

Assessment of eggplant germplasm genetic diversity using RAPD markers

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Abstract: Evaluation of genetic resources of eggplant (*Solanum melongena* L.) from different geographical areas using molecular markers (RAPD) is of great importance in the breeding process. A total of 90 polymorphic amplified products were obtained from 10 decameric RAPD primers, used to analyse the genetic diversity of 20 genotypes of eggplant (16 local and 4 genotypes of foreign origin). The highest polymorphism was determined using the OPAF-16 primer (70.83%). The number of detected bands ranged from 13 (OPF-04) to 24 (OPAF-16), while the average number of bands per primer was 17.2. The lengths of the amplified fragments ranged from 400 to 9 000 bp. The value of the Jaccard's genetic distance coefficient ranged from 0.095 to 0.35, and the dendrogram constructed using the UPGMA method showed that 16 local and 4 foreign genotypes were grouped into nine groups (clusters). Populations K1, K8/1, K19, K22, K25 and K38 represent genotypes that separated from the others and formed single clusters. The lowest value of the calculated genetic distance was 0.095 between domestic genotypes K13 and K12, which also showed morphological similarity in terms of shape and colour. On the other hand, the highest value of genetic distance was calculated between foreign genotypes K19 and K25 (0.35), K19 and K34 (0.34) and K19 and K38 (0.34). Genetically distinct genotypes identified using RAPD markers could be potential starting genetic material for crossing with other genotypes to obtain new and improved eggplant varieties.

Keywords: *Solanum melongena* L.; genetic resources; diversity; RAPD markers

Eggplant (*Solanum melongena* L.) is an agro-nomically and economically important member of the *Solanaceae* family. It is important as a source of various nutritional compounds, but also as a raw material for the pharmaceutical industry (Naee,

Ugur 2019). In 2021, world production of eggplants was over 58 million tonnes on more than 1.9 million ha, led by China with 63% of the total and India with 22% (FAOSTAT 2023). The presence of good fibre and various vitamins and minerals in fruits

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(rich source of iron, manganese) is of great benefit to human health. Eggplant also contains high phenolic contents that act as antioxidants (Stommel, Whitaker 2003; Caguiat, Hautea 2014). Eating foods containing certain flavonoids, including anthocyanins, helps reduce inflammatory markers that increase the risk of heart disease (Fallah et al. 2020). The colour of purple skin cultivars is due to the anthocyanin nasunin (Noda et al. 2000). The browning of eggplant flesh results from the oxidation of polyphenols, such as the most abundant phenolic compound in the fruit, chlorogenic acid (Prohens et al. 2007). Foods that contain antioxidants may help prevent a range of diseases. Various research shows that the eggplant extracts have superb healing effects on different disorders like burns, warts, inflammatory infections, gastritis, stomatitis and arthritis (Im et al. 2016). Chlorogenic acid shows anticarcinogenic functions by making apoptosis in many human cancer cells, such as leukaemia and lung cancer cells (Tajik et al. 2017).

Knowledge of the genetic divergence is important in both conventional and unconventional plant breeding. Morphological markers are influenced by the external environment and the variability that is estimated based on them does not always correspond to the variability at the genome level. Genetic, and especially molecular DNA markers have an advantage over classic phenotypic markers used in breeding, because they do not depend on the conditions of the external environment and can be detected at all stages of development. Studies of eggplant diversity included abundant and polymorphic markers such as RFLP (Doganlar et al. 2002; Isshiki et al. 2003), RAPD (Singh et al. 2006; Demir et al. 2010; Sifau et al. 2014) SSR (Nunome et al. 2009; Demir et al. 2010; Sunseri et al. 2010; Muñoz-Falcón et al. 2011; Caguiat, Hautea 2014) and AFLP markers (Sunseri et al. 2010). RAPD markers are particularly suitable for use in less known and analysed species, such as eggplant, because they can be applied without prior knowledge of the DNA sequence (Demir et al. 2010). Molecular DNA markers are particularly important in breeding for agronomically important traits that are otherwise difficult to control, such as resistance to diseases, insects, tolerance to biotic stress factors, quality parameters and quantitative traits. Random Amplified Polymorphic DNA (RAPD) markers were used to prove the genetic stability of regenerants obtained by secondary somatic embryogenesis in *broccoli* (Pavlović et al. 2023).

The aim of the investigation was to determine the genetic diversity of the analysed genotypes based on morphological and molecular parameters and to determine the best genotypes for the further selection process.

MATERIAL AND METHODS

Plant material. The research included 20 different genotypes that are part of the collection of the Institute for Vegetable Crops, Smederevska Palanka, Serbia. Sixteen genotypes originate from Serbia (K1, K3, K6, K7, K8/1, K10, K12, K13, K15, K16, K19, K20, K21, K34, K35, K36, K39), 2 from Netherlands (K22 and K25), 1 from Italy (K19) and one from Israel (K38). The trial was carried out on the experimental field of the Institute for Vegetable Crops, Smederevska Palanka according to a completely random block system in three repetitions. The area of the basic plot was 56 m². In each repetition, 10 plants of each genotype were placed in a row. The length of the rows was 4 m, the distance between the rows was 0.70 m, while the distance between the plants in the row was 0.40 cm. Morphological parameters: plant height, number of fruits per plant, length, width and weight of fruit and yield/hectare were monitored.

DNA extraction and RAPD amplification. For molecular characterization of selected genotypes 10 RAPD primers were used (Table 1) after DNA has been isolated from the leaves by modified CTAB method (Zhou et al. 1994). Quantity of extracted DNA were determined spectrophotometric (Agilent 8453, Santa Clara, CA, USA) while quality

Table 1. Sequence of RAPD primers used in eggplant genotypes analysis

	Primers	Primers sequence 5'→3'	Reference
1.	OPH-02	TCGGACGTGA	Demir et al. (2010)
2.	OPB-07	GGTGACGCAG	Demir et al. (2010)
3.	OPF-02	GAGGATCCCT	Kumchai et al. (2013)
4.	OPF-03	CCTGATCACC	Kumchai et al. (2013)
5.	OPF-04	GGTGATCAGG	Kumchai et al. (2013)
6.	OPC-05	GATGACCGCC	Singh et al. (2006)
7.	OPC-09	CTCACCGTCC	Singh et al. (2006)
8.	OPC-14	TGCGTGCTTG	Singh et al. (2006)
9.	OPB-01	GTTTCGCTCC	Moury et al. (2000)
10.	OPAF-16	TCCCGGTGAG	Moury et al. (2000)

was determined on 1% agarose gel electrophoresis (Pharmacia Biotech, Sweden). Each PCR reaction was performed in a 20 µl reaction mixture consisting of 1xPCR buffer, 0.3 mM dNTPs, 0.3 µM of each primer, 1.5 U of DreamTaq Green DNA Polymerase (Fermentas) and 50 ng of DNA. The amplification reaction was carried out by using thermal cycler (Mastecycler Eppendorf) with an initial denaturation at 94 °C for 3 min, followed by 40 cycles at 94 °C for 1 min, 37 °C for 1 min, 72 °C for 2 min and final extension at 72 °C for 10 minutes.

The amplified RAPD PCR products were separated on 2% (w/v) agarose (PeqGold Universal agarose) gel. The gel was run at 10 V/cm (Pharmacia Biotech, Sweden) in 1X TBE buffer and visualized with ethidium bromide under UV light (Vilber Lourmat, Germany). Amplification product size (bp) was determined by the position of bands relative to the DNA ladder (GeneRuler 1 kb DNA Ladder i GeneRuler 100 bp Plus DNA Ladder, Ready-to-Use, Fermentas). The amplified bands were recorded as 1 (band present) and 0 (band absent) in a binary matrix.

Data analysis. Statistical morphological data processing included determination of standard error, analysis of variance of one-factorial experiment and comparison of differences by LSD test ($P \leq 0.05$) (Statistica software, version 8.0). Jaccard's (1908) distance coefficient was used to determine the genetic

divergence of the analyzed populations based on the RAPD binary matrix:

$$GD_{ij} = 1 - (N_{ij} / (N_i + N_j - N_{ij}))$$

where: GD_{ij} – genetic distance coefficient; N_{ij} – presence of a band in both genotypes j (1,1); N_i – presence of a band in genotype i and absence in genotype j (1,0); N_j – presence of a band in genotype j and absence in genotype i (0,1).

DARwin 6.0 software was applied to construct a dendrogram for the 20 eggplant genotypes using neighbour-joining method, based on the dissimilarity matrix of allelic data by simple matching

RESULTS AND DISCUSSION

Analysis of morphological characteristics showed a significant difference between the tested genotypes (Table 2). The height of the plants was from 59.78 cm (genotype K3) to 85.62 cm (genotype K39). In 65% of the genotypes, the height was over 70 cm. The number of fruits per plant varied significantly from 3.53 in genotype K19 to even 10.27 in genotype K20. In 12 out of 20 genotypes, the number of fruits per plant was above 5. The length and width of the fruit varied depending on the shape of the fruit. The longest fruit length of 242.73 mm was recorded in genotype K36, which was also the narrowest with a width of 54.33

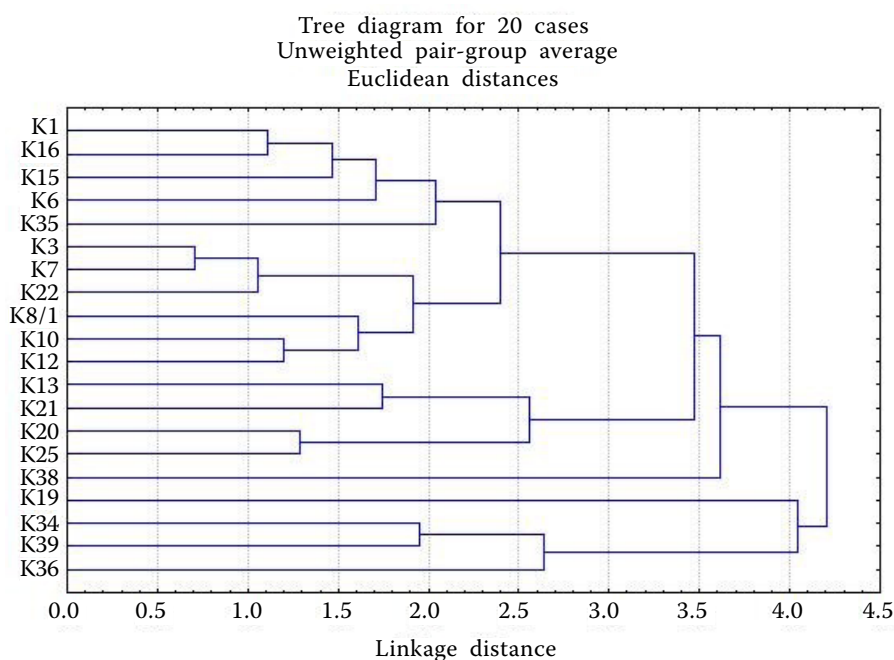


Figure 1. Dendrogram of 20 eggplant genotypes obtained by the UPGMA cluster method based on 6 morphological characteristics

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Table 2. Morphological parameters of analyzed genotypes

Genotype	Plant height (cm)	No. of fruits per plant	Fruit length (mm)	Fruit width (mm)	Fruit weight (g)	Yield (t/ha)
K1	74.13 ± 0.46	5.50 ± 0.32	171.33 ± 0.12	61.33 ± 0.64	197.33 ± 4.80	1.09 ± 0.04
K3	59.78 ± 0.71	5.57 ± 0.22	177.63 ± 0.90	70.73 ± 1.24	406.77 ± 7.42	2.27 ± 0.07
K6	83.62 ± 1.68	4.30 ± 0.21	192.63 ± 0.90	76.27 ± 0.55	294.63 ± 7.27	1.26 ± 0.06
K7	71.28 ± 0.44	6.47 ± 0.23	169.07 ± 0.59	84.27 ± 0.13	317.83 ± 5.34	2.05 ± 0.04
K8/1	64.23 ± 0.27	4.07 ± 0.07	136.10 ± 0.40	89.07 ± 0.72	426.97 ± 5.56	1.74 ± 0.02
K10	69.18 ± 1.11	4.83 ± 0.18	150.77 ± 0.66	69.33 ± 0.62	295.40 ± 14.18	1.47 ± 0.05
K12	65.60 ± 0.56	3.77 ± 0.03	155.28 ± 0.87	69.77 ± 0.19	399.53 ± 3.15	1.50 ± 0.02
K13	75.80 ± 0.58	9.20 ± 0.06	194.83 ± 0.41	56.63 ± 0.71	314.27 ± 8.13	2.96 ± 0.06
K15	74.40 ± 1.01	3.70 ± 0.12	176.30 ± 0.45	54.98 ± 1.22	339.40 ± 7.23	1.25 ± 0.02
K16	78.21 ± 0.62	4.63 ± 0.32	165.83 ± 0.17	70.75 ± 0.48	270.17 ± 7.52	1.38 ± 0.03
K19	67.33 ± 0.47	3.53 ± 0.13	135.67 ± 0.23	123.50 ± 1.75	601.17 ± 0.88	2.22 ± 0.05
K20	79.22 ± 0.26	10.27 ± 0.03	137.50 ± 0.86	68.47 ± 0.52	272.63 ± 9.63	2.77 ± 0.06
K21	65.28 ± 0.09	8.47 ± 0.15	217.47 ± 1.05	57.58 ± 1.29	379.40 ± 5.47	3.22 ± 0.03
K22	66.83 ± 0.61	4.97 ± 0.12	198.93 ± 1.33	67.57 ± 0.44	392.23 ± 6.09	1.95 ± 0.05
K25	79.23 ± 0.95	9.07 ± 0.29	158.13 ± 0.29	63.93 ± 1.16	358.57 ± 8.79	3.18 ± 0.05
K34	75.93 ± 1.82	7.83 ± 0.23	181.33 ± 0.69	84.30 ± 0.23	501.17 ± 2.91	4.04 ± 0.07
K35	70.73 ± 0.50	5.13 ± 0.09	196.70 ± 0.70	89.73 ± 0.96	301.70 ± 4.40	1.55 ± 0.03
K36	78.90 ± 0.28	5.03 ± 0.07	190.40 ± 1.79	111.87 ± 0.50	583.73 ± 9.74	2.97 ± 0.05
K38	85.18 ± 1.42	5.20 ± 0.10	242.73 ± 1.76	54.33 ± 0.81	355.73 ± 7.03	1.87 ± 0.04
K39	85.62 ± 0.55	7.27 ± 0.27	213.80 ± 2.40	79.63 ± 1.52	609.00 ± 4.91	4.27 ± 0.05
$\bar{x}$	73.53	5.94	178.12	75.20	380.88	2.25
Genotype	Genotype	Genotype	Genotype	Genotype	Genotype	Genotype
LSD _{0.05}	1.04	LSD _{0.05} 0.32	LSD _{0.05} 1.67	LSD _{0.05} 1.44	LSD _{0.05} 10.39	LSD _{0.05} 0.08
LSD _{0.01}	1.38	LSD _{0.01} 0.43	LSD _{0.01} 2.21	LSD _{0.01} 1.90	LSD _{0.01} 13.73	LSD _{0.01} 0.11

Values shown as mean value ± SE

mm, while the shortest (135.67 mm) and thickest (123.50 mm) was the fruit of genotype K19.

Fruit weight was from 197.33 g in K1 to 609 g in K39. In 14 genotypes, the weight of an individual fruit was less than 400 g. In 50% of the investigated genotypes, the yield was less than 2 t/ha, in 30% between 2 and 3 t/ha, in 10% between 3 and 4 t/ha and in 10% greater than 4 t/ha. The highest yield was recorded in genotype K39 (4.27 t/ha).

Figure 1 shows that 20 genotypes of eggplant were grouped into 5 clusters based on the average values of six morphological traits by similarity. Genotypes K1, K6, K15, K16 and K35, whose fruits were pear-sized, were grouped into one cluster. Genotypes with elongated fruits K13, K21 and K25 in the second cluster, while genotypes with large almost round fruits K19, K34, K36 and K39 grouped in the third. Genotype K38 was separated and formed a single fourth cluster, while genotypes K3, K7, K8/1,

K10, K12 and K22 with elongated, dark purple fruits formed fifth cluster.

RAPD amplification detected 172 bands, of which 90 bands were polymorphic (52.33%) and 82 monomorphic (47.67%) (Table 3). The highest polymorphism was found when using the OPAF-16 primer (70.83%). A slightly higher degree of polymorphism of 72.7% was detected during the analysis of 24 populations from different regions of Nigeria (Sifau et al. 2014), while in other studies this degree of polymorphism was lower compared to ours. Demir et al. (2010) obtained 29% of polymorphic bands by RAPD analysis of 19 populations of eggplant from Turkey, while a slightly higher level of polymorphism (31.81%) was detected in the work of Singh et al. (2006) on 28 local populations from India. Tiwari et al. (2009) analysed 19 populations of eggplant from India using 29 RAPD primers and detected 27.5% polymorphic bands. The number of detected bands was from

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Figure 3. Used genotypes different in colour and shape of the fruit

CONCLUSIONS

This study has successfully used morphological and RAPD analyses to characterize the genetic diversity and relationships among the eggplant genotypes. Genetically distinct genotypes identified using RAPD markers could be potential starting genetic material for crossing with other genotypes to obtain new and improved eggplant varieties. Genotypes from Netherlands K22, K25, together with genotypes K1 and K38 (from Israel) formed individual (single) clusters during genetic analyses, and which were also classified into different clusters based on morphological characteristics, represent good initial genetic material for crossing with other genotypes. Having in mind limitations concerning different climate zones of origin of examined genotypes as well as possibility of its alleviation using greenhouse environment for future growing, newly developed varieties and hybrids may be predominantly intended for: (a) growing in such conditions, (b) growing in open-field conditions, and (c) both purposes. Suitability of examined genotypes was mainly confirmed during the previous and ongoing research (data not shown), despite the doubts that were associated with the potentially inappropriate origin of certain genotypes (K22 and K25). Genotypes K12 and K13, which had the smallest genetic distance and at the same time the smallest distance based on morphological characteristics, should be excluded as potential starting material for further selection.

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