Exploitation of Plum Genetic Diversity to Identify Soil-Borne Fungi Resistance Rootstocks

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**ABSTRACT**

High genetic diversity increases the chances of obtaining varieties resistant to abiotic stress in breeding programs. Plum rootstocks that are resistant to disease can be used for the propagation of various Prunus species. This research was conducted to evaluate different myrobalan plum genotypes and their resistance to three pathogenic bacteria, i.e. Rosellinia necatrix, Verticillium dahlia, Phytophthora cactorum. It was also endeavored to identify resistant genotypes, their degree of resistance to disease, and the correlation between their morphological traits. The experiment spanned from 2016 to 2018. In the first phase of the experiment, the morphological traits of 5 superior genotypes of myrobalan plum were evaluated. These genotypes were, namely, Ahmad-beigloo, Anar-1, 4835, 4813 and G14. In the second phase of the experiment, the genotypes were examined for their degree of resistance to the three mentioned pathogenic fungi. This was performed in two separate groups of complete randomized designs, with five treatments (genotypes) and nine repetitions. According to the results, the seedlings showed a high level of diversity with respect to morphological traits and features relating to the seed. Furthermore, under the influence of the Rosellinia fungi, as the length of the shoot and root increased, the length and percentage of the necrosis decreased. The Anar-1 genotype exhibited the longest necrosis and the highest percentage of necrosis which makes it the most susceptible genotype to the fungus in one group. The genotype Ahmad-beigloo exhibited the shortest necrosis and the lowest percentage of necrosis, making it the most resistant genotype to the Rosellinia fungus, and was placed in a separate group. With respect to the degree of seedling resistance to Phytophthora and Verticillium, there were no significant differences among the genotypes.

**KEYWORDS**

Rosellinia; Phytophthora; Verticillium; stone fruit trees; resistance to disease

**Introduction**

Plums are one of the most important stone fruits in the world. There are more than 2000 species of plum which have European, Asian and American origins, but only several of them have economic value (Barrett et al., 2004). The most important breeding goal in plums worldwide is the development of cultivars that are resistant to cold winter, pathogens, disease and physiological disorders, along with good quality fruits (Topp et al., 2012). The myrobalan plum is a thorny tree with a fast growing habit. It has delicate branches and small fruits with medium quality. Its natural habitat...
ranges from the Balkans to Caucasus mountains in south-western Asia. In the Caucasus, central Asia and Crimea, there are wild varieties of *P. cerasifera* which are distributed locally and are used for fruit (Horvath et al., 2008). The myrobalan plums can be used as fresh table fruits or as canned products (Istrate, 2007). Local cultivars of myrobalan can be used as a rich source of germplasm for the breeding of new cultivars (Cosmulescu et al., 2018). The selection of genotypes is facilitated by the presence of high diversity in terms of growth, production and quality of fruits in this species (Cosmulescu et al., 2018). Myrobalan plums are also used as a valuable source for plum rootstocks (Lanauskas et al., 2020). Myrobalan plums (*Prunus cerasifera*) can be found in various places of the world and can be considered as a source of genetic breeding and resistance to very high or low temperatures, besides the capability of producing higher amounts of yield and precocity. Genetic diversity among plant populations can be a valuable source of genes, especially regarding their use in present and future breeding programs (Bouhadida et al., 2005). The realization of the extent and structure of genetic diversity can assist in the identification of plant features and their conservation in populations (Karimi et al., 2008). Some of the most important morphological traits that can help breeders in recognizing interspecies hybrids of plum trees are their leaves, flowers and fruits (Ertékin et al., 2006; Jakubowski, 2002). These characteristics are extensively used for revealing the genetic diversity of horticultural and agronomic products (Blazek, 2007). Plum genotypes which are adaptable to specific ecological conditions can be used extensively in breeding programs (Botu et al., 2010; Cosmulescu et al., 2010).

It is a necessary issue to conduct breeding programs on rootstocks of *Prunus* species (Gradziel, 2009; Moreno, 2004). In case *Prunus* species are planted in regions with pathogenic infestations or soil-borne disease, it is vital that the rootstocks be resistant to pathogens and soil-borne diseases in addition to the maintenance of scion growth and the amount of yield (Browne, 2017). Plum species (such as myrobalan) are used extensively as rootstocks because of their high level of resistance to biotic and abiotic stresses in comparison to other *Prunus* species (Moreno et al., 1995).

The ascomycete fungus *Rosellinia necatrix* is a soil-borne pathogen which attacks plant roots and it has been reported worldwide (Cruz et al., 2014). It can threaten a wide range of tree species such as almonds (*Prunus dulcis*), peach (*Prunus persica*), plums (*Prunus domestica*), apples (*Malus domestica*), pears (*Pyrus communis*), olives (*Olea europea*), cherries (*Prunus avium*), avocado (*Persea americana*), grapes (*Vitis vinifera*) and apricots (*Prunus armenica*) (Cruz et al., 2014; Kulshrestha et al., 2014). This fungus is responsible for the white root rot, the signs of which are a mixture of root rot and crown rot. These symptoms lead to leaf yellowing, wilting and leaf abscission. These outcomes ultimately reduce the canopy and, after several weeks of the appearance of the initial disease symptoms, the pathogen causes a rapid wilting of the plant and leads to its death (Aimi et al., 2002; Schena et al., 2002).

The *Phytophthora fungis* is another pathogenic agent which causes the rotting of roots, canopy, trunk and bark of *Prunus* species (Browne and Doster, 2002; Felix-Gastelum and Mircetich, 2005; Guzman et al., 2007). The difference among *Prunus* rootstocks in terms of their resistance to *Phytophthora* has been reported previously (Broadbent et al., 1996; Day, 1953; Elena and Tsipouridis, 2000). However, in many of the clonal rootstocks, there is little information about their susceptibility to *Phytophthora*. It has been reported that *Phytophthora niederhauseri* can cause infestations in almond rootstocks and hybrid rootstocks of peach × almond (Browne et al., 2015; Kurbetli and Değirmenci, 2012; Perez-Sierra et al., 2010).

*Armillaria* is a pathogenic agent which causes disease in *Prunus* species which are one of the most susceptible fruit trees to the root disease caused by this fungus (Baumgartner et al., 2011). The different species of *Armillaria* can cause the death of woody roots and then lead to their degradation. This destruction of the root through time can substantially reduce the amount of product and the growth capacity of the tree (Baumgartner, 2004) which ultimately leads to the death of the tree. The resistance of plum rootstocks to this disease have been evaluated in California on myrobalan rootstocks (*P. cerasifera* and *P. munsoniana*), in France on myrobalan rootstocks, mariana GF8-1 (*Prunus munsoniana*) (Guillaumin et al., 1989) and rootstocks which descend from at least one myrobalan parent (Baumgartner et al., 2018).
Based on the great importance of plum rootstocks and their resistance to disease, this research was carried out to evaluate several genotypes of myrobalan plums and their resistance to pathogenic fungi, i.e. *Rosellinia necatrix, Verticillium dahlia, Phytophthora cactorum*. This research aimed at identifying resistant genotypes in order to use them as rootstocks in the future. The correlation between morphological traits and the amount of resistance to disease were also gauged.

**Materials and Methods**

**Plant Material**

This research was carried out in the experimental orchard and the pathology greenhouse of the Horticultural Science Research Institute under Karaj condition, Iran during 2016–2018. This research consisted two stages. The first stage involved evaluating the morphological features of five superior genotypes of myrobalan plums which had been collected from various regions of Iran. These genotypes were, namely, Ahmad-beigloo 1, Anar-1, 4835, 4813 and G14. In the second phase of the experiment, these genotypes were examined in terms of their resistance to the pathogenic fungi. The second phase of the experiment was performed in two separate complete randomized designs, with five treatments (genotypes) and nine replications. For this purpose, after alleviating the chilling requirement of seeds and achieving germination, the seedlings were transplanted into a mixture of cocopeat, perlite and clay (1:2:1) inside plastic bags. These were relocated into the greenhouse. In this research, the quantitative traits were evaluated by giving scores to the samples according to the UPOV descriptor. The length and width of the leaf blade were measured. Furthermore, the length and the width of seeds were measured by a digital caliper, and the weight of seeds was measured to a precision of 0.1 grams. The volume of each seed was calculated via multiplying the length of the seed by the abdominal width and the lateral width of the seed.

**Obtaining the Strains of Fungus**

The pathogenic fungi were, namely, *Rosellinia necatrix, Verticillium dahlia, Phytophthora cactorum*. The pre-cultured strains of these fungi were obtained from the institute of horticultural research. These strains had grown in common culture media (dextrose-agar) and in the specific culture media of *Phytophthora* (which included the basal culture media of CMA, pimaricin 10 mg.L\(^{-1}\), ampicillin 250 mg.L\(^{-1}\) and rifampicin 10 mg.L\(^{-1}\)). A disk of 6 – 8 mm was separated from each strain of fungus and was then cultured separately on a Petri dish containing a specific culture media. Subsequently, the Petri dishes were placed in an incubator at 25°C for 3 – 5 days under dark or light/dark conditions.

**Obtaining the Inoculum and Inoculating the Crowns of Seedlings**

In order to prepare the inoculum of fungus, 60 grams of wheat grains were washed with distilled water and were imbied in water for 24 hours. Then, by removing the extra water, the seeds were autoclaved at a pressure of 1.2 atmosphere, 121°C for 60 minutes every three days. In the next stage, 8 disks were sampled from the edge of fungal colonies which had grown for three days on the PDA culture (Potato Dextrose Agar). Each of these disks measured 6 – 8 mm and were dropped into Erlenmeyer flasks which were placed in the incubator at 25°C under dark conditions. After 2 – 3 weeks of complete growth of the fungus on the wheat grains, the fungal inoculum was prepared for inoculation (Banihashemi and Moradi, 2004).

In order to inoculate the crowns of seedlings with pathogenic fungus, a scratch was made on the crown of each seedling at a depth of 3 cm in the soil. Each scratch measured 1 mm in depth and 1 cm in length. Then, with the help of a sterilized scalpel, 30 mL of the inoculum was placed around the crown and the main root of each seedling. The pots were maintained in an immersed state in water for 24 hours each week. In between the stages of immersion, the pots were irrigated according to their
usual needs. The control treatment was inoculated with sterilized wheat. During the stages of the experiment, the temperature of the greenhouse was 20–30°C and the temperature of the soil was 20–25°C. After inoculation, the pots were assessed on a daily basis and the appearance or progress of disease was recorded. The symptoms included yellowing, wilting and death of the seedling. Nearly two weeks after inoculation, some seedlings showed symptoms of disease and wilting. These were completely removed from the soil and the length of necrosis was measured. The crown of some infected seedlings were selected randomly and were taken to the laboratory to have them sampled for pathogenic disease.

**Identification and Isolation of the Fungi**

In order to identify and isolate the fungi, several segments of the infected seedlings, measuring 3–4 cm, were dissected and immersed in a solution of hypochlorite sodium (0.5–1%) for three minutes. These were then washed with sterile, distilled water and were dried with sterilized filter paper. Then, small segments measuring 0.5 cm were dissected from the middle parts of discolored regions and healthy regions. The segments were disinfected under a microbiological hood for 30 seconds in hypochlorite sodium solution (0.5%) or in ethanol (70%). The samples were washed three times in sterilized water and then dried with filter paper. The samples were cultured in Petri dishes containing the common culture media of PDA and the specific culture media of *Phytophthora* CMA (which consisted the basal culture media of CMA, pimaricin 10 mg.L\(^{-1}\), ampicillin 250 mg.L\(^{-1}\), rifampicin mg.L\(^{-1}\)). The Petri dishes were placed in dark or light/dark conditions for 3–5 days at 25°C and the fungus that had grown was sub-cultured in separate Petri dishes. The colonies that had grown were isolated by the Tip Hyphal method (Ivors, 2015).

**Data Analysis**

This experiment was carried out in a completely randomized design (CRD) with 5 treatments (genotypes) and five replicates. Analysis of variance (ANOVA) was performed using the SAS software (Version 9.2). Mean values were compared by Duncan’s multiple range test at 1 and 5% probability level. The data were normalized by a specific reference sample before principle component analysis (PCA), cluster analysis and bivariate correlation analysis. Cluster, PCA and spearman correlation analyses was conducted using SAS software (Ver. 9.2). The Ward method and spearman correlation coefficient were used for cluster and correlation analyses, respectively.

**Results**

**Morphological Evaluation of Plum Superior Genotypes**

The morphological evaluations of the superior plum genotypes showed significant variations and a substantial diversity among the seedlings (Table 1). The weakest branching was observed in the genotype 4835 and the other genotypes had an intermediate vigor of branching. In terms of the growth vigor, the genotypes Ahmad-beigloo 1, Anar-1 and 4835 had intermediate levels of growth vigor, whereas genotypes 4813 and G14 showed strong growth vigor. The genotype Ahmad-beigloo 1 showed a branched growth habit, whereas the other genotypes showed erect growth habits. There was a great diversity in terms of the width of leaf blade, so that genotype 4813 had the shortest blade, Anar-1 had an intermediate length of leaf blade, Ahmad-beigloo 1 and G14 had long leaf blades and 4835 had the longest. Other morphological traits of the genotypes are presented in Table 2.

The analysis of variance pertaining to qualitative traits of seeds showed that most of the genotypes were significantly different compared to each other in terms of the seed weight, length, lateral width, abdominal width, volume and germination percentage (P ≤ 0.01) (Table 3).
According to the comparison of mean values, the genotype G14 showed an average seed weight of 0.554 grams, which indicates the heaviest seed among the genotypes. The lowest value of seed weight was observed in the three genotypes, 4813, Anar-1 and Ahmad-beigloo 1, the seeds of which weighed 0.284, 0.346 and 0.356 grams, respectively. Furthermore, the G14 genotype yielded the longest seed (18.46 mm) among the genotypes. This is while the shortest seed length was yielded by Anar-1 (11.94 mm). The germination percentage was highest in genotype 4813 (62.25%). The lowest germination percentage was observed in Ahmad-beigloo 1 (37.5%) (Table 4).

**The Evaluation of Genotype Resistance to Pathogenic Fungi**

The analysis of variance showed that there was no significant difference in terms of genotype resistance to *Phytophthora* and *Verticillium*. On the other hand, the genotypes were significantly different
Table 4. Mean comparison of seed traits in some plum genotypes.

| Genotype     | Seed weight (gr) | Seed length (mm) | Seed lateral width (mm) | Seed ventral width (mm) | Seed volume (mm³) | Seed germination percentage (%) |
|--------------|------------------|------------------|-------------------------|-------------------------|-------------------|---------------------------------|
| G14          | 0.554a           | 18.46a           | 11.66a                  | 6.272bc                 | 1361.5a           | 49.74bc                         |
| 4835         | 0.464b           | 15.165b          | 12.02a                  | 6.916ab                 | 1271.3a           | 61.04ab                         |
| Ahmad-beigloo 1 | 0.336 c         | 13.754bc         | 9.792b                  | 6.122bc                 | 824.6b            | 37.50 c                         |
| Anar-1       | 0.346 c          | 11.494d          | 9.954bc                 | 7.336a                  | 788.3b            | 58.17ab                         |
| 4813         | 0.284 c          | 12.9 cd           | 8.898 c                 | 5.414 c                 | 623.1b            | 62.25a                          |

Table 5. Variance analysis of stem length, root length, necrosis length and percentage of necrosis in some plum genotypes treated with *Phytophthora cactorum*, *Verticillium dahliae* and *Rosellinia necatrix*.

| S.O.V. | df | N. cactorum | N. per (%) | N. dahliae | N. per (%) | N. necatrix | N. per (%) |
|--------|----|-------------|------------|------------|------------|-------------|------------|
| Genotype | 4  | 0.006**     | 0.221**    | 0.092**    | 0.098**    | 548.23**    | 3.02**     |
| Error   | 20 | 0.005       | 0.074      | 0.0063     | 15.47      | 0.41        | 0.11       |
| CV (%)  | 22.4 | 26.31          | 19.67      | 28.1       | 25.24      | 16.76       | 20.44      |

compared to each other in terms of the shoot height, root length, necrosis length and necrosis percentage when confronting the pathogenic fungus *Rosellinia necatrix* (P ≤ 0.01) (Table 5).

According to the results, the highest shoot length was observed in genotype 4813 (29.43 cm) when treated with *Rosellinia necatrix*. The shortest shoot height was observed in genotype Anar-1, 4835, G14 and Ahmad-beigloo 1. Furthermore, the longest root was observed in Ahmad-beigloo 1 and 4813, whereas the shortest roots were observed in genotypes Anar-1, 4835 and G14. The longest necrosis and the highest necrosis percentage were 3.6 cm and 34.22%, respectively, in the genotype Anar-1, whereas the shortest necrosis and the lowest necrosis percentage were 1.4 cm and 8.87%, respectively, in the genotype Ahmad-beigloo 1 (Table 6 and Figure 1).

**Cluster Analysis**

As the genotypes were analyzed by cluster analysis, it was revealed that genotypes Anar-1, 4835 and G14 had substantial similarities in comparison to the genotypes Ahmad-beigloo 1 and 4813. Furthermore, it was revealed that the 4835 and G14 had significant similarities. These two genotypes were categorized as weak and intermediate in terms of their resistance to pathogenic fungi and therefore had less genetic distances from Anar-1 which was the most susceptible genotype to fungal infestations. Genotypes Ahmad-beigloo 1 and 4813 were categorized in separate groups which indicates that they had a significantly different degree of resistance to fungi, compared to the other genotypes. They also showed the shortest necrosis and the lowest percentage of necrosis. The Ahmad-beigloo 1 and 4813 were the most resistant genotypes to the fungi. They can be used as successful genotypes where the soil is infested with *Rosellinia necatrix* (Figure 2).

Table 6. Mean comparison of stem height, root length, necrosis length and necrosis percentage in some plum genotypes under *Rosellinia* fungi treatment.

| Genotype     | Stem length (cm) | Root length (cm) | Necrosis length (cm) | Necrosis percentage (%) |
|--------------|------------------|------------------|----------------------|-------------------------|
| Anar-1       | 10.34b           | 10.81b           | 3.6a                 | 34.22a                  |
| 4835         | 11.76b           | 11.43b           | 3.11ab               | 27.58ab                 |
| G14          | 12.61b           | 13.26b           | 2.77b                | 22.49bc                 |
| 4813         | 29.42a           | 20.64a           | 3.05ab               | 15.83 cd                |
| Ahmad-beigloo | 14.44b           | 20.58a           | 1.4 c                | 8.87d                   |
The evaluation of morphological traits and the correlations between them showed that the extent of branching had a significant, positive correlation with the growth vigor of seedlings, the width of leaf blade and the length of the leaf blade tip. However, the amount of branching had a significant, negative correlation with the growth habit, the luminosity of leaf surface, the depth of the leaf-edge incision and the depth of the petiole pit. The growth vigor of seedlings showed a significant, positive correlation with the width and length of leaf blade, but had

**Correlation between Traits**

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a significant, negative correlation with the depth of the petiole pit. The growth habit of the tree had a significant, positive correlation with the intensity of green color on the leaf surface and the luminosity of the leaf surface. The length of the leaf blade had a significant, positive correlation with the width of the leaf blade, the length of the blade tip, the depth of the leaf-edge incision and the size of the petiole. The length of the leaf blade had a significant, negative correlation with the intensity of green color on the leaf surface and the luminosity of the leaf surface. The width of the leaf blade had a significant, positive correlation with the length of the blade tip, the depth of the leaf-edge incision and the size of the petiole, but had a significant, negative correlation with the intensity of green color on the leaf surface and the luminosity of the leaf surface. The length of the blade tip had a significant, positive correlation with the growth vigor, branching and the length and width of the leaf blade. There was a significant, positive correlation between the intensity of green color on the leaf surface and the luminosity of the leaf surface (Table 7).

The correlation of seed traits and root length, stem length, and size and percentage of necrosis in plants treated with *Rosellinia necatrix* showed that there was no significant difference between the seed traits and necrosis. In other words, seed size had no effect on the amount of resistance to the disease. The most important traits in determining the resistance to disease were stem height and root length. The correlation between necrosis length and root length revealed that a linear regression relationship does not exist between root length and necrosis length. The regression coefficient was very low ($R^2 = -0.130$). It seems that the root length cannot be a simple determinant of resistance to the pathogen, and many other factors are influential which require particularized examinations. Similar results were also observed in the correlation between necrosis length and stem height, and the regression coefficient between these two traits was $R^2 = -0.310$ (Figure 3).

**Table 7. Correlation between morphological traits in some plum genotypes, according Spearman test.**

| Trait Description | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|-------------------|---|---|---|---|---|---|---|---|---|----|----|----|----|
| Stem color        | 1 |   |   |   |   |   |   |   |   |    |    |    |    |
| Branching         | -0.03 | 1 |   |   |   |   |   |   |   |    |    |    |    |
| Tree vigor        | 0.06 | 0.3 * | 1 |   |   |   |   |   |   |    |    |    |    |
| Tree habit        | 0.2 ns | -0.4 | 0.2 | 1 |   |   |   |   |   |    |    |    |    |
| Length of Leaf blade | 0.03 | 0.02 | 0.2 | -0.1 | 1 |   |   |   |   |    |    |    |    |
| Width of Leaf blade | 0.07 | 0.4 * | 0.4 * | 0.2 | 0.4 * | 1 |   |   |   |    |    |    |    |
| Ratio length of leaf blade/ petiole | 0.3 ns | 0.04 | 0.04 | 0.2 | 0.2 | -0.1 | 1 |   |   |    |    |    |    |
| Angle of Leaf blade apex | 0.07 | 0.4 * | 0.4 * | 0.2 | 0.4 | 0.8 | 0.1 | 1 |   |    |    |    |    |
| Intensity of green color of Leaf blade upper side | 0.04 | 0.2 | 0.2 | 0.5 | -0.4 | -0.4 | 0.04 | -0.4 | 1 |   |    |    |    |
| Glossiness of Leaf blade upper side | 0.02 | -0.3 | 0.2 | 0.5 | -0.3 | -0.4 | 0.1 | -0.4 | 0.7 | 1 |   |    |    |
| Depth of incisions of margin | -0.1 | -0.3 | 0.05 | 0.1 | 0.5 | 0.5 | 0.1 | 0.5 | -0.5 | -0.4 | 1 |   |    |
| Length of Petiole | 0.3 ns | 0.2 | 0.07 | -0.1 | 0.5 | 0.3 * | 0.2 | 0.2 | -0.3 | -0.2 | 0.03 | 1 |    |
| Depth of petiole suture | 0.03 | -0.4 | 0.3 * | 0.03 | 0.1 | 0.1 | 0.02 | 0.1 | 0.4 | 0.4 * | -0.3 | 0.1 | 1 |
| Length of necrosis | -0.06 | 0.05 | 0.03 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | -0.3 | 0.2 | 0.2 | 0.2 |

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* ns: not significant, *: P < 0.05, **: P < 0.01, ***: P < 0.001.
There was a significant correlation between seed traits. The seed weight had a significant, positive correlation with seed size (i.e. seed length, lateral seed width, abdominal seed width and seed volume (Table 8).

### Discussion

The evaluation of germplasms and the identification of superior, promising genotypes is an initial step in introducing cultivars and commercial rootstocks (Khorami et al., 2018). Morphological indicators can be used extensively in breeding programs of fruit trees (Karimi et al., 2008). Iran is one of the centers of origin for *Prunus cerasifera*. It has a high genetic diversity that can potentiate the introduction of cultivars and rootstocks, especially those that are resistant to soil-borne fungal diseases (Horvath et al., 2008). According to the results of this study, a high level of diversity was observed among the morphological traits in plum genotypes. According to previous reports, wild plum cultivars are known to have high genetic variations in terms of their morphological features, besides a high structural similarity of genetic matter with other *Prunus* species (Kaufmane et al., 2002). The size of the leaf and its structure in fruit trees greatly depend on the variety or genotype (Goncalves et al., 2008). Cosmulescu et al. (2018) reported a high genetic diversity in terms of the morphological and pomological features of *Prunus cerasifera*, thereby suggesting a high potential of this species for selection and utilization (Cosmulescu et al., 2018).

The identification of cultivars in plums is traditionally performed according to the morphological traits. These may include their shape, color and size of leaves, fruits and seeds as well as stone adhesion to the fruit (Ilgin et al., 2009). Among all the traits that can be used in identifying the species, seed-related properties are one of the most reliable traits (Woldring, 2000). The seed size is very useful for identifying plum cultivars (Behre, 1978). The use of stone volume can assist in the identification of species and even varieties of plums (Nielsen and Olrik, 2001). In terms of seed-

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**Table 8. Correlation between seed traits with root, stem and necrosis traits in plum genotypes treated with *Rosellinia necatrix***

| Trait             | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Seed weight       | 1   |     |     |     |     |     |     |     |     |
| Seed length       | 0.86** | 1   |     |     |     |     |     |     |     |
| Seed lateral width| 0.93** | 0.79** | 1   |     |     |     |     |     |     |
| Seed ventral width| 0.26** | 0.04** | 0.27 ns | 1 |     |     |     |     |     |
| Seed volume       | 0.94** | 0.86** | 0.94** | 0.41* | 1   |     |     |     |     |
| Seed germination percentage | −0.21 ns | −0.25 ns | −0.08 ns | 0.05 ns | −0.13 ns | 1 |     |     |     |
| Shoot height      | −0.19 ns | −0.24 ns | −0.16 ns | 0.25 ns | −0.24 ns | 0.2 ns | 1 |     |     |
| Root length       | −0.55 ns | −0.19 ns | −0.36 ns | −0.28 ns | −0.29 ns | −0.3 ns | 0.35* | 1 |     |
| Necrosis length   | 0.01 ns | −0.2 ns | 0.02 ns | 0.22 ns | 0.05 ns | 0.3 ns | 0.02 ns | −0.34* | 1 |
| Necrosis percentage| 0.25 ns | −0.11 ns | 0.25 ns | 0.6 ns | 0.29 ns | 0.34 ns | −0.35* | −0.72** | 0.77** |

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**Figure 3.** Regression equation between root length with necrosis length (right) and stem height with necrosis percentage.
related characteristics, genotype G14 had the highest value of seed weight, seed length and abdominal width, and genotype 4813 had the lowest value of seed weight, abdominal width and lateral width of seed. Statistical methods like cluster analysis can be used as capable tools to gauge plant varieties (Aazami and Jalili, 2011). In this regard, based on morphological traits, G14 and 4835 genotypes had the closest relationship with each other, and 4813 and Ahmad-beigloo 1 had the closest relationship.

The categorization of cultivars and genotypes into separate groups can be based on qualitative traits. By using cluster analysis, it was revealed that the genotypes have high potentials for breeding purposes. These genotypes can be used as parental lines for hybridization programs, whereby new cultivars can be created. These genotypes are likely to have a high level of heterozygosity. They can potentially be useful in breeding programs which involve plum rootstocks and the myrobalan plum.

Genetic distance is commonly used as an indicator of genetic relatedness or distinction among cultivars and genotypes. Aazami and Jalili (2011) reported genetic variations among several Iranian plum genotypes based on morphological traits. These genotypes differed in terms of fruit size, bloom time, fertility, stone shape and stone adhesion to the fruit. They were divided into 4 groups; the first group consisted of distinctive fruits with round stones, and the fourth group consisted of oval fruits with ovate stones (Aazami and Jalili, 2011).

The correlation among qualitative traits showed that the factors affecting the growth of tree (seedling growth vigor, leaf width and leaf length) increased the ability of branching. Seedlings with erect growth habits had fewer branches than seedlings with descending or broad growth habits. As the leaf size increased, the growth vigor of seedlings increased and the depth of the petiole pit was reduced. The length of the leaf blade positively correlated with the width of the blade, the length of the leaf blade tip, the depth of leaf-edge incision and the petiole size. Ganji Moghadam and Khalighi (2007) reported a substantial relationship between growth vigor, canopy width and volume, and the size index in the Mahlab cultivar. Furthermore, leaves with longer blade tips had deeper leaf-edge incisions, leaf size, and growth vigor. As the intensity of the green color increased, the luminosity of the leaf surface increased.

A major objective in breeding plums worldwide is to create cultivars that are resistant to disease (Topp et al., 2012). The genetic harnessing of plants can be very important for their resistance to disease. This can reduce the use of chemicals in controlling the disease and in minimizing the costs of anti-pathogenic chemicals (Bruce et al., 1992). Therefore, gauging the diversity of genetic resources and the resistance of genotypes to plant diseases can be of prime significance in breeding programs (Rakonjac et al., 2014). Hartmann and Neumüller (2009) recognized significant step in the assessment of cultivar response to disease, which indicated the notion of diversity in this regard. Resistant genotypes can be used as parental sources in crossbreeding programs (Mitre et al., 2015). It has been reported that genotypes and various cultivars of Prunus can stage different responses to fungal infestations (Browne, 2017). In this research, the studied genotypes showed different levels of resistance to Rosellinia necatrix. The Ahmad-beigloo 1 was recorded as the most resistant genotype, whereas the Anar-1 was the most susceptible genotype to the Rosellinia fungi. Based on the results, as the shoot height increased, the root length increased likewise, whereas the necrosis percentage decreased. According to the results, the seed size had no effect on the degree of resistance to the disease.

In conclusion, this research suggests that the studied genotypes exhibit a high level of diversity. However, no significant difference was observed among the genotypes in terms of their resistance to Phytophthora and Verticillium. In contrast, the Rosellinia highlighted the differences among genotypes, among which the Anar-1 was identified as the most susceptible genotype, and the use of which as a rootstock is not recommended in areas with Rosellinia infestations. However, the Ahmad-beigloo 1 can be recommended as the most resistant rootstock for areas of infestations with this fungus.
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