. *The Egyptian Journal of Experimental Biology (Botany)*, *16*(2), 129-139. DOI:10.5455/egyjebb.20200721080720

Hussein, B. A., & Osman, N. H. (2020). Assessment of genetic diversity of some cowpeas (*Vigna unguiculata* L.) cultivars grown in Egypt based on Start Codon-Targeted (SCoT) markers. *Plant Archives*, *20*(2), 8660-8666.
Igwe, D. O., Afiukwa, C. A., Ubi, B. E., Ogbu, K. I., Ojuederie, O. B., & Ude, G. N. (2017). Assessment of genetic diversity in *Vigna unguiculata* L. (Walp) accessions using inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) polymorphic markers. *BMC Genetics*, *18*(98), 1-13. https://doi.org/10.1186/s12863-017-0567-6
Jena, R. C., Agarwal, K., Ghosh, T. S., & Chand, P. K. (2017). Population structuring of selected mungbean landraces of the Odisha State of India via DNA marker-based genetic diversity analysis. *Agri Gene*, *3*, 67–86. https://doi.org/10.1016/j.aggene.2016.11.007
Jiang, G.-L. (2015). Molecular Marker-Assisted Breeding: A Plant Breeder's Review. In: Al-Khayri, J. M., Jain, M. S., Johnson, D. V. (eds). *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools*. Cham: Springer. 431–472.
Jiménez, O. R. (2019). Common Bean (*Phaseolus vulgaris* L.) Breeding. In: Al-Khayri, J., Jain, S., Johnson, D., (eds). *Advances in Plant Breeding Strategies: Legumes*. Cham: Springer. 151-200. https://doi.org/10.1007/978-3-030-23400-3_5
Luo, C., He, X.-H., Chen, H., Ou, S.-J., & Gao, M.-P. (2010). Analysis of diversity and relationships among mango cultivars using Start Codon Targeted (SCoT) markers. *Biochemical Systematics and Ecology*, *38*(6), 1176–1184. https://doi.org/10.1016/j.bse.2010.11.004
Marzooghian, A., Moghaddam, M., Valizadeh, M., & Kooshk, M. H. (2013). Genetic diversity of common bean genotypes as revealed by seed storage proteins and some agronomic traits. *Plant Breeding and Seed Science*, *67*, 125-137. https://doi.org/10.2478/v10129-011-0075-1
Myers J.R., & Kmiecik K. (2017). Common Bean: Economic Importance and Relevance to Biological Science Research. In: Pérez de la Vega M., Santalla M., Marsolais F. (eds). *The Common Bean Genome*. Compendium of Plant Genomes. Springer, Cham. https://doi.org/10.1007/978-3-319-63526-2_1
Osman, S. A., & Ali, H. B. M. (2021). Genetic relationship of some *Pisum sativum* subspecies using different molecular markers. *Jordan Journal of Biological Sciences*, *14*(1), 65-74.
Paetkau, D., Calvert, W., Stirling, I., & Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, *4*(3), 347-354. https://doi.org/10.1111/j.1365-294X.1995.tb00227.x
Petrovičová, L., Balážová, Ž., Vivodík, M., & Gálová, Z. (2017). Detection genetic variability of *Secale cereale* L. by scot markers. *Potravinarstvo*, *11*(1), 197-202. https://doi.org/10.5219/726
Pipan, B., & Meglič, V. (2019). Diversification and genetic structure of the western-to-eastern progression of European *Phaseolus vulgaris* L. germplasm. *BMC Plant Biology*, *19*(442), 1-16. https://doi.org/10.1186/s12870-019-2051-0
Pour-Aboughadareh, A., Ahmadi, J., Mehrabi, A. A., Etminan, A., & Moghaddam, M. (2017). Assessment of genetic diversity among Iranian *Triticum* germplasm using agro-morphological traits and start codon targeted (SCoT) markers. *Cereal Research Communications*, *45*(4), 574–586. https://doi.org/10.1556/0806.45.2017.033
Rasmussen, S. K. (2020). Molecular Genetics, Genomics, and Biotechnology in Crop Plant Breeding. *Agronomy*, *10*(3), 439. https://doi.org/10.3390/agronomy10030439
Rayan, W. A., & Osman, S. A. (2019). Phylogenetic relationships of some Egyptian soybean cultivars (*Glycine max*, L.) using SCoT marker and protein pattern. *Bulletin of the National Research Centre*, *43*(161), 1-10. https://doi.org/10.1186/s42269-019-0197-4
Sadek, M. S. E., & Ibrahim, S. D. (2018). Genetic relationships among maize inbred lines as revealed by start codon targeted (SCoT) analysis. *Journal of Innovations in Pharmaceutical and Biological Sciences*, *5*(1), 103-107.
Shekhawat, J. K., Rai, M. K., Shekhawat, N. S., & Kataria, V. (2018). Start codon targeted (SCoT) polymorphism for evaluation of genetic diversity of wild population of *Maytenus emarginata*. *Industrial Crops and Products*, *122*, 202–208. https://doi.org/10.1016/j.indcrop.2018.05.074
Singh, S. K., Jadhav, P. V., Nandanwar, R. S., Patil, A. N., Wandhare, M., & Naik, R. M. (2018). Molecular Characterization of Vegetable-Type Pigeonpea Genotypes Using SCoT Markers. *International Journal of Current Microbiology and Applied Sciences*, (special issue-6),1471-1477.
Šustar-Vozlič, J., Maras, M., Javornik, B., & Meglič, V. (2006). Genetic Diversity and Origin of Slovene Common Bean (*Phaseolus vulgaris* L.) Germplasm as Revealed by AFLP Markers and Phaseolin Analysis. *Journal of the American Society for Horticultural Science*, *131*(2), 242–249. https://doi.org/10.21273/jashs.131.2.242
Vidak, M., Carović-Stanko, K., Barešić, A., Grdiša, M., Šatović, Z., & Liber, Z. (2017). Microsatellite markers in common bean (*Phaseolus vulgaris* L.). *Journal of Central European Agriculture*, *18*(4), 902–917. https://doi.org/10.5513/JCEA01/18.4.1983
Vivodík, M., Balážová, Ž., Gálová, Z., & Petrovičová, L. (2017). Genetic Diversity Analysis of Maize (*Zea mays* L.) Using SCoT Markers. *Journal of Microbiology, Biotechnology and Food Sciences*, *6*(5), 1170–1173. 10.15414/jmbfs.2017.6.5.1170-1173
Wang, J., Sun, P., Li, Y., Liu, Y., Yu, J., Ma, X., Sun, S., Yang, N., Xia, R., Lei, T., Liu, X., Jiao, B., Xing, Y., Ge, W., Wang, L., Wang, Z., Song, X., Yuan, M., Guo, D., Zhang, L., Zhang, J., Jin, D., Chen, W., Pan, Y., Liu, T., Jin, L., Sun, J., Yu, J., Cheng, R., Duan, X., Shen, S., Qin, J., Zhang, M-c., Paterson, A. H., & Wang, X. (2017). Hierarchically Aligning 10 Legume Genomes Establishes a Family-Level Genomics Platform. *Plant Physiology*, *174*(1), 284–300. https://doi.org/10.1104/pp.16.01981
Weber, J. L. (1990). Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics*, *7*(4), 524-530. https://doi.org/10.1016/0888-7543(90)90195-Z
Weir, B. S. (1990). *Genetic data analysis*. Sinauer Associated. Sunderland, UK : Massachussets. 445 p. ISBN 0 87893 871 00 87893.
Yeken, M. Z., Emiralioğlu, O., Çiftçi, V., Bayraktar, H., Palacioğlu, G., & Özer, G. (2022). Analysis of genetic diversity among common bean germplasm by start codon targeted (SCoT) markers. *Molecular Biology Reports*. https://doi.org/10.1007/s11033-022-07229-z
Zhang, J., Xie, W., Wang, Y., & Zhao, X. (2015). Potential of Start Codon Targeted (SCoT) markers to estimate genetic diversity and relationships among Chinese *Elymus sibiricus* accessions. *Molecules*, *20*(4), 5987–6001. https://doi.org/10.3390/molecules20045987
Zheng, C., Boer, M. P., & van Eeuwijk, F. A. (2019). Construction of Genetic Linkage Maps in Multiparental Populations. *Genetics*, 212(4), 1031–1044. https://doi.org/10.1534/genetics.119.302229