# O 8. GENETIC RELATIONSHIPS AMONG PRUNUS SP IN EX SITU COLLECTION IN ALBANIA

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**ABSTRACT:** The genus *Prunus* consist of fruit tree species of high economic value, well known for their edible fruits. Its germoplasm is represented by a large number of cultivars, breeding selections and rootstocks. The present study considers genetic diversity and relationships among four different *Prunus* species used for breeding new cultivars and rootstocks. Fifty-five accessions of *Prunus domestica*, *P. armeniaca*, *P. avium* and *P. persica* from *ex situ* collection in Albania were analysed using RAPD molecular markers. Prunus accessions were grouped into three clades, according to the unweighted pair group method with arithmetic mean (UPGMA) dendrogam constructed based on molecular data, with a mean similarity of 43%. The obtained results were supported by principal coordinate analysis (PCoA) which clearly differentiated Prunus sp. into three similar groups. The results demonstrated that *P. domestica* and *P armeniaca* were more closely related among analysed species, their accessions were clustered in the same clade. The understanding of genetic relationships among *Prunus* sp. contribute significantly to breeding and effective utilisation of *Prunus* germplasm resources held in the *ex situ* collection.

***Keywords****: Prunus sp., Genetic relationships, RAPD, Albania*

## 1. INTRODUCTION

The genus *Prunus* belong to Rosaceae family and comprises around 400 species of tree and shrubs (Bouhodida et al. 2007). It includes fruit tree species of high economic value, well known for their edible fruits, such as peaches, apricots, plums, cherries, etc. Albania is considered a heartland of many cultivars belonging to *Prunus* in the South-East of the Balkans. This rich diversity of cultivars and biotypes is spread out over the country and many of the cultivars are particularly well adapted to local ecological conditions (Cakalli et al. 2007).

The number of planted trees was increased over years while increased the interest on cultivating cultivars of favourable traits and the demand of their products. *Prunus* germplasm in Albania is represented by a large number of cultivars, selections and rootstocks as well their hybrids, mainly distributed in the Central region. It is maintained in the collections that aims the selection and breeding new *plum* cultivars, one of them is the Agricultural Technologies Transfer Centre (ATTC) in Vlora. However, the data on molecular diversity of the *Prunus* species within these collections are missing. The lack of this genetic background affects the output and costs of breeding programs.

Classification within the genus *Prunus* was based on morphologic traits, mainly considering the fruit characteristics (Aradhya et al. 2004). The development and the use of DNA based techniques had overcome the drawbacks related to the application of morphological characters, such as their availability and being influenced by environmental conditions, for genetic diversity and phylogenetic relationship studies among plant species.

Randomly amplified polymorphic DNA(RAPD) markers have been extensively used in the investigation of genetic diversity and evaluation of genetic relationships within different *Prunus* species, *P. armeniaca* (Mariniello et al. 2002; Ercisli et al. 2008), *P avium* (Di Vaio et al. 2015; Berindean et al. 2016; Antic et al. 2020), *P. persica* (Zheng, 2007; Melgoza et al. 2009; Dervishi et al. 2019), *P. domestica* (Shimada et al. 2004) as well as among them (Liu et al. 2006; Athanasiadis et al. 2013). Although advanced molecular techniques were developed, RAPD is still in use for estimation of genetic variability of different plant species, due to their low cost and efficiency compared with other methods (Antic et al. 2020).

The characterisation of *Prunus* sp. is the prerequisite for the development of effective breeding strategies (Athanasiadis *et al.* 2013). The present study aims the molecular characterisation and evaluation of genetic relationships among most important *Prunus* species used for breeding new cultivars and rootstocks held in *ex situ* collection of ATTC in Vlore in Albania. Increasing our knowledge on genetic diversity and relationships of these fruit species might be useful for germplasm utilization in the breeding programmes.

## 2. MATERIAL AND METHOD

The plant material was provided by the ex-situ collection of the ATTC (Agricultural Technologies Transfer Centre). Fresh leaves were collected from a total of 55 accessions from four important *plum* species; *Prunus persica* (20), *Prunus armeniaca* (9), *Prunus avium* (16), and *Prunus domestica* (10). The list of analysed *Prunus* sp. genotypes is given in table 1.

The genomic DNA isolation was done using 120 mg fresh leaves following CTAB (cetyltrimethylammonium bromide) protocol as described by Kump and Javornik, (1996). The DNA quantity and quality was assessed using UV spectrophotometric method and by 0.8 % agarose gel. The DNA was adjusted to 4 ng/µl concentrations and stored to -4°C.

**Table 1**. List of analysed *plum* genotypes

| Species | No | Cultivar name | Species | No | Cultivar name |
|---|---|---|---|---|---|
| | 1 | Cardinal | | 31 | E Zeze |
| *Prunuspersica* | 2 | Coronet | *Prunusavium* | 32 | Roze |
| | 3 | Dixired | | 33 | Italiane |
| | 4 | Fairhaven | | 34 | Napolonbishteshkurter |
| | 5 | Nay Crest | | 35 | Napolonbishtegjate |
| | 6 | Nemaguard | | 36 | Vishnje |
| | 7 | Redtop | | 37 | Bukje |
| | 8 | Rubira | | 38 | Carzy Star |
| | 9 | Springcrest | | 39 | Celeste |
| | 10 | Andross | | 40 | New Star |
| | 11 | Baby Gold 6 | | 41 | Burlat |
| | 12 | Baby Gold 7 | | 42 | Sweet Early |
| | 13 | Percoco di Turi | | 43 | Moro di casano |
| | 14 | Percoco di Novembre | | 44 | Sciena |
| | 15 | Maria Serena | | 45 | Big Star |
| | 16 | PercocoPrecoce | | 46 | Uknown |
| | 17 | Vivian | *Prunusarmeniaca* | 47 | Tsunami |
| | 18 | Nektarina | | 48 | Sankastres |
| | 19 | Arm King | | 49 | Magicot |
| | 20 | Fantasia | | 50 | Bulida |
| *Prunusdomestica* | 21 | Black Diamont | | 51 | Prima |
| | 22 | Tcsan | | 52 | Antonio Errani |
| | 23 | Black-Amber | | 53 | Rubistar |
| | 24 | Sugar | | 54 | Bora |
| | 25 | Stanley | | 55 | Spink Blush |
| | 26 | V1.1 | | | |
| | 27 | V2.1 | | | |
| | 28 | V3.1 | | | |
| | 29 | V1.2 | | | |
| | 30 | V2.2 | | | |

The 55 plum genotypes were amplified by a total of six random decamer RAPD markers (OPA 07, OPA 17 OPB 01, OPAG 04, OPJ 04, OPJ 12). The RAPD markers specific sequences are given in table 2.The amplification was carried out in a final volume reaction of 15 µl, containing 20ng DNA template, 1xPCR buffer, 2mM $MgCl_2$, 0.2mM dNTPs, 0.2µl primer (Operon Technologies) and 0.3U of *Taq* polymerase. The amplifications were performed in the PCR conditions as follow, initial denaturation at 94°C for 1.5 min, then 36 cycles of 30s at 94°C denaturation, 45s at 36°C annealing, polymerisation for 1 min at 72°C and the final elongation step on 72°C for 5 min. RAPD-PCR products were analized on agarose gel1.5% in 1xTAE buffer at 8V $cm^{-1}$, stained in ethidium bromide and visualized with transilluminator under UV light.

The amplified bands sizes were determined against a DNA standard of 100bp and scored as present (1) or absent (0). The provided data were used to construct a binary matrix, on which Dice's similarity coefficient (Dice, 1945) were calculated. The genetic relationships among four *Prunus* species under study were evaluated and visualized in a dendrogram constructed by applying UPGMA (unweighted pair group method using arithmetic average) method using NTSYS v.2.2 software (Rohlf, 2000). PCoA analysis was performed using GenAlEx software.

The efficiency of RAPD markers in our analysis was assessed based on the total number of bands (TNB), the number of polymorphic bands (NPB), and the percentage of polymorphic bands (PPB).

## 3. RESEARCH FINDINGS

A total of 56 fragments were scored among all plum genotypes for the six selected RAPD markers and were used to estimate genetic relationships among them. The fragments size ranged from 300-2000bp, the mean number of polymorphic fragments per primer resulted 9.3 ranging from 8 to 11 in OPAG04, OPB01 and OPA07, respectively (Table 2).

**Table 2**. Approximate range of amplified fragments and RAPD polymorphism data

| Locus | TBN | PBN | Fragment size (bp) |
|---|---|---|---|
| OPB01 | 8 | 8 | 1500-400 |
| OPAG04 | 8 | 8 | 2000-400 |
| OPJ04 | 10 | 10 | 2000-300 |
| OPJ12 | 10 | 10 | 2000-300 |
| OPA17 | 9 | 9 | 2000-300 |
| OPA07 | 11 | 11 | 1500-300 |
| **Mean** | 9.33 | 9.33 | - |

Genetic similarities between pairs of accessions were calculated according to Dice's coefficient. The mean genetic similarity among analysed genotypes was 43%, ranging from 9 - 97%. The present study revealed remarkable genetic diversity among the analysed *Prunus* sp genotypes.

Based on the provided RAPD profiles the analysis of genetic relatedness analysis among plum genotypes was performed by applying Dice's similarity coefficients and UPGMA method of clustering. Fifty five plum genotypes were grouped in three main clades in the dendrogram constructed in cluster analysis (Figure 1). The most distant were three genotypes; one genotype of *P. armeniaca* 'Rubistar' and two of *P. domestica* 'Sugar' and V2.1 which are clustered together. The first group comprised all genotypes (20) of *P. persica*, the second group comprises *P. avium* genotypes (16), and the third group comprises the genotypes of species *P. armeniaca* (8) and *P. domestica* (8) divided in two subgroups within this second group. Genotypes of *P. persica* showed to be more distant among all species under study.

To obtain a better understanding of the genotypes relationship, principal coordinate analysis (PCoA) was conducted. (Figure 2). The PcoA analysis revealed a total of 51.3% of variation. The first and the second principal coordinates account for 31.9 % and 12.3 % of the total variation, respectively. The genotypes of different species were clearly grouped in accordance with UPGMA clustering, into three groups.

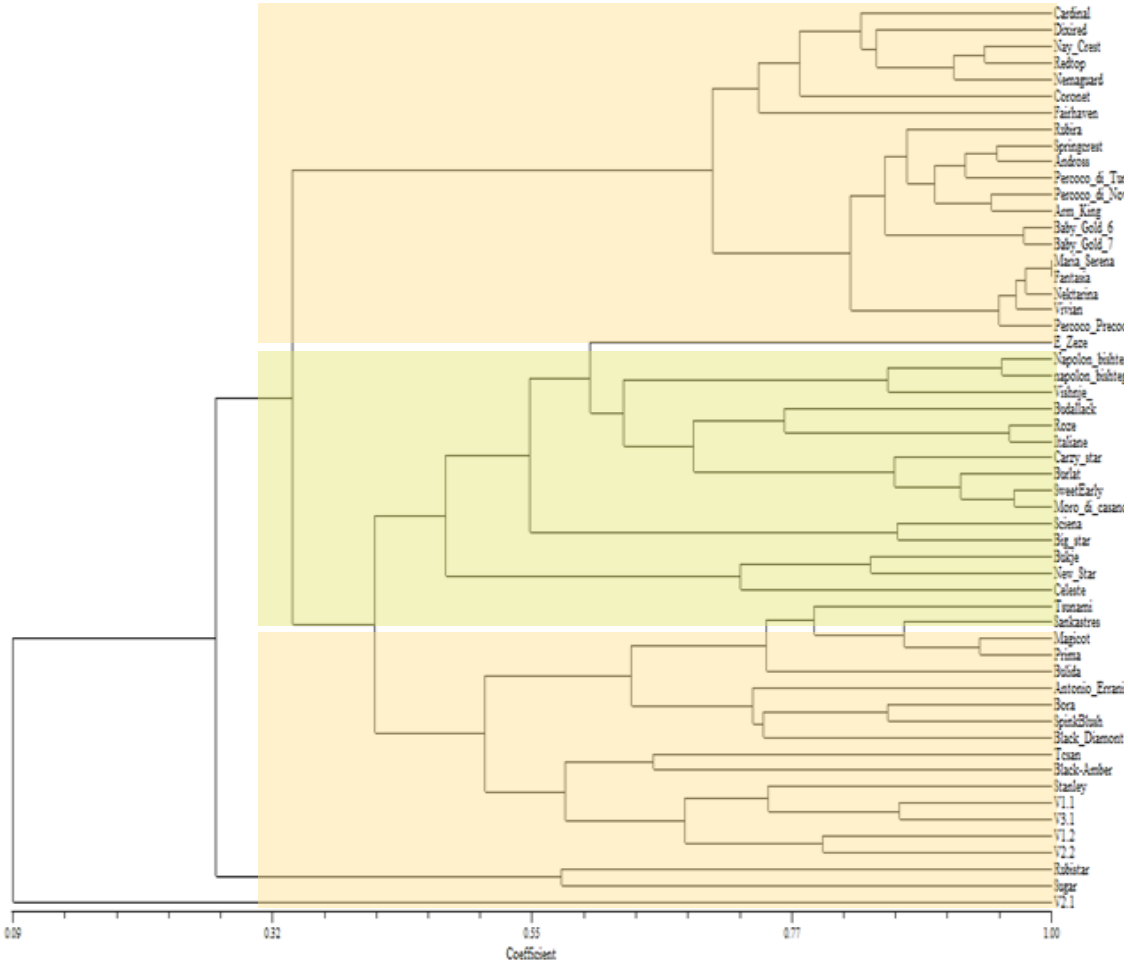


**Figure 1**. Dendrogram representing genetic relationships among 55 Prunus sp genotypes based on RAPD data

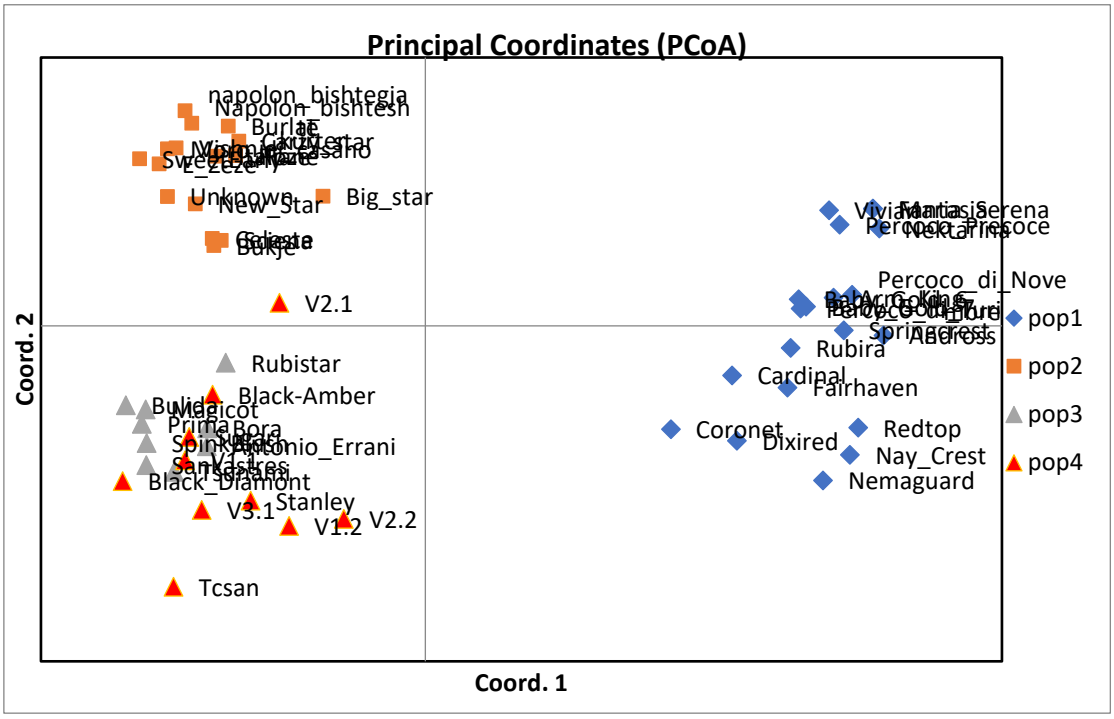


**Figure 2**. Principal coordinate analysis (PcoA0 of 55 Prunus sp genotypes Pop 1- *P. persica*; Pop 2-*P. avium*; Pop 3-*P. armeniaca*; Pop 4- *P. domestica*

## 4. CONCLUSION AND DISCUSION

In the current study, RAPD markers were used to evaluate genetic diversity and relationships among four important *Prunus* species used for breeding new cultivars and rootstocks in the *ex situ* collection of ATTC, Vlore, Albania. The RAPD technique proved to be efficient and a practical approach for the evaluation of genetic diversity and relationships among different *Prunus* species, in congruence with previous studies (Athanasiadis et al. 2013).

The present study revealed remarkable genetic diversity among *Prunus* sp genotypes, the mean similarity of43% among genotypes suggests their potential usefulness in breeding programs aimed by the collection institution. The genotypes of *P. armeniaca* and *P. domestica* were more closely related among analysed species.

The UPGMA cluster analysis which was supported also by PCoA analysis showed that the distribution of genotypes across groups mostly represented botanical classification within the genus *Prunus*. The genotypes of the species *P. armeniaca* and *P. domestica* were more closely related among analysed species. The understanding of genetic relationships among *Prunus* sp. will help significantly in breeding and effective utilisation of *Prunus* germplasm resources held in the *ex situ* collection.

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