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STUDIUL DIVERSITĂȚII GENETICE LA GENOTIPURI DE PRUN UTILIZÂND MARKERI SRAP

STUDY OF GENETIC DIVERSITY IN PLUM GENOTYPES USING SRAP MARKERS

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Abstract

The hexaploid European plums (*Prunus domestica* L. and *Prunus domestica* subsp*. insititia* (L.) C.K.Schneid.) are main traditional fruit tree culture in Romania and represent an economically important fruit species with limited information on its genetic structure. Our objective was to fingerprint 33 cultivars using four Sequence-Related Amplified Polymorphism (SRAP) primer pairs to estimate the genetic relationships among local and international cultivars. The primer pairs amplified a total of 70 alleles ranging from 14 to 21 alleles per marker. A wide range of fragment length was detected among the accessions, from 65 to 2000 bp. The mean number of alleles per primer combination was 17.5, with the most alleles obtained with SRAP80 (21 alleles), range between 65-1800 bp and the fewest alleles (14) were obtained with SRAP95, range between 150-2000 bp. The neighbor-joining dendrogram, based on Rogers genetic distance, of the plum germplasm studied, consisted of two main clusters of different sizes: 23 entries were grouped into cluster 1 and the remaining 10 entries were grouped into cluster 2. It is interesting that cultivars representing plum species with 6x and 2x ploidy levels were clustered together. Another interesting aspect observed refers to plum genotypes clustered closely on the dendrogram according to their pedigree, such as 'Dani' (P14) - 'Tita' (P15), 'Roman' (P7) - 'Early Rivers' (P9), 'Zamfira' (P6) - 'Pescăruș' (P33). Accessions within the Romanian landrace/old autochthonous cultivars group were not clustered together. Regarding the PCA, the first two principal axes accounted for 12.91% (CP1) and 10.46% (CP2) of the total variation, respectively, together explaining 23.37% of the total variability. Our results showed that SRAP markers represent valuable tools for genetic diversity study on *Prunus domestica*. To our knowledge, this is the first study using SRAP markers for characterization of *P. domestica* germplasm. In the future, this molecular genetic information can be combined with phenological and biochemical data to identify genes, quantitative trait loci (QTL) and molecular markers that can be used to improve the plum crop breeding program.

Cuvinte cheie: prun, markeri SRAP, diversitate genetică, dendrograma.
Key words: plum, SRAP markers, genetic diversity, dendrogram.

1. Introduction

In Romania, in 2021, the plum orchards covered an area about 66700 ha with an average yield about 6050 kg/ha (https://insse.ro). In the traditional growing areas for plum several species of *Prunus* are present in spontaneous or semi-cultivated status (*Prunus domestica* L., *Prunus domestica* subsp*. insititia* (L.) C.K.Schneid*, Prunus cerasifera* Ehrh. and *Prunus spinosa* L.) (Botu et al., 2010). An analysis of the pedigrees of plum cvs. developed in Romanian breeding programs shows that the most are descended from 'Tuleu gras', 'Renclod Althan', 'Anna Späth', 'Stanley' and 'Early Rivers', called 'ancestors' (Butac, 2021). European plum is a less-investigated species in the *Prunoideae* subfamily because of the complex structure of its genome (Makovics-Zsohár et al., 2017). To date, only a limited number of molecular genetic diversity analyses have been done within *P. domestica* (Gregor et al., 1994; Makovics-Zsohár et al., 2017). As a result, there is a need for assessment of the genetic diversity and structure of more European plum cvs. using different molecular markers, such as Sequence-Related Amplified Polymorphisms (SRAP). SRAP markers have high levels of polymorphism and reproducibility (Li and Quiros, 2001) indicating that they should be a useful system for investigating plum genetic diversity. In addition, these markers are not species-specific.

The goal of this work was to fingerprint 33 European plums accessions using SRAP markers to assess the polymorphism level.

2. Material and methods

2.1. Plant materials

Leaf tissue from 30 *P. domestica* modern, traditional and landrace/old autochthonous, one rootstock *P. domestica* subsp. *insititia*, and two diploid species, totaling 33 accessions which were collected in two locations (Mărăcineni- Research Institute for Fruit Growing Pitesti and Bucharest - University of Agronomic Sciences and Veterinary Medicine of Bucharest), and provided by University of Agronomic Sciences and Veterinary Medicine of Bucharest (Table 1).

2.2. DNA extraction

Genomic DNAs were isolated from frozen leaf tissue ground with nitrogen liquid, based on CTAB method (Ciuca et al., 2020). DNA concentrations (ng/µl) of all *Prunus* samples were measured at Abs 260/280 (nm) using a spectrophotometer (Beckman Coulter DU 730). All genomic DNAs were stored at -20°C.

2.3. SRAP analysis

DNA amplification was performed with four combinations of SRAP primers (SRAP 20; SRAP 24; SRAP 80; SRAP 95) (Table 2) selected from Elshafei et al. (2013). PCR reactions were carried out in a total volume of 20 µl, which contained 30 ng of template DNA, 1x buffer DreamTaq Green PCR Master Mix (Thermo Scientific), 10 µM from each primer in a ProFlex (Applied Biosystem) thermocycler system. The PCR reaction had two main stages. Stage I (5x cycles): 3 min of initial denaturation at 94°C, followed by denaturation at 94°C for 1 min, then 1 min annealing at 35°C, then followed by 2 min extension at 72°C for 5 cycles. Stage II (35x cycles): denaturation 94°C for 1 min, 1 min annealing at 50°C and 2 min extension at 72°C followed by a final extension at 72 °C for 7 min. A hold at 20°C ended the reactions. After PCR amplification, the samples were loaded in 1.5 % agarose gel (Cleaver Scientific routine use), Tris– Boric acid–EDTA buffer, run for 2 h at 120 V and DNA bands were visualized by ethidium bromide staining. Fragment lengths were estimated by comparison with a DNA ladder. Gels were visualized in the gel documentation system UVITEC HD6.

2.4. Data analysis

Alleles obtained as a result of SRAP analysis were recorded in a binary system, with “1” denoting the presence of the product for a given genotype, and “0” denoting its absence. The neighbor-joining algorithm was used to construct a dendrogram based on Rogers distance using the BIO-R software (Biodiversity analysis with R for Windows) Version 3.0 (Pacheco et al., 2016).

3. Results and discussions

In 33 plum accessions, amplification of genomic DNA was successful in each of the four SRAP primer pairs. Example gel images are provided in Figure 1. Altogether, the primer pairs produced a total of 70 alleles ranging from 14 to 21 alleles per locus. A wide range of fragment lengths was detected among the accessions, from 65 to 2000 bp. The mean number of alleles per primer combination was 17.5, with the most alleles obtained with SRAP80 (21 alleles), ranging between 65-1800 bp and the fewest alleles (14) were obtained with SRAP95, ranging between 150-2000 bp. To our knowledge, this is the first study using SRAP markers for the characterization of *P. domestica* germplasm.

Çakir et al. (2021) found 10.4 mean number of alleles per locus in 66 plum genotypes of *P. cerasifera* using 47 SRAP marker combinations.

The dendrogram was obtained using Archaeopteryx software (Figure 2). The neighbor-joining dendrogram of the plum germplasm studied consisted of two main clusters of different sizes: 23 entries were grouped into cluster 1 and the remaining 10 entries were grouped into cluster 2. In cluster 1, the 23 plum genotypes were differentiated into 5 sub-clusters. Also, in this cluster was observed that Centenar-Bucharest (P31) is similarly with 'Čačanska Lepotiča' (P26) and 'Centenar'-Pitești (P4) with 'Elena' (P30), 'Vinete românești' (P3), 'Brumarii' Voinesti (P27) and 'Gras ameliorat' (P13) were grouped into the same group, Grase românești' (P2) was grouped with 'Stanley' (P20), 'Tuleu gras' (P1) was grouped with 'Romanța' (P8) and 'Haganta' (P22). In cluster 2, the 10 plum genotypes were separately into three sub-clusters. One comprised only the diploid species ('Black Diamond'-P21 and 'Lama'-P23). Another sub-cluster contained the following genotypes: 'Timpurii de Țurlești' (P16), 'Topend' (P24) and 'Gemenea' (P29). The bigger sub-cluster comprised of five genotypes, emphasizing the similarity between 'Scolduș' (P17) and 'Jojo' (P18), 'Centenar'-Pitești (P5), 'Centenar'-Bucharest (P28) and 'Renclod Althan' (P12), too. It is interesting that these cultivars, from the cluster 2, representing plum species with 6x and 2x ploidy levels were clustered together.

Another interesting aspect observed, refers to plum genotypes clustered closely on the dendrogram according to their pedigree, such as 'Dani' (P14) - 'Tita' (P15), 'Roman' (P7) - 'Early Rivers' (P9), 'Zamfira' (P6) - 'Pescăruș' (P33). Accessions within the Romanian landrace/old autochthonous

cultivars group were not clustered together. The summary of genetic diversity based on SRAP markers is presented in Table 3.

Principal Component Analysis - PCA (Figure 3) confirmed the information provided by the dendrogram and also supplied further details. The first two principal axes accounted for 12.91% (CP1) and 10.46% (CP2) of the total variation, respectively, together explaining 23.37% of the total variability.

Comparison of these results suggests that there is genetic diversity in Romanian plum trees collection and analyses of more SRAP markers could provide a better and more sure differentiation.

4. Conclusions

Our results showed that SRAP markers represent valuable tools for genetic diversity study on *P. domestica*.

There was no distinct genetic clustering in the germplasm analyzed according to geographical origin, however a high level of diversity was observed in the collection.

In the future, this molecular genetic information can be combined with phenological and biochemical data to identify genes, quantitative trait loci (QTL) and molecular markers that can be used to improve the plum crop breeding program.

Acknowledgements

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Tables and Figures

Tabel 1. Plant material used in this study

No.	Accessions	Presumed species	Pedigree	Country of origin	Cultivar status*
P1	Tuleu gras	*Prunus domestica*	Unknown	Romania	L
P2	Grase românești	*P. domestica*	Unknown	Romania	L
P3	Vinete românești	*P. domestica*	Unknown	Romania	L
P4	Centenar - Pitești**	*P. domestica*	Tuleu gras x Early Rivers	Romania	T
P5	Record - Pitești**	*P. domestica*	Renclod violet o.p.	Romania	T
P6	Zamfira	*P. domestica*	Anna Spath x Renclod Althan	Romania	M
P7	Roman	*P. domestica*	Tuleu gras x Early Rivers	Romania	M
P8	Romanța	*P. domestica*	Stanley x Vâlcean	Romania	M
P9	Early Rivers	*P. domestica*	Unknown	England	T
P10	Reinen Claude Violette	*P. domestica*	Unknown	France	T
P11	Anna Späth	*P. domestica*	Unknown	Hungary	T
P12	Reine Claude d'Althan	*P. domestica*	Unknown	Czech Republic	T
P13	Gras ameliorat	*P. domestica*	Grase romanesti (auto pollination)	Romania	T
P14	Dani	*P. domestica*	Tuleu gras x Grase românești	Romania	M
P15	Tita	*P. domestica*	Tuleu gras stones irradieted 2014	Romania	M
P16	Timpurii de Țurlești	*P. domestica*	Unknown	Romania	L
P17	Scolduș	*P. insititia*	Unknown	Romania	R
P18	Jojo[IC]	*P. domestica*	Ortenauer x Stanley	Germany	M
P19	Milenium (sin. Dara)	*P. domestica*	Unknown	Romania	M
P20	Stanley [IC]	*P. domestica*	d'Agen x Grand Duke	USA	T
P21	Black Diamond	*P. salicina*	Unknown	USA	M
P22	Haganta	*P. domestica*	Cacanska najbolja x Valor	Germany	M
P23	Lama	*P. cerasifera* var. *pissardii*	Hybrid 9-250 (*P. cerasifera* var. *pissardii*) open pollination	Belarus	M
P24	Topend	*P. domestica*	Čačanska Najbolja x Valor	Germany	M
P25	Vinete românești	*P. domestica*	Unknown	Romania	L
P26	Čačanska lepotica [IC]	*P. domestica*	Wangenheimer x Besztercei	Serbia	T
P27	Brumării Voinești	*P. domestica*	Unknown	Romania	L
P28	Record - București**	*P. domestica*	Renclod violet o.p.	Romania	T
P29	Gemenea	*P. domestica*	Unknown	Romania	L
P30	Elena	*P. domestica*	Tuleu gras x Stanley	Romania	M
P31	Centenar - București**	*P. domestica*	Tuleu gras x Early Rivers	Romania	T
P32	Silvia	*P. domestica*	Renclod Althan x Early Rivers	Romania	T
P33	Pescăruș	*P. domestica*	Renclod Althan x W. Spath	Romania	T

IC-International reference cultivar; *(L - landrace/old autochthonous cultivar; M - modern cultivar; R - rootstock; T – traditional cultivar) ** source from Research Institute for Fruit Growing Pitesti, Romania/ University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV), Romania; o.p. = open-pollination.

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Table 2. SRAP markers used in this study and PCR reaction stages

Marker	Primers sequence	PCR reaction stages	
SRAP 20	Forward: TGAGTCCAAACCGGTCC	94°C-3 min	1X
	Reverse: GACTGCGTACGAATTTGA	94°C-1 min	5X
SRAP 24	Forward: TGAGTCCAAACCGGTCC	35°C-1 min	
	Reverse: GACTGCGTACGAATTTGC	72°C-2 min	
SRAP 80	Forward: TGAGTCCAAACCGGACC	94°C-1 min	35X
	Reverse: GACTGCGTACGAATTTGC	50°C-1 min	
SRAP 95	Forward: TGAGTCCAAACCGGAAG	72°C-2 min	
	Reverse: GACTGCGTACGAATTGAC	72°C-7 min	1X

Table 3. Diversity analysis summary

Percent of polymorphic loci	0.814286
Expected Heterozygosity (HE)	0.322588
Standard deviation for HE	0.019294
Observed Heterozygosity (HO)	0
Standard deviation for HO	0
Number of effective allele (Ae)	1.544569
Standard deviation for Ae	0.043262
Shannon diversity Index (ShanIn)	0.707392
Standard deviation for ShanIn	0.033328

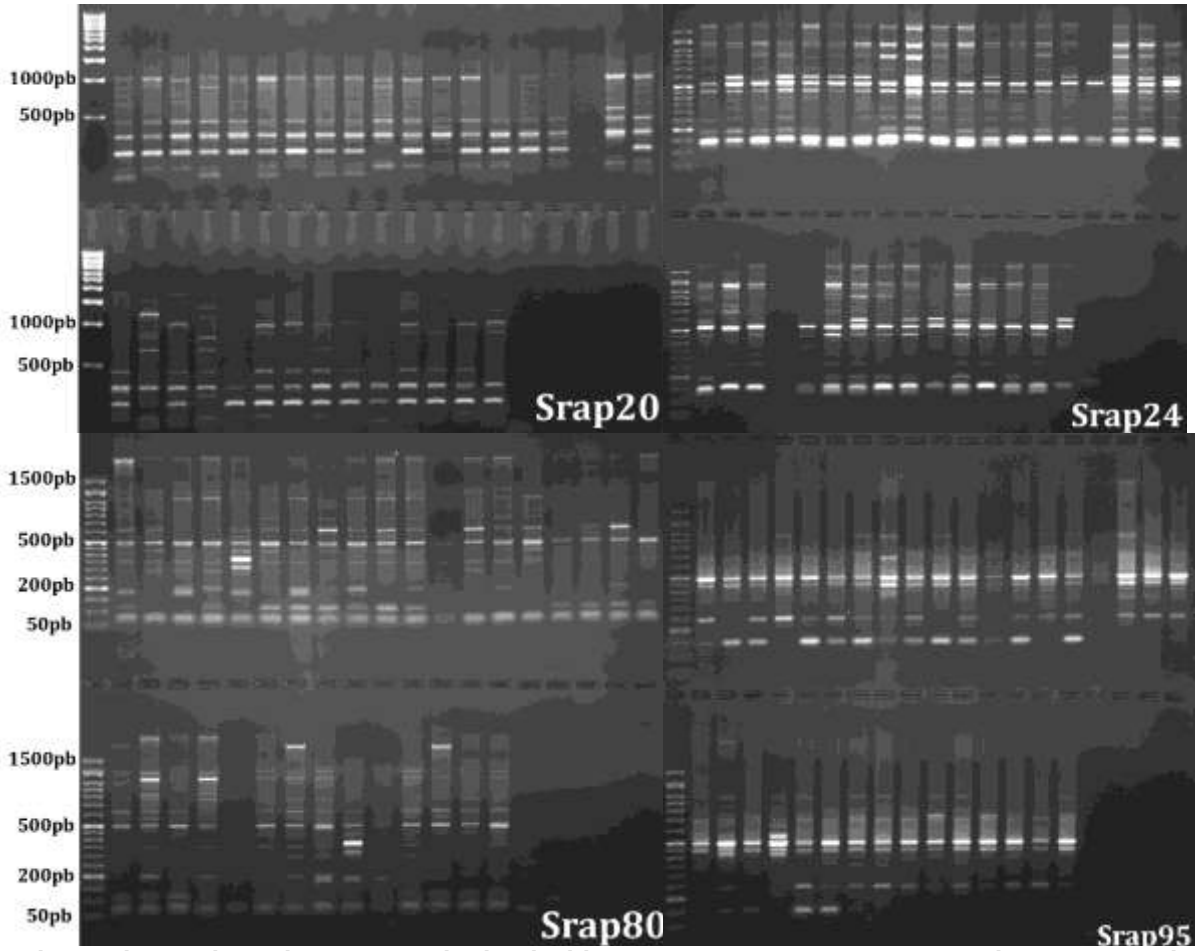

Fig. 1. Electrophoresis patterns obtained with SRAP20, SRAP24, SRAP80 and SRAP95 markers

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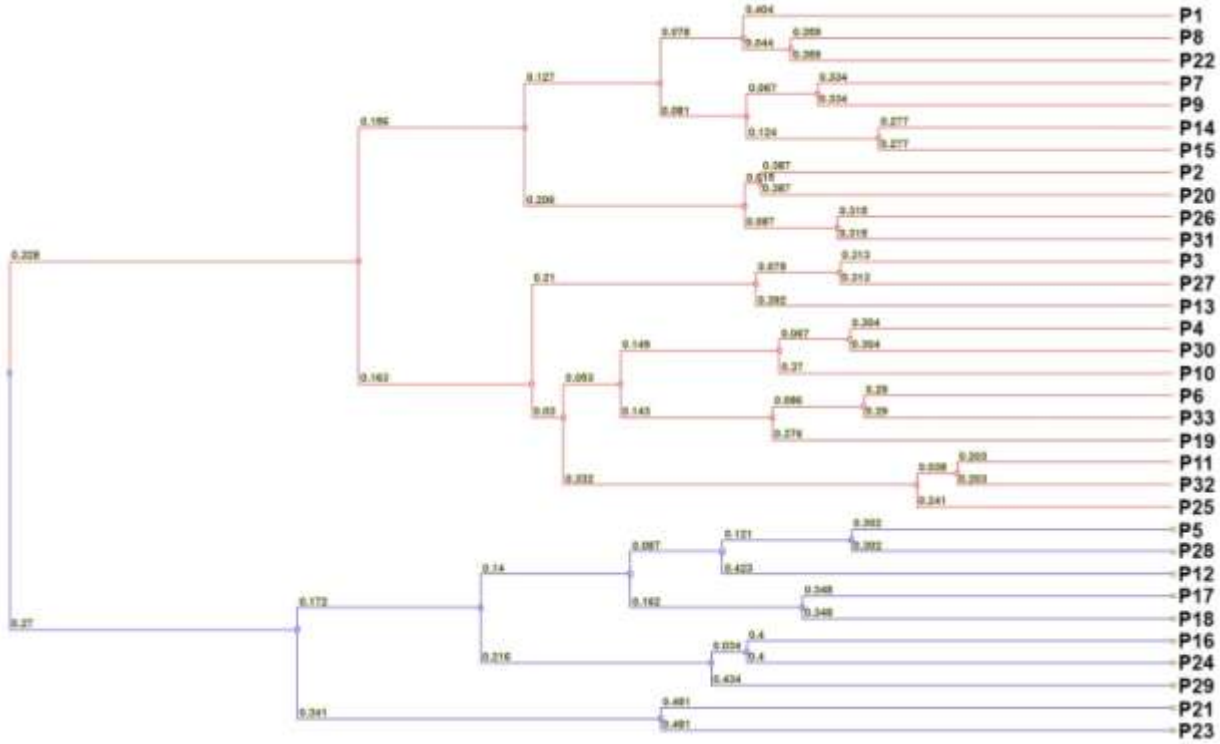

Fig. 2. Dendrogram -The differentiation between 33 plum genotypes based on SRAP analysis and Rogers's genetic distance

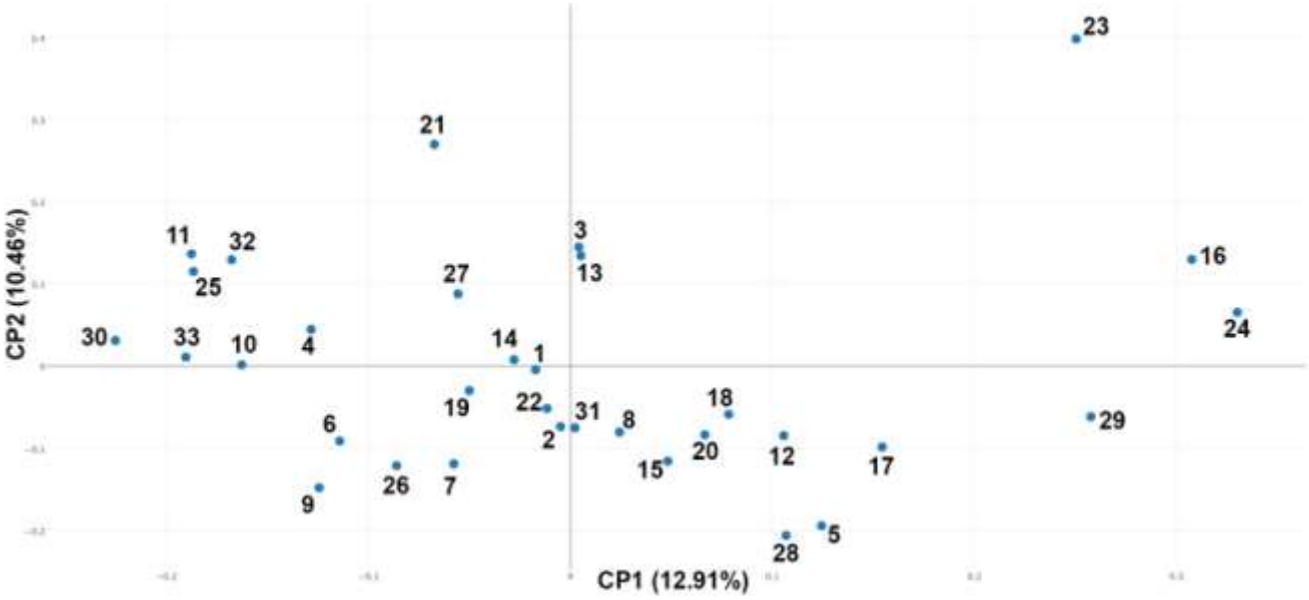

Fig. 3. PCA-Distribution of 33 plum cultivars on the two first principal component analysis axes determined from SRAP genotyping.