Scientific Papers. Series A. Agronomy, Vol. LXVII, No. 2, 2024
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

USING RAPD MARKERS TO ESTABLISH DNA FINGERPRINT AND TO STUDY THE GENETIC VARIABILITY DISCRIMINATION BETWEEN TWO ROMANIAN POTATO VARIETIES

Floriana Maria STEFAN[1], Ioana BERINDEAN[2], Andreea TICAN[1], Ionuț RACZ[2]

[1]National Institute of Research and Development for Potato and Sugar Beet Brasov,
2 Fundaturii Street, Brasov, Romania
[2]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca,
3-5 Calea Manastur, Cluj-Napoca, Romania

Corresponding author email: ioana.berindean@usamvcluj.ro

Abstract

In order to optimize the potato breeding program in Romania, molecular research was developed so that we can have the way of expressing the genetic potential of the new breeding creations. This study aimed to establish a unique DNA fingerprint for two new Romanian potato cultivars from NIRDPSB Brasov, using RAPD molecular markers for genetic variability discrimination between them. Amplification was carried out with 17 arbitrary primers, but only 14 produced amplified sequences. OPC 08 produced the most banding patterns. The level of similarity between these two varieties of potato has approximately the same value, suggesting a stronger degree of relationship between varieties. Although the examined varieties have different genetic origins, their shared geographical origin and belonging to the same species, this can explain nearly identical similarity coefficient between them and to use of only two varieties for discrimination of genetic diversity can do a high degree of similarity. Discrimination between varieties was limited by the RAPD technique, we recommend using a large number of varieties or a more precise technique for improved accuracy.

Key words*: breeding, potato, PCR, RAPD, genetic diversity, discrimination.*

INTRODUCTION

The intensification of agriculture as a whole has led to significant genetic erosion, as well as soil depletion and environmental damage.

Currently, globally but also in Romania, remarkable results in potato breeding domain were obtained focusing on counteraction tendency of negative effects caused by the hyper-intensification of agriculture, by raising the novelty degree of techniques, proposed to be used in breeding programs (Stefan, 2012). Potato varieties must fulfill the requirements of the market and consumer preferences, as well as to show good agronomic performance in several environments and wide adaptation to productive systems, all thaw, potato needs to deal with some complicated issues that make potato breeding a special case in genetic improvement of crops (Munoz et al., 2018).

The variety represents the most important link in obtaining high and constant productions, without a continuous and progressive increasing of material and energy costs. In 2014, the 6th edition of The World Catalogue of Potato Varieties it appeared, which includes the description of more than 4500 potato varieties and about 1900 wild species, which are cultivated in more than 100 countries around the world (Hermeziu et al., 2020).

The large-scale cultivation of the potato is not only a matter of theoretical interest but is an integral part of diet of a large population worldwide (Caliskan et al., 2022).

The introduction and development of new potato cultivars (*Solanum tuberosum* L.) has been an important strategy to increase crop productivity of this important staple food, fourth after rice, wheat, and corn (Rocha et al., 2010). Breeding is the key factor in a complex equation that determines both agricultural production and the standard of civilization (Hermeziu et al., 2015).

Identification of crop plants, studies in their genetic diversity and relationships is crucial for the development of breeding programs (Onamu et al., 2016). The increasing number of potato cultivars and the importance of their choice make necessary to strengthen users guarantees concerning purity and identity (Yasmin et al., 2006), because due to Moisan-Thiéry et al.,

2001, the identification mainly based on phenotypic characters implies crop inspection at different stages and is often doubtful.
Molecular markers based on the deoxyribonucleic acid (DNA) sequence are more reliable in this regard (Raghunathachari et al., 2000). DNA fingerprinting analysis is defined by applying marking techniques at the molecular level, to identify cultivars. This technique has entered the spotlight in recent years, because of two multilateral agreements: the Intellectual Property Rights related to varieties' marketing (TRIPs) and the Convention on Biological Diversity (CBD). To implement the dispositions of the two conventions, there is a need to dispose by identity and ownership of the obtained genotypes (Sunil, 2000).
The developments of molecular genetics resulted in several procedures based on DNA for detecting genetic polymorphism (Collares et al., 2004). Randomly amplified polymorphic DNA (RAPD) is a technique based on the amplification of discrete regions of the genome by polymerase chain reaction (PCR), with short oligonucleotide primers of arbitrary sequence (Williams et al., 1990). Using DNA fingerprinting it was possible to distinguish between true mutations and epimutations, such as those caused by changes in DNA methylation (Vagnarelli et al., 1995).
This technique is being used successfully to identify, characterize, and estimate genetic divergence of potato cultivars (Rocha et al., 2002), being a simple and inexpensive method for accessing the variability at DNA level compared to other methods (Singh et al., 2021).
The aim of the study was to establish a unique DNA fingerprint for two new Romanian potato cultivars developed at the National Institute of Research and Development for Potato and Sugar Beet Brasov (NIRDPSB Brasov), employing RAPD molecular markers for molecular discrimination between them.
The main limitation in potato clonal selection is the time taken to obtain elite genotypes (Melo, 2011). Thus, the results will be useful for potato breeding programs, by increasing accuracy and safety, within the applied selection schemes. At the same time, the genetic base used in applied genetics research is diversified.

MATERIALS AND METHODS

Within the activities of NIRDPSB Brasov, pota.to breeding works are constantly oriented towards the creation of new potato varieties. Thus, the molecularly characterized biological material in this paper, it is represented by two new mid early potato varieties, respectively Cezarina (Figure 1) and Ervant (Figure 2), patented and registered in The Romanian Official Catalogue of cultivated plant varieties, in the year 2019.
The method of obtaining these varieties is sexual hybridization, followed by clonal selection. During breeding process, varieties were subjected to progressive selection (in vegetation and at harvest), in accordance with breeding objectives (morphological aspect, resistance to diseases and pests, tolerance to thermo-hydric stress and production capacity).
The molecular analysis was developed in the Genetics Laboratory of the Agriculture Faculty from University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

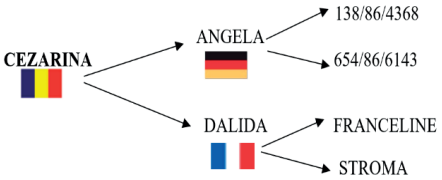

Figure 1. Genealogy of Cezarina potato variety

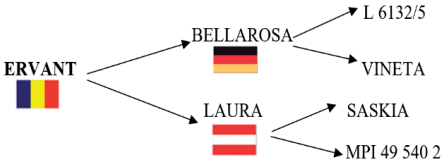

Figure 2. Genealogy of Ervant potato variety

DNA extraction was performed with the protocol described by Lodhi et al. (1994) modified and improved by adding ascorbic acid and diethyldithiocarbamic acid (DIECA), by Pop et al. (2003). The appreciation of purity and quantity of DNA was carried out with NanoDrop100 spectrophotometer. The molecular markers used were RAPD type. The DNA fingerprint obtained through the RAPD technique being able to become one of the most

useful methods for variability discrimination between varieties.

A number of 17 decanucleotide primers were used for amplification, produced by Operon Technologies, Inc. USA (Table 1). The primers used to amplify the DNA sequences had a G-C nucleotide content between 50 and 80%.

Table 1. Primers used in DNA amplification

Crt. no.	Primer name	The nucleotide sequence of the primers (5'- 3')
1	OPAB 11	GTGCGCAATG
2	OPAB 10	CTGCTGGGAC
3	OPB 11	GTAGACCCGT
4	OPC 08	TGGACCGGTG
5	OPC 10	TGTCTGGGTG
6	OPA 20	GTTGCGATCC
7	OPE 14	TGCGGCTGAG
8	OPB 08	GTCCACACGG
9	OPB 09	TGGGGGACTC
10	AB 11	CAATCGCCGT
11	OPA 04	AATCGGGCTG
12	OPA 18	AGGTGACCGT
13	OPB 10	CTGCTGGGAC
14	OPB 17	AGGGAACGAG
15	OPA 01	CAGGCCCTTC
16	OPAB 12	GTGCGCAATG
17	OPC 09	TGGACCGGTG

Electrophoresis of amplified DNA products in agarose gel. Amplified DNA fragments were separated on 1% agarose gel with TAE buffer 1% and stained with safe-green (5 µl/100 ml gel) (Bioline). Throughout the whole process, keeping the voltage and current intensity constant is essential for a correct electrophoresis. Gels were run for 1.5 h at 80 V and visualized under UV light (UVP BioImaging Systems). Each product was visualized on gel with 100 bp DNA Step Ladder molecular marker (Fermentas) to calculate the bands sizes.

Images capture of electrophoresis gels and data analysis. The visible bands, with a certain predetermined intensity, were included in the analyses. These were automatically detected using the TotalLab 100 program. This program determines the size of DNA fragments by comparing them with a DNA standard (100 bp DNA Step Ladder). After comparison, it was possible to determine the size of each amplified fragment in base pairs (bp).

For the analysis of genetic diversity using dominant markers, a method is employed that involves calculating genetic distances between the analyzed taxa. Based on these distances, phenotypic trees are subsequently constructed. These trees can provide insights into how taxa are grouped, depending on their similarities or differences. The analysis and interpretation of the data was carried out using the PAST (Paleontological Statistics) program (Hammer and Harper, 2001) and Jaccard similarity coefficient. Dendrograms are typically constructed using outcomes derived from dominant markers, along with diverse distance or similarity coefficients among the analyzed taxa. The Jaccard similarity coefficient is a coefficient used in the analysis of genetic diversity and the construction of phylogenetic trees (Jaccard, 1901).

RESULTS AND DISCUSSIONS

DNA sample isolation results

DNA purity is one of the most important factors in the reproducibility of the RAPD method. Only using DNA template with high purity ensures reproducible results by the RAPD method. If the DNA is of adequate quality, the RAPD fingerprints will be identical in repetitions. The producer (www.promega.ro) recommends a good-quality of DNA when the report A260/A280 is between 1.7-2.0. Values lower than 1.7 indicate impurity with proteins, while value exceeding 2.0 indicate impurity with other contaminants, such as RNA. In his study, Piskata et al. (2019) has the same recommendation. According to Kasem et al., 2008, high-quality DNA is defined by predominantly featuring long, high molecular weight fragments, displaying an A260/280 ratio ranging between 1.8 and 2.0 and exhibiting an absence of contaminants such as polysaccharides and phenols.

The results obtained for DNA quality are discussed in association with the usefulness for downstream applications and availability of the DNA source in the target study (Lucena-Aguilar et al., 2016).

In our study the purity and quantity of DNA, gave as the following values, shown in Table 2.

Table 2. Purity and amount of DNA resulting from the application of the isolation protocol

Variety name	DNA purity 260/280	Amount of DNA isolated (ng/µl)
Cezarina	1.92	49.50
Ervant	1.88	48.01

As can be seen, the purity of the isolated DNA falls between the limits recommended by the literature, so it could be safely used for the next step, PCR amplification. Also, the amount of isolated DNA is considered to be optimal to continue molecular analyses.

DNA fingerprinting results using RAPD primers

DNA fingerprinting, referred to as DNA typing, DNA profiling or genotyping is a technique employed to distinguish individuals based on unique patterns present in their DNA samples (Adnan et al., 2023). These methods have been widely employed and continue to be utilized extensively for various studies from humans to animals, plants and fungus.

Also, was used to infer familial relationships and conducting phylogenetic studies between humans (Katki et al., 2010; Madboly et al., 2021), to determine the sex of individuals, as well as paternity/maternity and close kinship in divers animals (Chambers et al., 2014) or between different variety of crop plants (Adnan et al., 2023), genotypic distributions in natural populations of blackberries and raspberries (Nybom and Schaal, 1990), in sports of 'Red Delicious' apples (Nybom, 1990) or in establishment to paternity analysis in apples "*Malus* x *domestica*" (Nybom and Schaal, 1990), in rice, where this methods use oligonucleotide probes specific for simple repetitive DNA sequences (Ramakishana et al., 1994) or in plant and fungi (Weising et al., 1995). Application of oligonucleotide probes for DNA fingerprinting is a sensitive tool for genome diagnosis in cultivated beet (Schmidt et al., 1993). Vosman et al. (1992) used DNA fingerprinting for identification of highly polymorphic DNA regions in tomato, also the list can continue with Nidudur and Sanjeet (2022) which showed the relevance of DNA fingerprinting in crop improvement. To select genotypes in terms of sexual type, color and size of the fruit, Vázquez and his team (2016) used different PCR molecular markers.

In our study the RAPD molecular markers used were able to identify and amplify DNA fragments, specific to each variety studied. Thus, from 17 RAPD primers, the following amplified unique DNA sequences: OPAB 11, OPAB 10, OPB 11, OPC 08, OPC 10, OPA 20, OPB 09, AB 11, OPA 04, OPA 18, OPB 10, OPB 17, OPA 01 and OPAB 12. The primers OPE 14 and OPC 09 did not amplify any fragment and OPB 08, amplified only in Ervant cultivar. Primer AB 11 was not considered for analysis because the program did not identify the amplified bands due to their low intensity (Figure 3 and Figure 4). Morales et al. (2011), used 40 RAPD markers, of which only 11 were polymorphic, the rest of primers did not amplify or presented low amplification quality and were not considered in the final data analysis.

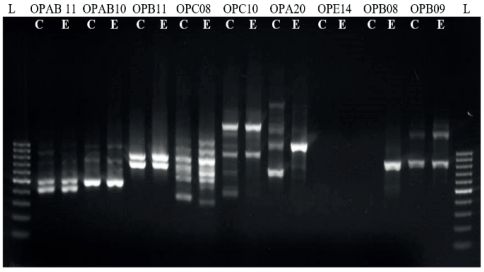

Figure 3. Amplification of DNA fragments using 9 RAPD primers for varieties Cezarina (C) and Ervant (E)

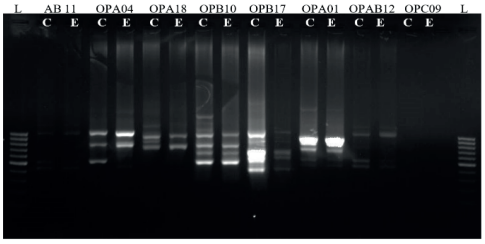

Figure 4. Amplification of DNA fragments using 8 RAPD primers for varieties Cezarina (C) and Ervant (E)

The other RAPD primers are considered polymorphic, succeeding in differentiating the two varieties, due to different values between the amplified fragments.

Seven RAPD primers, including OPB 10, were utilized by Sandhya et al. (2018), to assess the diversity both within and among twelve populations of three mushroom species: *Ganoderma lucidum*, *Leucoagaricus* sp. and *Lentinus* sp., where the size band was between 320 and 2400 pb, while in our study, it ranged from 543 to 1200 bp in the Cezarina variety and 543 to 1085 bp in the Ervant variety with four identical bands size in varieties (see Table 3). Also, this primer generated an appropriate amplified fragment in size, between 718 and

1259 pb to identified different varieties in *Mentha* sp. (Berindean et al., 2021).

Table 3. DNA fingerprints obtained using RAPD molecular markers

Primer RAPD	Amplified band size (bp)	
	CEZARINA	ERVANT
OPAB 11*	441	441
	404	404
OPAB 10*	928	928
	712	712
	441	441
OPB 11*	801	801
	690	690
OPC 08	928	1088
	766	862
	690	766
	566	626
	333	566
	-	348
OPC 10	1354	1354
	837	862
	606	-
	404	-
OPA 20	1607	-
	1088	1088
	606	-
OPB 08	-	800
OPB 09	-	1234
	837	837
OPA 04	1085	1085
	822	823
	543	-
OPA 18	956	1042
	927	-
	822	822
OPB 10	1200	-
	1085	1085
	822	822
	738	738
	543	543
OPB 17	1085	-
	738	738
	619	650
	483	-
OPA 01*	956	956
	822	822
OPAB 12	1114	1114
	619	-
	543	-
Total bands	**38**	**30**
Total bands used in analysis	**31**	**23**

*Monomorphic primers

The OPC 10 primer in our study generated four polymorphic bands in Cezarina variety (range from 404 to 1354 pb) and only two polymorphic bands in Ervant cultivar (range from 862 and 1354). As we can see the last band size amplified is the same in both varieties. The same primer did not generate banding patterns for estimate genetic diversity present in eight varieties of soybean, even if when it was in pair with OPD 4 or OPD 14 (Ramavtar et al., 2019).

The RAPD primer OPC 08 produced the most banding patterns, with five for the Cezarina variety and six for the Ervant variety, and the size of the bands ranged between 333 and 928 bp, respective 348 and 1088 bp. This primer shows two specific bands for both varieties, 566 pb and 766 pb respectively. The average number of polymorphic bands/germplasms for the OPC 08 was 3.05 and has shown a particular fragment of 750 bp to Safedvelchi and Elavazhai cultivars (commercially grown banana), within the research led by Hinge and his team (2022).

The following RAPD primers that generate only one amplified pattern are OPA 20 amplified only one band with a size of 1088 bp in the Ervant variety, but the same band it was amplified in Cezarina variety too (one of the three), while OPB 09 amplified a band of 837 bp in size in the Cezarina variety, which appear in Ervant variety, too. Additionally, the OPAB 12 primer amplified only one band with a size of 1114 bp in the Ervant variety, but the same band also appeared in the Cezarina variety.

Also, as seen in Table 3, four of the RAPD primers used (OPAB 11, OPAB 10, OPB 11, OPA 01) are considered monomorphic, because they failed in differentiating the two potato varieties by amplifying fragments, so they were not considered for analysis.

Only clear and reproducible bands were counted, so from a total of 38 fragments amplified in the Cezarina variety, 31 were considered relevant for analysis. Similarly, from the 30 fragments amplified in the Ervant, only 23 were considered for analysis.

Table 3 shows the fragments (genetic fingerprints) in base pairs (bp) obtained after PCR amplification and using the RAPD molecular marker method for discrimination of potato genetic diversity. Each band amplified with RAPD primers was manually scored as present (1) or absence (0).

The level of similarity between these two varieties of potato was analyzed with Jaccard coefficient.

The genetic distance obtained among the varieties has approximately the same value (0.578 and 0.580) suggesting a stronger degree of relationship between varieties (Table 4).

Table 4. Jaccard`s correlation between the genetic distance of the varieties

Coefficient	Cezarina	Ervant
Cezarina	1	0.580
Ervant	0.578	1

Based on genetic distance, the dendrogram shows us a high degree of similarity between the varieties, even though varieties are different from a genetic origin perspective (Figure 5).
The current potato breeding process generally starts with planting tens of thousands of genetically unique individuals, with only ~1% retained because they meet expectations for market class based on visual criteria.

Figure 5. Phylogenetic tree generated by computing genetic distances among RAPD primers, utilizing the Jaccard similarity coefficient and PAST software

In contrast to other raw material crops, there has been no improvement in genetic potato yield over the last 100 years, primarily due to the highly heterozygous autotetraploid genome of commercial potato (Agha et al., 2023).
Given the complex genetic structure of the cultivated potato (2n=4x=48) and the strong segregation of characters in the descendants obtained by sexual hybridization, but also due to the linkage phenomenon, often unfavorable, it can reach a high degree of similarity between cultivars within a collection.
The random amplified polymorphic DNA technology is a powerful tool for searching for species-specific markers and can detect slight intraspecific differences with great accuracy, it is confirmed by the larger number of studies carried out at the molecular level on many species.
The use of RAPD primers was thought to be a useful method for distinguishing between newly bred cultivars, breeding lines and potato genetic resources (Seo et al., 2001).
However, the RAPD technique can generate multiple polymorphic bands between varieties. But this polymorphism does not reflect the difference between specific sequences for each cultivar, even if they belong to the same species. Kim et al. (2021) in his study showed genetic divergence between the China elite local lines and foreign sources of *Ricinus communis* L. and the overall genetic variation was not extremely large despite the geographical distance.
The potato varieties we examined (Ervant and Cezarina) have different genetic origin, but they have the same geographical origin and also, they are from the same species, so this can explain why the similarity coefficient is nearly identical between them. And the limited number of varieties used (only two varieties), to generate discrimination of genetic diversity leads to obtaining a high degree of similarity. RAPD analysis stands out as the most cost-effective and straightforward method for DNA genotyping, making it well-suited for routine analysis with multiple samples (Dhurva et al., 2012). In our case, RAPD markers has not been able to discriminate at the genomic level. For more and exactly results is needed to use a higher number of varieties and molecular techniques more accurate, like SSR. In Hussein et al.'s study (2023), the comparison of SSR and RAPD markers for genetic diversity in some mango cultivars led to the opinion that applying SSR markers yielded significantly higher values for other genetic diversity parameters when compared to RAPD markers.

CONCLUSIONS

Using DNA analysis methods allowed for obtaining a genetic fingerprint characteristic of each genotype.
Out of the 17 RAPD primers used, 13 successfully amplified a distinct DNA sequence in the examined varieties. Two primers, OPE 14 and OPC 09, did not produce any fragments. Another primer, OPB 08, exclusively amplified in the Ervant cultivar, while the AB 11 primer was not considered for analysis due to the low intensity of its amplified bands. Fragments obtained after amplification vary in size and number from one primer to another. The primer that generated the most bands, both for Cezarina and Ervant was OPC 08.
The Jaccard coefficient yielded a genetic similarity of approximately equal values (0.578 and 0.580) between Ervant and Cezarina, signifying a robust connection between the varieties. This observation is further supported by the dendrogram obtained, reinforcing the indication of a strong relationship. Even though the genetic fingerprinting technique employed produced DNA polymorphic fragments, it is unable to discriminate between the varieties at

the genomic level. For more and exactly results is needed to use a higher number of varieties or a more precise technique for improved accuracy.

REFERENCES

Agha, H. I., Shannon L. M., Morrell P. L. (2023). Unloading potatoes: Potato breeding moves forward with only half the genome, *Cell Genomics* 3(6), 100343, ISSN 2666-979X, https://doi.org/10.1016/j.xgen.2023.100343.

Aydin, A., Kocak, M. Z., Kulak, M. (2023). DNA fingerprinting of crop plants. In *Genomics, Transcriptomics, Proteomics and Metabolomics of Crop Plants* (pp. 229-247). Academic Press.

Berindean, I. V., Geabot, I., Stoie, A., Muntean, L., Racz, I., Ona, A., Varban, D. (2021). Use of RAPD Markers for Identification of Different Varieties in *Mentha* sp. *Hop and Medicinal Plants*, XXIX (1-2).

Calıskan, M. E., Bakhsh, A., Jabran, K. (2022). *Potato production worldwide.* Elsevier Academic Press, eBook ISBN 9780128229262.

Chambers, G. K., Curtis, C., Millar, C. D., Huynen, L., Lambert, D. M. (2014). DNA fingerprinting in zoology: past, present, future. *Investigative genetics*, 5(1), 1-11, https://doi.org/10.1186/2041-2223-5-3.

Collares, E. A. S., Choer, E., Pereira, A. S. (2004). Characterization of potato genotypes using molecular markers. *Pesq. agropec. bras.*, 39(9), 871-878.

Dwivedi, S., Singh, S., Chauhan, U. K., Tiwari, M. K. (2018). Inter and intraspecific genetic diversity (RAPD) among three most frequent species of macrofungi (*Ganoderma lucidum*, *Leucoagricus* sp. and *Lentinus* sp.) of Tropical Forest of Central India. *Journal of Genetic Engineering and Biotechnology*, *16*(1), 133-141.

Gauchan, D. P., Brinegar, C., Hada, A., Singh, D. (2012). Molecular characterization of Nepali potato cultivars using Randomly Amplified Polymorphic DNA (RAPD) markers. *Journal of Advanced Laboratory Research in Biology*, *3*(2), 72-75.

Hammer, Ø., Harper, D. A. (2001). Past: paleontological statistics software package for educaton and data analysis. *Palaeontologia electronica*, 4(1), 1.

Hermeziu, R., Chiru, S. C., Hermeziu, M., Stefan, M. (2015). New potato varieties created at the National Institute of Research and Development for Potato and Sugar Beet Brasov. *Magazine Hortus*, 14, 129-132.

Hermeziu, R., Hermeziu, M., Ștefan, M., Barascu, N., Chelmea, C. (2020). Ervant variety-new result of potato breeding research at NIRDPSB Brasov. *Annals of the University of Craiova Series Biology, Horticulture, Food products processing technology, Environmental engineering*, XXV(LXI), 382-387, ISSN 1453-1275.

Hinge, V. R., Shaikh, I. M., Chavhan, R. L., Deshmukh, A. S., Shelake, R. M., Ghuge, S. A., Dethe, A. M, Suprasanna, P., Kadam, U. S. (2022). Assessment of genetic diversity and volatile content of commercially grown banana (*Musa* spp.) cultivars. *Scientific Reports* 12(1), 7979, https://doi.org/10.1038/s41598-022-11992-1

Hussein, M. A., Eid, M., Rahimi, M., Filimban, F. Z., Abd El-Moneim, D. (2023). Comparative Assessment of SSR and RAPD markers for genetic diversity in some Mango cultivars. *PeerJ*, 11, e15722.

Jaccard, P. (1901). Étude comparative de la distribution florale dans une portion des Alpes et des Jura. *Bulletin de la Société vaudoise des sciences naturelles*, 37, 547–579.

Kasem, S., Rice, N., Henry, R. (2008). DNA extraction from plant tissue DNA extraction from plant tissue. In: *Plant genotyping II: SNP technology*. Wallingford UK: CABI, 2008. p. 219-271.

Katki, H. A., Sanders, C. L., Graubard, B. I., Bergen, A. W. (2010). Using DNA fingerprints to infer familial relationships within NHANES III households. *Journal of the American Statistical Association*, 105(490), 552-563.

Kim, H., Lei, P., Wang, A., Liu, S., Zhao, Y., Huang, F., Meng, F. (2021). Genetic diversity of castor bean (*Ricinus communis* L.) revealed by ISSR and RAPD markers. *Agronomy*, *11*(3), 457.

Lodhi, M. A., Ye, G. N., Weeden, N. F., Reisch, B. I. (1994). A Simple and Efficient Method for DNA extraction from Grapevine Cultivars and Vitis Species. *Plant Molecular Biology Reporter*, 12(1), 6-13.

Lucena-Aguilar, G., Sánchez-López, A. M., Barberán-Aceituno, C., Carrillo-Ávila, J. A., López-Guerrero, J. A., Aguilar-Quesada, R. (2016). DNA source selection for downstream applications based on DNA quality indicators analysis. *Biopreservation and biobanking*, *14*(4), 264-270, doi: 1089/bio.2015.0064.

Madboly, A. G., Adrees, H. A., Al-Aziz, A., Samer, H., Alhusseini, N. F., & Haggag, O. G. (2021). DNA Fingerprinting for Determination of Phylogenetic Tree: A Genomic Study on Samples of Egyptian and Iraqi Populations. *Zagazig Journal of Forensic Medicine*, 19(1), 74-90.

Melo, D. S., Brasil, C. A., Pinto, P., Peixouto, L. S., Gonçalves, N. D., Assis, J. C. (2011). Early selection of full-sib potato families. *Ciênc. agrotec., Lavras*, 35(6), 1101-1109.

Mingfu, M. L. (2001). DNA: Methods for Preparation. *Encyclopedia of Life Sciences*, Nature Publishing, https://doi.org/10.1038/npg.els.0002606.

Moisan-Thiéry, M., Le H. Y., Kerlan, M. C. (2001). Potato cultivars identification using molecular markers. *Acta Horticulturae*, 546, 471-477.

Morales, R. G. F., Resende, J. T. V., Faria, M. V., Andrade, M. C., Resende, L. V., Delatorre, C. A., Silva, P. R. D. (2011). Genetic similarity among strawberry cultivars assessed by RAPD and ISSR markers. *Scientia Agricola*, 68, 665-670.

Muñoz, M. A., Kalazich, J. C., Folch, C. V., Orena, S. V., Winkler, A. (2018). The Use and Impact of Biotechnology in Potato Breeding: Experience of the Potato Breeding Program at INIA, Chile. In *Potato-From Incas to All Over the World.* DOI: 10.5772/intechopen.72961.

Nidudur, S. H., Sanjeet, S. S. (2022). DNA fingerprinting and its applications in crop improvement: *A Pharma Innovation Journal*, SP-11(5), 792-797, ISSN (E): 2277-7695.

Nybom, H., Schaal, B. A. (1990). DNA "fingerprints" reveal genotypic distributions in natural populations of blackberries and raspberries (Rubus, Rosaceae). *Am J Bot*., 77, 883-888, 10.2307/2444504.

Nybom, H., Schaal, B. A. (1990). DNA "fingerprints" applied to paternity analysis in apples (*Malus x domestica*). *Theor Appl Genet.,* 79, 763-768.

Nybom, H. (1990). DNA fingerprints in sports of 'Red Delicious' apples. *HortScience*, 25, 1641-1642.

Onamu, R., Legaria, J., Rodríguez, J. L., Sahagùn, J., Pèrez, J. (2016). Molecular characterization of potato (*Solanum tuberosum* L.) genotypes using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. *African Journal of Biotechnology*, 15(22), 1015-1025, ISSN 1684-5315.

Piskata, Z., Servusova, E., Babak, V., Nesvadbova, M., Borilova, G. (2019). The Quality of DNA Isolated from Processed Food and Feed via Different Extraction Procedures. *Molecules*, 24, 1188.

Poets, A., Silverstein, K., Pardey, P. G., Hearne, S., Stevenson, J. (2020). DNA fingerprinting for crop varietal identification: Fit-for-purpose protocols, their costs and analytical Implications.

Pop, R., Ardelean, M., Pamfil, D., Gaboreanu, I. M. (2003). The Efficiency of Different DNA Isolation and Purification in Ten Cultivars of *Vitis vinifera*. *Bul. USAMV-CN*, 59, 259-261, ISSN 1454-2382.

Raghunathachari, P., Khanna, V. K., Singh, U. S., Singh, N. K. (2000). RAPD analysis of genetic variability in Indian scented rice germplasm (*Oryza sativa* L.). *Current Sci.,* 79, 994-998.

Ramakishana, W., Lagu, M. D., Gupta, V. S., Ranjekar, P. K. (1994). DNA fingerprinting in rice using oligonucleotide probes specific for simple repetitive DNA sequences. *Theoretical and Applied Genetics*, 88, 402-406.

Rocha, B. H. G., Augustin, E., Pereira, A. S., Fortes, G. R. L., Peters, J. A. (2002). Caracterização de cultivares de batata através de marcadores moleculares. *Revista Científica Rural*, 7, 42-51.

Schmidt, T., Boblenz, K., Metzlaff, M., Kaemmer, D., Weising, K., Kahl, G. (1993). DNA fingerprinting in sugar beet (*Beta vulgaris*) - identification of double-haploid breeding lines. *Theoretical and Applied Genetics*, 85, 653-657.

Seo, H. W., Yi, J. Y., Cho, H. M., Park, Y. E., & Oh, S. E. (2001). Discrimination of potato varieties by random amplified polymorphic DNA analysis. *Horticultural Science & Technology*, 19(1), 29-33.

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(3), 243-249.

Singh, A., Singh, G. S., Shukla, G., Verma, S., Kumar, A., Rani, P., Nguyen, D. H. M., Panthum, T. (2021). Assessment of genetic diversity using RAPD molecular marker in potato varieties. *International Journal of Botany Studies*, 6(1), 92-101.

Stefan, M. (2012). New research directions in potato breeding from Romania. *Magazine Potato in Romania*, 21(1), 26-31.

Sunil, A. (2000). Plant DNA fingerprinting: an overview. *AgBiotechNet*, 2, ABN 046, 1-5.

Vagnarelli, P., Raimondi, E., Coelli, C., Fattorini, P., Dorigo, E., De Carli, L. (1995). DNA fingerprint analysis for the detection of induced mutations in mammalian cells in culture. *Cytotechnology*, 19, 137-142, doi: 10.1007/BF00749768. PMID: 22359013.

Vázquez Calderón, M., Zavala León, M. J., Sánchez Teyer, L. F., Mijangos Cortés, J. O., Ortiz García, M. M., Fuentes Ortiz, G., Santamaría, J. M. (2016). Validation of the use of molecular markers of sex and color in hybrids derived from crosses of Maradol x creole papaya. *Revista mexicana de ciencias agrícolas*, *7*(4), 767-780.

Vosman, B., Arens, P., Ruskortekaas, W., Smulders, M. J. M. (1992). Identification of highly polymorphic DNA regions in tomatoes. *Theor Appl Genet*., 85, 239-244.

Weising, K., Nybom, H., Wolff, K., Meyer, W. (1995). *DNA Fingerprinting in Plants and Fungi*. Boca Raton: FL CRC press, Inc. United States, ISBN: 0-8493-8920-8.

Williams, J. G. K., Kubelik, A.R., Livak, K. J., Rafalski, J.A., Tingey, S.V. (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.,* 18, 6531-6535.

Yasmin, S., Islam, M. S., Nasiruddin, K. M., Alam, M.S. (2006). Molecular Characterization of Potato Germplasm by Random Amplified Polymorphic DNA Markers. *Biotechnology*, 5(1), 27-31, ISSN: 1682-296X.

Yuan, D., Qin, H., Chen, D., Chen, J. (2021). Genetic diversity analysis of Chinese Leishmania isolates and development of L. donovani complex-specific markers by RAPD. *BMC Infectious Diseases*, 21(1), 1-10.

*https://www.promega.ro/.