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MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY STUDIE OF SOYBEAN (*GLYCINE MAX* L.) CULTIVARS USING RAPD MARKERS

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ABSTRACT

Soybean (*Glycine max* L.) is an important crop plant which contains a high amount of oil and protein. The main goal of the work was to use 13 RAPD (Random Amplified Polymorphic DNA) markers to study genetic polymorphism in a set of 28 soybean genotypes and to construct a dendrogram from the obtained results, based on which we will recommend genotypes for further breeding. In total, using 13 RAPD markers, we determined 108 fragments in a set of 28 soybean genotypes with an average number of 8.31 fragments per genotype. The number of fragments varied from 6 (OPB-08, OPE-07) to 12 (SIGMA-D-01). Of the total number of 108 fragments, 70 were polymorphic with an average number of 5.38 polymorphic fragments per genotype. The number of polymorphic fragments varied from 3 (OPE-07, OPF-14) to 9 (SIGMA-D-01). The average percentage of fragment polymorphism was 63.68% and ranged from 42.86% (OPF-14) to 83.33% (OPB-08). Diversity index (DI) values ranged from 0.710 (OPA-03) to 0.846 (OPD-08) with an average value of 0.763. A dendrogram prepared based on hierarchical cluster analysis using UPGMA algorithm separated 28 soybean genotypes into four clusters. The applied RAPD markers proved to be a suitable tool for detection of the genetic diversity of soybean genotypes. Based on the constructed dendrogram, it is possible to select suitable varieties for marker-assisted breeding.

Keywords: soybean, RAPD, dendrogram, polymorphism, UPGMA

INTRODUCTION

Soy (Glycine max L.) is relatively the cheapest source of protein. Soybean seeds contain up to 40-42% protein, the oil content is at the level of 18-20% and soluble carbohydrates are up to 11%. It follows from the above that soybean seeds are nutritionally interesting for the production of various types of food, but also for the production of animal feed (**Devi *et al.*, 2012**). The wild and cultivated soybeans showed significant phenotypic diversity but the small reproductive difference, and they have very similar genomes in both its size and content **(Singh and Hymowitz, 1999)**. A potential source of protein and oil makes soybeans a large share in human nutrition, and also improves soil fertility therefore; soybean is also an important crop for research **(Priestera *et al.*, 2012)**. From the point of view of the possibility of improving the useful properties of agriculturally and food-important agricultural crops, it is important to evaluate their genetic variability. Knowledge of the genetic diversity of soybean can help breeders and geneticists understand the structure of the germplasm, on the basis of which it would be possible to expand the genetic basis for obtaining new soybean varieties with improved characteristics in the process of marker-assisted reproduction **(Priestera *et al.*, 2012)**.
Genetic diversity studies are usually tapped due to molecular markers. Molecular markers are an excellent method to disentangle phylogenetic association between species and population. One of the technical advances in the field of detecting genetic variability in plants has been the development of different types of polymerase chain reaction (PCR). The RAPD method, also known as random amplification polymorphic DNA, allows the amplification of discrete fragments of the genome, while prior knowledge of their sequences is not necessary. After electrophoretic separation, DNA fragments can be used as a genetic marker to determine the differences between individual plant genotypes **(Shuangxia *et al.*, 2008).** Among molecular methods or markers, RAPD are sensitive to detect variability among individuals of species. RAPD method is costeffective and can work with limited sample quantities. In addition to this, RAPD can amplify and target genomic regions with potential and several markers **(Esfandani-Bozchaloyi *et al.*, 2017)**. Soybean genetic diversity was analyzed using several DNA techniques, such as: RAPD **(Wahyudi *et al.*, 2020; Nkongolo *et al.*, 2020)**, Microsatellite markers (SSR) **(Mihaljevic, *et al.*, 2020; Kumar *et al.*, 2022)**, Inter Simple Sequence Repeat (ISSR) **(Monpara *et al.*, 2017)**, Target region amplification polymorphism (TRAP) (**Kim *et al.*, 2020)**, Restriction fragment length polymorphism (RFLP) **(Lee *et al.*, 2002)** and Amplified Fragment Length Polymorphism (AFLP) (**Zargar *et al.*, 2017).**

The RAPD technique has been used to study the polymorphism of many plant species, such as: phaseolus **(Gjorgieva *et al.*, 2012)**; pea **(Choudhury *et al.*, 2007)**; castor **(Vivodík *et al.*, 2014; Vivodík *et al.*, 2015)**, patchouli (**Pandey *et al.*, 2022**), carnation **(Sharma *et al.*, 2022)**, cassia **(Eldemerdash *et al.*, 2022)**, maize **(Balážová *et al.*, 2017)**, rye **(Petrovičová *et al.*, 2015)**, wheat (**Kuťka Hlozáková *et al.*, 2016)**, amaranthus **(Štefúnová *et al.*, 2015)**, sorghum **(Ruiz-Chután *et al.*, 2019)**.
The main goal of the work was to use 13 RAPD markers to study genetic polymorphism in a set of 28 soybean genotypes and to construct a dendrogram from the obtained results, based on which we will recommend genotypes for further breeding.

MATERIAL AND METHODS

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 Plant Genomic DNA Purification Mini Kit 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.
Amplification of RAPD fragments was performed according to **Gajeraa *et al.*, (2010)** using decamer arbitrary primers (Table 2). A total volume of 25 µl of the reaction mixture contained 100 ng of DNA, 12.5 µl of Master Mix (Genei, Bangalore, India) and 10 pmol of primer. DNA amplification was performed in a thermocycler (Biometra, Germany) programmed as follows: initial DNA denaturation at 94°C for 5 min, followed by 42 cycles of denaturation at 94°C for 1 min, primer annealing at 38°C for 1 min, synthesis of the new DNA strand at 72°C for 1 min and a final step at 72°C for 5 min. Amplified DNA products were separated by horizontal gel electrophoresis in 1.5% agarose in 1 x TBE buffer at a constant voltage of 100 V for approximately 1 hour. Ethidium bromide was used as an intercalating agent in the gel. Evaluation of the gels was performed under a UV lamp using the UVP PhotoDoc-It® system and evaluated by the program GelAnalyzer.

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

Data analysis

The presence (1) or absence (0) of polymorphic reproducible markers was assessed in the obtained DNA profiles. The resulting binary matrix was used to construct a dendrogram. The representation of the genetic relatedness of the analyzed varieties in the form of a dendrogram was carried out based on a hierarchical cluster analysis using the UPGMA algorithm (Unweighted Pair Group Method using arithmetic averages) according to the Jaccard coefficient of genetic similarity using a specific SPSS Professional Statistics module version 17, a statistical package of SPSS programs for Windows. Genetic diversity was expressed in relative values in the range 0-25. For the assessment of the polymorphism between genotypes ricin and usability RAPD markers in their differentiation we used diversity index (DI) **(Weir, 1990)**, the probability of identity (PI) **(Paetkau *et al.*, 1995)** and polymorphic information content (PIC) **(Weber, 1990)**.

Table 2 List of RAPD primers **(Gajeraa *et al.*, 2010)**

Primers	Primer sequence (5´-3´)	Molecular weight range (bp)
OPA-02	TGCCGAGCTG	400-2800
OPA-03	AGTCAGCCAC	330-870
OPA-13	CAGCACCCAC	370-1800
OPB-08	GTCCACACGG	530-1550
OPD-02	GGACCCAACC	280-1850
OPD-07	TTGGCACGGG	360-1440
OPD-08	GTGTGCCCCA	260-1700
OPD-13	GGGGTGACGA	160-1800
OPE-07	AGATGCAGCC	300-1940
OPF-14	TGCTGCAGGT	190-1850
SIGMA-D-01	AAACGCCGCC	280-1350
SIGMA-D-14	TCTCGCTCCA	350-900
SIGMA-D-P	TGGACCGGTG	300-3000

RESULTS AND DISCUSSION

In total, using 13 RAPD markers, we determined 108 fragments in a set of 28 soybean genotypes with an average number of 8.31 fragments per genotype (Figure 1), (Table 3). The number of fragments varied from 6 (OPB-08, OPE-07) to 12 (SIGMA-D-01). Of the total number of 108 fragments, 70 were polymorphic with an average number of 5.38 polymorphic fragments per genotype. The number of polymorphic fragments varied from 3 (OPE-07, OPF-14) to 9 (SIGMA-D-01). The average percentage of fragment polymorphism was 63.68% and ranged from 42.86% (OPF-14) to 83.33% (OPB-08). Diversity index (DI) values ranged from 0.710 (OPA-03) to 0.846 (OPD-08) with an average value of 0.763. For all 13 RAPD markers, the DI values were higher than 0.700, which indicates a high degree of polymorphism of the analyzed soybean genotypes. Polymorphic Information Content (PIC) values ranged from 0.639 (SIGMA-D-14) to 0.754 (OPE-07) with an average value of 0.698. In this case, 8 RAPD markers had a calculated PIC value higher than 0.700 and 5 RAPD markers had a PIC value lower than 0.700. The PIC values also speak of the good usability of RAPD markers for soybean identification and differentiation. Probability of identity (PI) values ranged from 0.004 (OPA-03) to 0.070 (OPD-08) with an average value of 0.028 (Table 3).

Lower average PIC values were obtained by many authors for example **Perić *et al.* (2014), Jain *et al.* (2017)** and **Wahyudi *et al.* (2020)** who studied soybean genotypes using RAPD markers. The genetic diversity of a set of soybean genotypes was analyzed by **Perić *et al.* (2014)** using 33 RAPD primers. Of the mentioned number, 21 RAPD markers showed a clear DNA profile of sufficient intensity, which were suitable for evaluating the genetic similarity between the analyzed soybean genotypes. PIC values for all polymorphic primers ranged from 0.027 - 0.7359 with a mean of 0.3887. RAPD fragments from 13 polymorphic RAPD primers with PIC>0.30 were considered as informative. **Jain *et al.* (2017)** used a total 20 RAPD primers of which 18 amplified 164 bands and all were polymorphic. The Polymorphic Information Content (PIC) values ranged from 0.126 (OPP-01) to 0.399 (OPF-19) with an average of 0.295. Jaccard‟s similarity coefficient values ranged from 0.12 to 0.70 with an average of 0.41. **Wahyudi *et al.* (2020)** studied the genetic diversity of soybean mutant genotypes, using twenty OPA primers to amplify RAPD fragments. A total of 105 DNA bands were detected, of which 92 bands were polymorphic. Polymorphic information content (PIC), effective multiplex ratio (EMR) and marker index (MI) were calculated to evaluate DNA polymorphism. The PIC values ranged from 0.20 to 0.50 with a mean of 0.42, with the lowest PIC value shown by primer OPA-19 and the highest PIC value generated by OPA-01, OPA-05, OPA-13, OPA-14 and OPA- 20.

Panjoo *et al.*, (2014) determined the same percentage of polymorphism as us (63.68%). The genetic variability of the soybean set was assessed by 10 RAPD primers, which produced a total of 103 bands, of which 62 were polymorphic and 41 monomorphic. The percentage of polymorphism between individual primers ranged from 36% to 78% with an average of 60%. Primers B15, E19 and D15 produced the highest number of polymorphic bands (each primer with 13 amplified bands), while primers D10 and OPY10 produced the lowest polymorphic bands (each primer with 6 bands). **Perić *et al.* (2014)** determined a lower percentage of polymorphism while detecting together 107 fragments, of which 46 (43 %) were polymorphic.

These authors determined a higher percentage of polymorphism than we did: **Jain *et al.* (2017), Sharma *et al.* (2018)** and **Wahyudi *et al.* (2020)**. **Jain *et al.* (2017)** were obtained 164 amplified bands of which all were polymorphic and showed 100% polymorphism. **Sharma *et al.* (2018)** tested a total of 19 RAPD markers on soybean genotypes, which generated 83 unambiguous polymorphic amplification DNA fragments, the size of which ranged from 225–3630 bp. The total number of bands formed per primer ranged from 2 (primer OPC 12) to 10 (primer OPC 19) with an average of 5.92 bands per primer. The number of polymorphic bands lying between 0 and 7 with polymorphism range from 0% (OPC 12 and OPD 19) to 100% (OPC 19 and OPD 4). In total, 74.69% of bands were polymorphic. **Wahyudi *et al.* (2020)** analyzed a set of EMS soybean mutated genotypes for the detection of genetic diversity, applying twenty OPA RAPD primers. The percentage of polymorphism ranged from 33.30% to 100%, with a mean of

85.80%. OPA-19 contains the lowest percentage of polymorphism, generating only 1 polymorphic band out of 3 of all DNA fragments. On the other hand, 11 primers (OPA-2, OPA-07, OPA-10, OPA-11, OPA-12, OPA-13, OPA-14, OPA-15, OPA-16, OPA-18, OPA-20) showed 100% polymorphism. The authors recommend calculating PIC, EMR and MI values for DNA polymorphism detection and to identify the primer that is most informative.

Nkongolo *et al.* (2020) studed the level of genetic variation among soybean (*G. max*) accessions from different countries using RAPD markers. Genomic DNAs from 108 soybeans accessions from 11 different gene pools were analyzed using five RAPD primers (OPA 11, Pinus 23, UBC 377, UBC186, Grasse 8). The average level of polymorphic loci detected with the RAPD primers was 35%. The highest polymorphic index among accessions was 48.00% in France, followed by accessions from Hungary with 43.30%. Over all, the lowest polymorphic index was 29.90% (Canada), 26.29% (Netherlands) and 24.74% (China). At the primer level, primers UBC 186 and Grasse 8 generated the most number of bands (41) followed by UBC 377 with 40 bands. The lowest number of amplified product was observed with primer Pinus 23. The highest level of polymorphic loci (46.30%) was detected with primer UBC 186 and the lowest with UBC 377.

A dendrogram prepared based on hierarchical cluster analysis using UPGMA algorithm separated 28 soybean genotypes into four clusters (Figure 2). Cluster 1 contains 6 soybean genotypes, 2 of which come from the USA (Anoka, Holt) and one each from Canada (Cesar), Slovakia (Zora), France (Kador) and the Czech Republic (Dacota). Cluster 2 consists of 3 genotypes, of which 2 come from North America, one from the USA (Maverick), one from Canada (Bristol) and the Comet genotype is of unknown origin. Cluster 3 consists only of the Ugo genotype, which originates from Canada. Cluster 4 is the largest of all 4 clusters and contains a total of 18 soybean genotypes, which are divided into 2 subclusters (4A and 4B). Subcluster 4A contains 2 soybean genotypes from Canada (Baron and Gaillard), one genotype from the Czech Republic (Chmelarova Brnenska) and one genotype from Japan (Ishigo Wase). Subcluster 4B is the most diverse and contains 4 genotypes from Canada (Primus, Mario, Korada and Cardiff), 3 genotypes from the Czech Republic (Sluna, Polanka and Jihomoravska Zluta), 3 genotypes from France (Fred, Armor and Recor), one genotype from the USA (Canton) and the ZSSR (Khabarovskaja) and 2 genotypes of unknown origin (Anko and Lokus). In subcluster 4 B, we could not distinguish between 2 Czech genotypes (Polanka and Jihomoravska Zluta), which probably have the same genetic origin, using 13 RAPD markers. To distinguish them, it would be necessary to use more RAPD markers or other DNA markers. On the basis of the obtained results, we can conclude that with the help of 13 RAPD markers, we failed to distinguish the genotypes based on their genetic origin. To better distinguish soybean genotypes, it would be advisable to use other DNA techniques, such as SSR, SCoT, TRAP and EBAP technique.

Similarly, many authors were able to differentiate genotypes of soybean using RAPD markers. **Jain *et al.* (2017)** constructed a dendrogram in which the 24 soybean genotypes analyzed divided into 3 major clusters with a similarity coefficient of 0.29. The RAPD methods displayed genetic variation among 24 soybean genotypes and phylogenetic tree was showing a relationship among them. **Wahyudi *et al.* (2020)** constructed a dendrogram in which the analyzed soybean genotypes were divided into 2 main clusters. The wild-type soybean became the first group, which act like an outgroup. The second group was consist of all soybean mutants, which can be separated into 3 subgroups. The similarity index and clustering method were performed to ascertain the degree of genetic relationship among soybean mutants and wild-type.

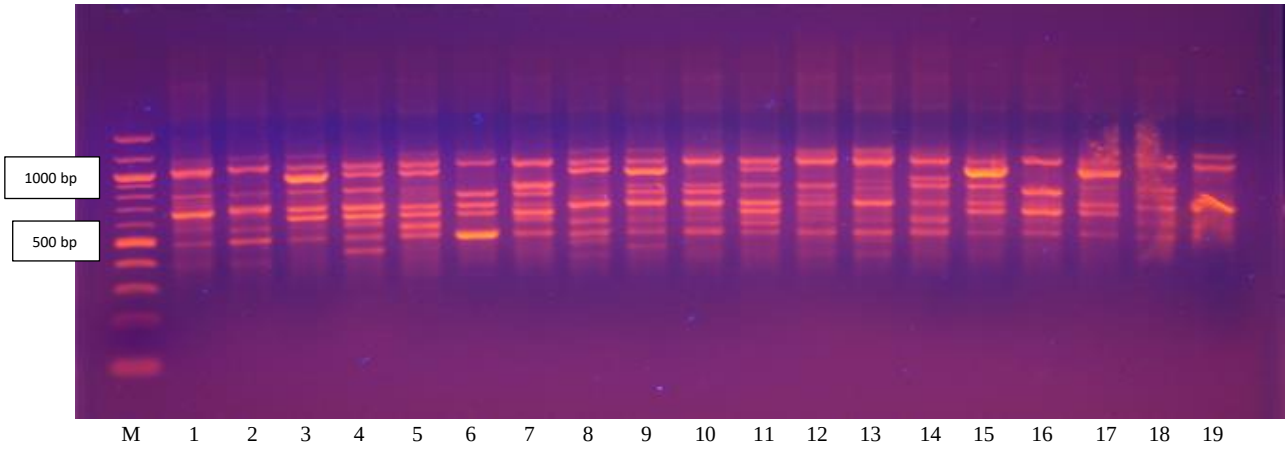

Figure 1 RAPD profiles of bands in soybean genotypes (OPA-02). M- Quick-Load ® 2-Log DNA ladder and 1-19 are analyzed genotypes of soybean (Table 1)

Table 3 The statistical characteristics of the 13 RAPD markers used in 28 soybean genotypes

Primer	Total number of fragments	Number of polymorphic fragments	Percentage of polymorphism	DI	PIC	PI
OPA-02	8	5	62.50	0.729	0.659	0.015
OPA-03	7	4	57.14	0.710	0.700	0.004
OPA-13	7	4	57.14	0.780	0.721	0.010
OPB-08	6	5	83.33	0.715	0.701	0.008
OPD-02	9	6	66.66	0.816	0.730	0.038
OPD-07	7	4	57.14	0.714	0.659	0.015
OPD-08	9	6	66.66	0.846	0.711	0.070
OPD-13	11	8	72.73	0.810	0.709	0.010
OPE-07	6	3	50.00	0.825	0.754	0.062
OPF-14	7	3	42.86	0.812	0.745	0.059
SIGMA-D-01	12	9	75.00	0.731	0.693	0.039
SIGMA-D-14	10	7	70.00	0.711	0.639	0.019
SIGMA-D-P	9	6	66.66	0.717	0.649	0.021
Total	**108**	**70**		-	-	-
Average	**8.31**	**5.38**	**63.68**	**0.763**	**0.698**	**0.028**

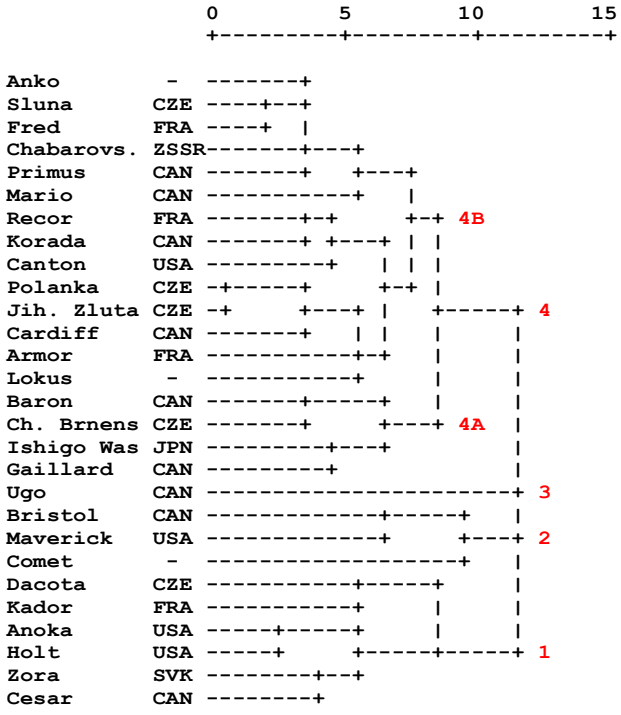

Figure 2 Dendrogram of 28 soybean genotypes prepared based on 13 RAPD markers

CONCLUSION

The main goal of the work was to use 13 RAPD markers to study genetic polymorphism in a set of 28 soybean genotypes and to construct a dendrogram. In total, using 13 RAPD markers, we determined 108 fragments in a set of 28 soybean genotypes with an average number of 8.31 fragments per genotype. The number of fragments varied from 6 (OPB-08, OPE-07) to 12 (SIGMA-D-01). Of the total number of 108 fragments, 70 were polymorphic with an average number of 5.38 polymorphic fragments per genotype. The number of polymorphic fragments varied from 3 (OPE-07, OPF-14) to 9 (SIGMA-D-01). Based on the obtained results, we can conclude that with the help of 13 RAPD markers, we failed to distinguish soybean genotypes based on their genetic origin. To better distinguish soybean genotypes, we would suggest using a combination of several DNA techniques, such as SSR, TRAP, SCoT or EBAP technique. Knowledge regarding the genetic variability of studied soybean genotypes may provide information important for the improvement of existing soybean cultivars in the breeding process, as well as the preservation and maintenance of soybean germplasm resources.

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REFERENCES

Balážová, Z., Vivodík, M. and Gálová, Z. (2017). Evaluation of molecular diversity of central european maize cultivars. *Emirates Journal of Food and Agriculture*, *28*(2), 93-98. https://doi.org/10.9755/ejfa.2015-05-204.

Devi, K. N., Singh, L. N. K., Singh, M. S., Singh, S. B., Singh, K. K. (2012). Influence of sulphur and boron fertilization on yield, quality, nutrient uptake and economics of soybean (*Glycine max*) under upland Cconditions. *The Journal of Agricultural Science*, *4*, 1-10.

Eldemerdash, M. M., El-Sayed, A. S. A., Hussein, H. A., Teleb, S. S. and Shehata, R. S. (2022). Molecular and metabolic traits of some Egyptian species of *Cassia* L. and *Senna* Mill (Fabaceae-Caesalpinioideae). *BMC Plant Biology*, *22*, 205-220. https://doi.org/10.1186/s12870-022-03543-7

Esfandani-Bozchaloyi, S., Sheidai, M., Kalalegh, M. (2019). Comparison of DNA extraction methods from Geranium (*Geraniaceae*). *Acta Botanica Hungarica*, *61*(3-4), 251-266.

Gajeraa, B. B., Kumara, N., Singha, A. S., Punvara, B. S., Ravikirana, R., Subhasha, N., Jadejab, G. C. (2010). Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. *Industrial Crops and Products*, *32*, 491-498.

Gjorgieva, D., Kadifkova-Panovska, T., Mitrev, S., Kovacevik, B., Kostadinovska, E., Bačeva, K. & Stafilov, T. (2012). Assessment of the genotoxicity of heavy metals in *Phaseolus vulgaris* L. as a model plant system by Random Amplified Polymorphic DNA (RAPD) analysis. *Journal of Environmental Science and Health, Part A*, *47*(3), 366-373. https://doi.org/10.1080/10934529.2012.645784

Choudhury, P. R., Tanveer, H., Dixit, G. P. (2007). Identification and detection of genetic relatedness among important varieties of pea (*Pisum sativum* L.) grown in India. *Genetica*, *130*, 183–191. https://doi.org/10.1007/s10709-006-9005-9

Jain, R. K., Joshi, A. and Jain, D. (2017). Molecular Marker Based Genetic Diversity Analysis in Soybean [*Glycine max* (L.) Merrill] Genotypes. *International Journal of Current Microbiology and Applied Sciences*, *6*(6), 1034-1044. https://doi.org/10.20546/ijcmas.2017.606.119

Kim, D. G., Lyu J. I., Lee, M. K., Kim, J. M., Hung, N. N., Hong, M. J., Kim, J. B., Chang-Hyu Bae, Ch.H. and Kwon, S. J. (2020). Construction of Soybean Mutant Diversity Pool (MDP) Lines and an Analysis of Their Genetic Relationships and Associations Using TRAP Markers. *Agronomy*, *10*(253), 1-16. https://doi.org/10.3390/agronomy10020253

Kumar, S. P. J., Susmita, C., Sripathy, K. V., Agarwal, D. K., Pal, G., Singh, A. N., Kumar, S., Rai, A. K., Simal-Gandara, J. (2022). Molecular characterization and genetic diversity studies of Indian soybean (*Glycine max* (L.) Merr.) cultivars using SSR markers. *Molecular Biology Reports*, *49*, 2129-2140. https://doi.org/10.1007/s11033-021-07030-4

Kuťka Hlozáková, T., Gálová, Z., Gregová, E., Vivodík, M., Balážová, Ž., Miháliková, D. (2016). RAPD analysis of the genetic polymorphism in european wheat genotypes. *Potravinárstvo, 10*(1), 1-6. https://doi.org/10.5219/520

Lee, M. E., Grau, C. R., Lukaesko, L. A. & Lee, I. M. (2002). Identification of aster yellows phytoplasmas in soybean in Wisconsin based on RFLP analysis of PCR-amplified products (16S rDNAs), *Canadian Journal of Plant Pathology*, *24*(2), 125-130. https://doi.org/10.1080/07060660309506986

Mihaljevic, M. Ž., Šarčevic, H., Lovric, A., Andrijanic, Z., Sudaric, A., Jukic, G., Pejic, I. (2020). Genetic diversity of European commercial soybean [*Glycine max* (L.) Merr.] germplasm revealed by SSR markers. *Genetic Resources and Crop Evolution*, *67*, 1587-1600. https://doi.org/10.1007/s10722-020-00934-3

Monpara, J., Chudasama, K. and Thaker, V. (2017). ISSR studies on small and large seed varieties of *Glycine max. Journal of Pharmacognosy and Phytochemistry*, *6*(6), 1652-1656.

Nkongolo, K., Alamri, S., Michael, P. (2020). Assessment of Genetic Variation in Soybean (*Glycine max*) Accessions from International Gene Pools Using RAPD Markers: Comparison with the ISSR System. *American Journal of Plant Sciences*, *11*, 1414-1428. https://www.scirp.org/journal/ajps

Paetkau, D., Calvert, W., Stirling, I., Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, *4*, 347-354.

Pandey, S. K., Baruah, J., Paw, M., Sarma, N., Begum, T., Saikia, S. & Lal, M. (2022). Molecular Diversity and Chemical Composition Among Induced Mutation Lines of *Pogostemon cablin* (Blanco) Benth. Using RAPD and ISSR Marker. *Journal of Essential Oil Bearing Plants*, *25*(2), 456-466.

Panjoo, M., Nazarian-Firouzabadi, F., Ismaili, A. and Ahmadi, H. (2014). Evaluation of Genetic Diversity among Soybean (*Glycine max*) Genotypes, Using ISJ and RAPD Molecular Markers. *Journal of Plant Physiology and Breeding*, *4*(2), 55-65.

Perić V., Nikolić, A., Babić, V., Sudarić, A., Srebrić, M., Đorđević, V. and Mladenović Drinić, S. (2014). Genetic relatedness of soybean genotypes based on agromorphological traits and RAPD markers, *Genetika*, *46*, 839- 854. https://doi.org/10.2298/gensr1403839p

Petrovičová, L., Gálová, Z., Balážová, Ž., Vivodík, M., Wójcik-Jagła, M., Rapacz, M. (2015). Assessment of RAPD polymorphism in rye (*Secale cereale* L.) genotypes. *Journal of Microbiology, Biotechnology and Food Sciences*, *4* (special issue 2), 94-97. https://doi.org/10.15414/jmbfs.2015.4.special2.94-97

Priestera, J. H., Gea, Y., Mielkea, R. E., Horsta, A. M., Moritzb, S. C., Espinosae, K., Gelbf, J., Walkerg, S. L., Nisbetb, R. M., Ani, Y., Schimelb, J. P., Palmere, R. G., Hernandez Viezcasc, J. A., Zhaoc, L., Gardea-Torresdeyc, J. L., Holdena, P. A. (2012). Soybean susceptibility to manufactured nanomaterials with evidence for food quality and soil fertility interruption. *Proceedings of the National Academy of Sciences U S A*, *109*, 2451–1456. https://doi.org/10.1073/pnas.1205431109

Ruiz-Chután J. A., Salava, J., Janovská, D., Žiarovská, J., Kalousová, M., Fernández, E. (2019). Assessment of genetic diversity in Sorghum bicolor using RAPD markers. *Genetika*, *51*(3), 789-803. https://doi.org/10.2298/GENSR1903789R

Sharma, P., Nath, A. K., Dhiman, S. R., Dogra, S., Sharma, V. (2022). Characterization of carnation (*Dianthus caryophyllus* L.) genotypes and gamma irradiated mutants using RAPD, ISSR and SSR markers. *South African Journal of Botany*, *148*, 67-77. https://doi.org/10.1016/j.sajb.2022.04.012

Sharma, R., Sharma, S., Kumar, S. (2018). Pair-wise combinations of RAPD primers for diversity analysis with reference to protein and single primer RAPD in soybean. *Annals of Agrarian Science*, *16*, 243-249. https://doi.org/10.1016/j.aasci.2018.03.002

Shuangxia, J., Ramesh, M., Huaguo, Z., Lili, T., Zhongxu, L., Yanxin, Z., Xianlong, Z. (2008). Detection of somaclonal variation of cotton (*Gossypium hirsutum*) using cytogenetics, flow cytometry and molecular markers. *Plant cell Reports, Berlin, (8)*, 1303-1316.

Singh, R. J. and Hymowitz, T. (1999). Soybean genetic resources and crop improvement. *Genome*, *42*, 605-616.
Štefúnová, V., Bežo, M., Žiarovská, J. and Ražná, K. (2015). Detection of the genetic variability of Amaranthus by RAPD and ISSR markers. *Pakistan Journal of Botany*, *47*(4), 1293-1301.
Vivodík, M., Balážová, Ž., Gálová, Z. (2014). DNA analysis of ricin using RAPD technique. *Journal of Microbiology, Biotechnology and Food Sciences*, *3* (special issue 2), 181-183.
Vivodík, M., Balážová, Ž., Gálová, Z., Chňapek, M., Petrovičová, L. (2015). Study of DNA polymorphism of the castor new lines based on RAPD markers. *Journal of Microbiology, Biotechnology and Food Sciences*, *4* (special issue 2), 125-127. https://doi.org/10.15414/jmbfs.2015.4.special2.125-127
Wahyudi, D., Hapsari, L., Sundari. (2020). RAPD Analysis for Genetic Variability Detection of Mutant Soybean (*Glycine max* (L.) Merr). *Journal of Tropical Biodiversity and Biotechnology*, *5*(1), 68-77. https://doi.org/10.22146/jtbb.53653
Weber, J. L. (1990). Informativeveness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics*, *7*, 524-530.
Weir, B. S. (1990). Genetic data analysis. Methods for discrete population genetic data. pp. 377. ISBN 0-87893-872-9
Zargar, M., Romanova, E., Trifonova, A., Shmelkova E. and Kezimana, P. (2017). AFLP-analysis of genetic diversity in soybean [*Glycine max* (L.) Merr.] cultivars Russian and foreign selection. *Agronomy Research*, *15*(5), 2217-2225. https://doi.org/10.15159/AR.17.066