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Evaluation of the Behavior of Native Iranian Almond Species as Rootstocks

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Abstract: Iran is one of the most important regions for origin and diversification of wild almond species in the world. Over 20 species, naturally distributed in many regions, have been identified to date in Iran. These can be used as rootstocks in different *Prunus* species such as almond or peach due to their adaptability to severe (drought) environmental conditions and resistance to some pests and diseases. Results showed that *P. eburnea* had the most stem biomass, and *P. scoparia* the least leaf area and the largest root system among the three species, which can indicate better adaptation to drought conditions. The correlations between the measured traits suggest that the relationship between shoot and root morphology is unique for each species. In addition, results indicated that *P. eburnea* had the highest seed germination percentage and *P. elaeagnifolia* the lowest. Finally, there was significant difference between wild rootstocks in grafting success, with *P. scoparia* and *P elaeagnifolia* showing the best behavior.

Keywords: Almond, *Prunus*, rootstock, breeding, drought resistance.

INTRODUCTION

Almond [*Prunus dulcis* (Mill.) D.A. Webb syn. *P. amygdalus* (L.) Batsch] is a species of genus *Prunus* subgenus *Amygdalus* (Rosaceae, subfamily Prunoideae) that is commercially grown worldwide (Kester and Gradizel 1996; Martínez-Gómez et al., 2007). The cultivated almond is thought to have originated in the arid mountainous regions of Central Asia (Grasselly 1976a; Ladisinsky, 1999). In this area, several wild species are also found growing throughout Southwest and Central Asia (Fig. 1). Over 30 species described by botanists may represent subspecies or ecotype (Browicz and Zohary1996; Grasselly 1976b; Kester et al., 1991; Kester and Gradziel 1996). Iran is as a site of origin of almond and is extremely rich in wild almond species (Etemadi and Asadi, 1999; Ghahreman and Attar 1999; Sorkheh et al., 2009). These wild species provide an enlarged pool of available germplasm and suitable characteristics such as late bloom and self-fertility and resistance to drought, salinity and low winter temperatures (Denisov 1980, 1988). In addition, related almond species demonstrate a greater resistance to abiotic and biotic stresses, and so represent valuable germplasm sources for breeding (Gradziel et al., 2001; Sorkheh et al., 2009).

On the other hand, some of these species have been used directly as rootstocks for almond, usually in non-irrigated conditions. In several parts of Iran, grafting of cultivated almonds on wild almonds has a 50-60 year old record (Mortazavi, 1986) (Fig. 1). *P. scoparia* (Spach) C.K. Schneid., *P. elaeagnifolia* (Spach) Fritsch, and *P. eburnea* (Spach), naturally distributed in many regions of Iran, have be used in arid and semi-arid areas to control soil erosion and water sheds (Khatamsaz, 1983; Sabeti, 1994). *P. webbii* was a species used as dwarf rootstock for cultivated almonds in Yugoslavia and Turkey (Dimitrovski and Ristevski, 1973). Other examples include the use of *P. spartioides* (Spach) C. K. Schneid in Iran (Gentry, 1956); *P. bucharica* (Korsh.) Hand.-Mazz. (Evreinoff, 1952; Jadrov, 1970) and *P. fenzliana* Fritsch (Denisov 1980) in Russia; and *P. kuranica* (Korsh.) Kitam. and *P. argentea* (Lam.) Rehder (Grasselly 1976b) or *P dehiscens* Koehne and *P. kotschyi* (Boiss. et Hohen.) Nab. (Grasselly 1992) in France. *P. petunnikowii* has also been described as exceptionally resistant to drought and crown gall (Evreinoff, 1952). In addition, wild species have been used as rootstock in other fruit trees such as pistachio (Baninasab and Rahemi, 2007), walnut (McGranhan and Forde,

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1985) cherry (Day, 1951) and pear (Westwood, 1981). The aim of this work was the evaluation of the behavior of three native Iranian almond species as rootstock, including the study of morphological characteristics of shoot and root and the evaluation of germination rate and grafting take of cultivated almonds.

MATERIALS AND METHODS

Wild almond species studied, included in the genus *Prunus* and subgenus *Amygdalus*, were *P. elaeagnifolia* (Spach) Fritsch (section *Euamygdalus)*, *P. eburnea* Spach (section *Lycioides*) and *P. scoparia* Spach (section *Spartioides*) (Grasselly, 1976b; Kester and Graziel, 1991; Zohary, 1988). This plant material was collected in the region of Firoozabad of Fars Province in Iran (Fig. 1). Twenty nuts of the three wild almond species for each experiment were stratified at 4-6 ºC for 30 days. Then, nuts were sown in 5 kg black plastic bags filled with a 1:1:1 (v/v) mixture of fine sand, leaf mould and soil. The bags were then transferred to the glasshouse, under natural photoperiod for the whole period of experiment, with an average temperature of 24 ±2 and 17±2 ^{o}C for day and night, respectively.

In the first assay, four months after sowing, seedlings were removed from the containers and the root system was carefully washed. The following seedling and root system data were recorded: seedling height (in cm) and diameter (in mm); internode length (cm) (the distance between the two nodes in middle of seedling); leaf number; and total leaf area (cm^2) using a leaf area meter (Delta-T devices, Ltd., Cambridge UK). Leaf dry weight (g) was also measured after drying samples for 72 h at 70^{o} C. In addition, root number, root length (cm), and root diameter (mm) using the Delta-T scan Image Analysis system were scored. Finally, stem and root biomass percentages (%) were also calculated as dry weight/fresh weight × 100.

In the second assay, the seeds of these species were mechanically scarified and then soaked in water for 48h for seed germination. Then, nuts were mixed with peat-mass (3:1), peat-moss: seed (V/V). Germination observation was performed every 15 days during 60 days. Percentage of germinated seeds was calculated for all of the days. The pots were then transferred to the glasshouse, under natural photoperiod for the whole period of experiment, with an average temperature of 24 and 17 (±2) ºC, for day and night, respectively. Grafting of seedlings was performed 8 months after seed sowing in the glasshouse using the herbaceous cleft grafting technique. Before grafting, seedling height and seedling diameter (5 cm from soil level) were measured. Bud woods were cut from the/an almond "Shahrood-8" cultivar planted at the Agricultural Research Center of Fars Province, Iran. After grafting, glasshouse temperature and relative humidity were regulated at 25± 5 ºC and 75± 3%, respectively. When the emerged scion shoot reached about 3-5 cm in length, grafting was recorded as "successful."

In both assays, the experiments were carried out in a completely randomized design (CRD) with 4 replications and 5 plants per replication in each wild species studied. The data were statistically analyzed using SAS package, and the means were compared using Duncan's Multiple Range Test (DMRT) at p = 0.05 level. Data recorded as percentages were analyzed after appropriate statistical transformation. Correlation analysis was also performed between measured traits at the p= 0.05 and 0.01 levels using SAS software (SAS, 1989).

RESULTS AND DISCUSSION

The analysis of variance showed a significant difference among species for most of the shoot and root traits evaluated, and differences for most traits showed high variability among these species (Tale 1). *P. scoparia* had the tallest (77.00 cm) and *P. eburnea* the shortest height (21.09 cm) with a similar diameter and internode length from 0.72 cm (*P. eburnea*) to 1.15 cm (*P. scoparia*). Leaf number was also very different between the three assayed species, from 93 (*P. scoparia*) to 283 (*P. eleagnifolia*). Leaf area was significantly different between *P. elaeagnifolia* (226.4 cm^2) and *P. eburnea* (120.3 cm^2), which both had larger leaf areas compared to *P. scoparia* (75.3 cm^2). Finally, stem biomass ranged from 49.87 % in the case of *P. elaeagnifolia* to 58.43 % in *P. eburnea*. On the other hand, the characters of the root system have also been determined, and the results showed that the *P. scoparia* seedling had a larger root system (6,178.5 mm) with a higher number of roots than the other two species. In addition, the average root diameter and percentage of root biomass was significantly different between *P. scoparia* (1.54 mm and 47.02% respectively), *P. elaeagnifolia* (1.49 mm and 50.92%) and *P. eburnea* (1.63 mm and 42.51%).

Leaf area values agree with previous results obtained by Khatsmsaz (1983) and Sabeti (1994). In addition, the lesser number of leaves and lower leaf area observed in *P. scoparia* indicated more drought tolerance compared to other wild species (Rieger and Duemel, 1996). On the other hand, knowledge of root system morphology and variation can be important in selection and development of rootstocks and is effective in orchard management. The larger root system observed in *P. scoparia* could be a very good trait for the use of this species as rootstock and can indicate a better adaptation to drought. In addition, the lower number of roots in *P. eburnea* was similar to that of Tworkoski and Scorza (2001), who found that dwarf rootstock had a lesser number of roots than other species. It is probable that lower leaf areas in *P. scoparia* caused less transportation of carbohydrate to root and decreased the root number in this species. Also roots are the main source of cytokinins and gibberellins (Arteca, 1996), and transportation of these substances from root to shoot via the xylem caused more stem internode elongation in *P. scoparia* compared to *P. eburnea*.

The correlation between the studied traits is shown in Table 2. Several shoot characteristics were significantly correlated with root characteristics. In *P. scoparia*, seedling diameter was significantly correlated with leaf dry weight, stem biomass and root biomass. Root number was positively correlated with internode length, stem biomass, and leaf dry weight. In *P. elaeagnifolia*, seedling height was positively correlated with seedling diameter, internode length, leaf dry weight, stem and root biomass. In *P. eburnea* root length was positively correlated with stem biomass and negatively correlated with internode length. Root biomass was positively correlated with stem biomass, stem diameter and stem height. In general, *P. scoparia* had the fastest growing seedlings with strong root systems. *P. elaeagnifolia* seedlings showed moderate growth of stem and root systems, while *P. eburnea* seedlings were slow-growing with the smallest root systems.

The correlations between the measured traits suggest that the relationship between shoot and root morphology is unique for each species. Seedling diameter, root number, and total root length in *P. scoparia*; seedling height, root number, root diameter and leaf in *P. elaeagnifolia*; and internode length, leaf area, total root length and root diameter in *P. eburnea*, were found to be important evaluation criteria when selecting material for commercial rootstock or environmental restoration potential.

Results showed significant differences between the germination percentages of the species (Table 3). The highest germination percentage was in *P. eburnea* (79%) and the lowest in *P. elaeagnifolia* (35%). In addition, graft-take percentage of *P. scoparia*, *P. elaeagnifolia* and *P. eburnea* rootstocks are shown in Table 3. The results showed that the difference in grafting success between three rootstocks was significant. However, *P. scoparia* and *P. eburnea* had the highest (40%) and lowest (35%) grafting-take percentages, respectively.

In existing literature, there are few studies on the germination ability of almond species. Khalil and Qrunfleh (2000) report maximum germination percentage of *P. arabica* (43%) by chilling seeds for 45 days, while unchilled seeds did not germinate. Chilling decreased plant growth inhibitors in *P. arabica* seeds. The high germination rate of *P. eburnea* may be explained by a genetic difference between the species. The seeds of *P. elaeagnifolia* may have a dormant embryo with a higher chilling requirement or higher concentrations of plant growth inhibitors than other species. It's probable that environmental factors such as temperature, humidity and elevation when the fruits set and developed had an effect on germination percentage. (Hartman et al., 1997).

According to the results, *P. scoparia* had fast growing seedlings (with the longest and thickest stems) with a strong root system (highest root number and greatest length), and the highest grafting success. *P. elaeagnifolia* seeds had the lowest germination percentage, but seedlings had suitable growth in stem with viable grafting success. *P. eburnea* seeds had the highest germination percentage, but seedlings were slow-growing (shortest and thinnest stemmed seedlings) with weak root systems (lowest root length and number), and had the lowest grafting success. *P. scoparia* is thus better and more suitable for use as rootstock.

There are several factors affecting grafting success, and in this study, these factors, except genetics material, were minimized as much as possible in order to compare only the species effect. However, in some cases, amygdalin, a cyanogenic glycoside produced by rootstock and or by the scion bud, deposits between the rootstock and bud, and as a result the callus bridge does not occur and causes lower grafting take (Hartman et al., 1997). In conclusion, the grafting experiment demonstrated that there is variability between almond rootstock species if investigated in almond species. Furthermore, species studied may provide an important source of rootstock germplasm for the future.

Finally, in this study seedling height and diameter of wild species were measured before grafting (Table 3). The seedling height was significantly different between the three species (Table 1). At the end of the experiment, *P. scoparia* had the tallest (53.47 cm) and *P. eburnea* the shortest height (15.91 cm). This was demonstrated in nature by Sabeti (1994). Another characteristic taken into consideration was tree growth capacity as determined by seedling diameter. Seedling diameter is essential to allow early budding and transplanting in the orchard. Seedling diameter was not significantly different between *P. elaeagnifolia* and *P. eburnea*, whereas the differences were significant between these two species and *P. scoparia*. However, *P. scoparia* had the thickest seedling diameter (3.84 mm). Seedling diameter of *P. scoparia* was similar to that reported by Baninasab and Rahemi (2007).

In addition to genetic factors, sprouting time of seedlings grown in the glasshouse may affect seedling growth. For example, we observed that the sprouting time of *P. scoparia* was earliest, followed in order by *P. elaeagnifolia* and *P. eburnea*. *P. scoparia* seems, therefore, to produce the best seedling (according to seedling height and diameter) among the tested wild almond species. It is possible that the better root system (measured by root number and length) in *P. scoparia*, compared to *P. eburnea* and *P. elaeagnifolia*, resulted in more vigorous seedlings in *P. scoparia* than in the other two species.

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Table 1. Shoot and root traits evaluated in the three Iranian wild almond species assayed as rootstocks.

Shoot and root traits*	*P. scoparia*	*P. elaeagnifolia*	*P. eburnea*
Seedling height (cm)	77.00 a	39.58 b	21.09 b
Seedling diameter (mm)	2.74 a	2.67 a	2.39 a
Internode length (cm)	1.15a	0.91 b	0.72 b
Leaf number	93 c	283 a	188 b
Total leaf area (cm^2)	75.3b	226.4 a	120.3 a
Leaf dry weight (g)	0.33 c	0.72 a	0.64 b
Stem biomass (%)	53.83 b	49.87 b	58.43 a
Number of roots	272 a	211 b	139 c
Total root length (mm)	6,178.5 a	4,693.4 b	3,286.5 c
Average root diameter (mm)	1.54 ab	1.49 b	1.63 a
Root biomass (%)	47.02 a	50.92 a	42.51 b

* For each trait (row), means with the similar letters are not significantly different at 5% level by DMRT.

Table 2. Correlation between morphological traits in the three Iranian wild almond species assayed as rootstocks.

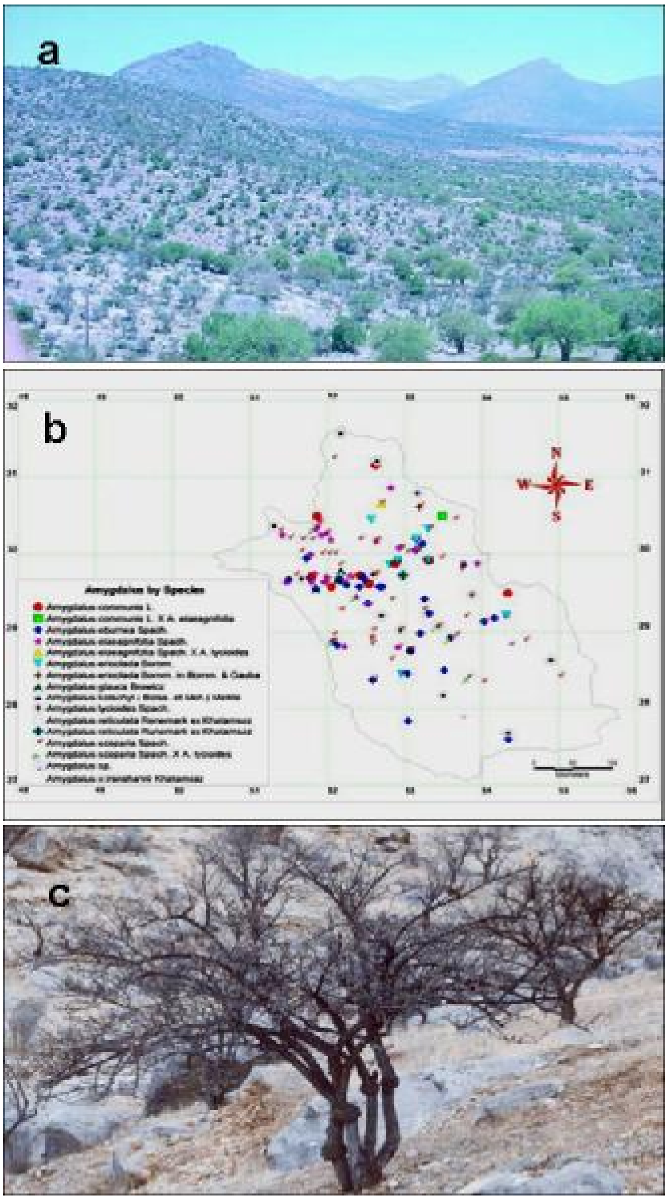
Trait	Seedling height	Seedling diameter	Internode length	Leaf area	Leaf dry weight	Stem biomass	Root length	Root diameter	Root number	Root biomass
					P. scoparia					
Seedling height	1.00									
Seedling diameter	0.53	1.00								
Internode length	0.73^{**}	0.23	1.00							
Leaf area	-0.12	0.20	0.41^{*}	1.00						
Leaf weight	-0.15	0.55^{*}	0.95	0.15	1.00					
Stem biomass	0.27	0.50^{**}	0.47	0.59	0.81^{*}	1.00				
Root length	0.15	0.21	0.31	-0.11	0.54^{**}	0.49^{*}	1.00			
Root diameter	0.21	0.02	0.50^{**}	0.40	-0.38	0.27^{*}	-0.35	1.00		
Root number	0.02	0.22	0.78^{**}	0.07	0.58^{**}	0.52	0.99^{**}	0.44	1.00	
Root biomass	0.52	0.34^{**}	0.48	0.41	0.70	0.85	0.97	0.97	0.47	1.00
					P. elaeagnifolia					
Seedling height	1.00									
Seedling diameter	0.47^{*}	1.00								
Internode length	0.32^{**}	0.27	1.00							
Leaf area	0.13	0.11	0.67	1.00						
Leaf weight	0.47^{*}	0.64^{**}	0.19	0.35	1.00					
Stem biomass	0.65^{**}	0.66^{**}	0.45	0.41^{**}	0.78^{**}	1.00				
Root length	0.30	0.20	0.61	0.51^{*}	0.49^{*}	0.47^{*}	1.00			
Root diameter	0.13	-0.06	0.31	-0.35	0.71^{**}	0.53^{*}	-0.33	1.00		
Root number	0.39	0.39	0.45	0.43	-0.35	-0.19	0.65^{**}	-0.17	1.00	
Root biomass	0.42	0.72^{**}	0.39	0.79	0.27	0.87	0.56^{**}	0.12	0.19	1.00
					P. eburnea					
Seedling height	1.00									
Seedling diameter	0.52	1.00								
Internode length	0.15^{**}	0.95	1.00							
Leaf area	-0.38	-0.02	-0.28	1.00						
Leaf weight	0.48^{*}	0.80^{**}	0.42	0.19	1.00					
Stem biomass	0.62^{**}	0.72^{**}	0.37	0.15	0.51^{*}	1.00				
Root length	0.14	0.43	-0.95^{**}	0.34	0.38	0.52^{**}	1.00			
Root diameter	0.20	0.08	-0.76^{**}	-0.31	0.20	0.13	0.45	1.00		
Root number	0.26	0.01	-0.07	0.27	0.05	0.16	0.30	-0.16	1.00	
Root biomass	0.25^{*}	0.52^{**}	0.47	0.04	0.28	0.87^{**}	0.90	0.19	0.12	1.00

*,** Significant at $P < 0.05$ and 0.01, respectively

Table 3. Germination percentage, grafting success and seedling characteristics in the three Iranian wild almond species assayed as rootstocks.

Wild species *	Seed germination (%)	Grafting success (%)	Diameter (mm)	Height (cm)
P. scoparia	64 b	60 a	3.84 a	53.47 a
P. elaeagnifolia	35 c	50 b	3.51 b	32.32 b
P. eburnea	79 a	35 c	3.40 b	15.91 c

Figure 1. General view (a) and GIS picture (b) of almond wild species located in Firoozabad region of Fars Province in Iran. Cultivated almond grafted on wild almond species *P. scoparia* in the Fars Province (c).



* For each wild species (row), means with the similar letters are not significantly different at 5% level by DMRT