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Full Length Research Paper

Seed germination and seedling establishment of some wild almond species

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Wild almond species are important genetic resources for resistance to unsuitable condition, especially drought stress. They have been used traditionally as rootstocks in some areas of Iran. So far, 21 wild almond species and 7 inter species hybrids have been identified in Iran. To study seed germination and seedling establishment of some of these species, three separate experiments were designed. In the first experiment, the application of gibberellic acid (GA_3) (0, 250, 500 and 750 ppm) for 24 h was studied on germination characteristics of four wild almond accessions after stratification at 5 ± 0.5°C in Perlite media. Germination percentage, index vigor and root initiation factors were different in almond accessions, but were not affected by hormonal treatments. In the second experiment, seeds of another six wild almond accessions were stratified to compare their germination ability. Germination percentage, index vigor and root initiation were different among accessions significantly. In the last experiment, the establishment and vigor of 14 accessions from eight almond species have been evaluated in plastic bags in outdoor conditions. Two ecotypes of *Prunus* spp. had the highest stem diameter and length at all growing stages.

Keywords: *Amygdalus*, germination percentage, index vigor, root initiation, stem length, stem diameter.

INTRODUCTION

Almond is one of the oldest tree nut crops, and represents the largest production of any commercial nut products in the world (Kester and Gradziel, 1996) and in Iran (Imani and Hasani, 2005). Wild almond species grow in subtropical Mediterranean climates with mild wet winters and warm dry summers (Kester and Gradziel, 1996). Some wild species also grow in arid and semi-arid regions usually with bushy and dwarf growth habit. They are usually resistant to unsuitable conditions, especially drought stress (Imani et al., 2006). The resistant species have been traditionally used as rootstock for almond and other *Prunus* species like plum, prune, peach and almond (Zeinalabedini et al., 2002; Rahemi, 2002; Rahemi and Yadollahi, 2006). Antecedent of using almond species as rootstock in Iran (Fars province) is about 300 years ago (Madani et al., 2006). At present, more than 5000 ha of almonds on wild rootstocks are located in Fars, Kerman, Boushehr and Hormozgan provinces in Iran (Rahemi and Yadollahi, 2006).

Species *Prunus spartioides*, *Prunus spinosissima* (Gentry, 1956), *Prunus scoparia*, *Prunus elaeagnifolia* (Rahemi and Yadollahi, 2006), *Prunus eriocalda* and *Prunus horrida* have been used as rootstock for almond in rainfed conditions in Southwest of Iran. Evreinov (1952) also introduced *Prunus bucharica* as an appropriate rootstock for almond in rainfed condition. *Portea nana* L. is a thorny bush that is quiet resistant to drought and well compatible with almond cultivars as dwarf rootstock in Turkey (AK et al., 2001). *Prunus webbii* Spach. are used as dwarf rootstock for almond, nectarine

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and peach in Turkey. Seedlings of *P. webbii* have 30 to 50% less growth than *Prunus communis* and its scions cultivars showed a similar reduction in vigor (AK et al., 2001). This species are also used as rootstock for almond in Yugoslavia (Kester and Gradziel, 1996). *Prunus arabica* Oliv., as a rootstock in South East of Jordan, increased fruit production of peaches, nectarines and other stone fruits (Khalil and Al-Eisawi, 2000). Khalil and Al-Eisawi (2000) proposed that native rootstocks are adapted to local environments and tolerate prevailing harsh conditions better than any other introduced unadapted rootstocks. Kester and Gradziel (1996) mentioned the possibilities of improving rootstocks by direct selection of other almond species or hybridization with almond cultivars.

The distribution of wild almond species has been studied in different parts of Iran, but their seed germination has not been well studied. Seed germination of *Prunus* species are influenced by dormancy. The seed coat and embryo dormancy prevent seed germination. Stratification is the traditional method for breaking the embryo dormancy. It has been known that gibberellins play a significant role in breaking seed dormancy (Garcia-Gusano, et al., 2004). Gonzalez-Copeda (1975) described the hormonal nature of the dormancy mechanism in almond seeds. Grigorian (1972) and Kester et al. (1977) indicated an optimum stratification period of 8 to 10 weeks in some almond cultivars and Grasselly (1977) determined 30 to 50 days of stratification for 8 wild almond species. *P. arabica* seeds were stratified for different durations and significantly high germination percentage was obtained at 45 or 60 days, while the non stratified ones did not germinate. Soaking seeds in gibberellic acid (GA_3) with concentrations of 750, 1000 or 1500 ppm increased germination percentage significantly when combined with stratification (Khalil and Al-Eisawi, 2000). However seed germination percentage of *P. scoparia* showed no significant differences at the tested GA_3 concentrations (Rouhi et al., 2003).

Heidari et al. (2008) used stratification and scarification on two wild almond genotypes (*P. scoparia* and *P. webbii*) and concluded that mechanical removal of seed endocarp before stratification is more efficient than chemical scarification in both species.

In this work, effects of 4 gibberellins concentrations and stratification were studied on germination percentage, index vigor and root initiation factors of four and six accessions of Iranian wild almonds in two separate experiments. Also, a study was done to determine seedling establishment percentage and stem length and diameter of 14 accessions from 8 wild almond species.

MATERIALS AND METHODS

In all the experiments, fully mature half sib seeds of almond species (Table 1) were collected in August, from wild almond trees grown in their habitats in four provinces (Fars, Kerman, West Azarbaijan and Kordestan) of Iran. The exocarp and mesocarp were dried and separated from the shell. Seeds were soaked in 20% sodium chloride (Floater test) to separate empty endocarps (Baninasab and Rahemi, 2006), and then soaked in water for 48 h.

In the first experiment, nuts (with intact endocarps) of four wild almond accessions were disinfected with 10% sodium hypochlorite (5.25% active chloride) for 10 min and soaked in 4 different GA_3 solutions 0 (control), 250, 500 and 750 ppm for 24 h. In the second experiment, seeds of six wild almond accessions were disinfected as before and immersed in water for 48 h. In both experiments, complete randomized designs with 3 replicates (Ten seeds in each replication) were used. Seeds were placed in humid perlite in drained plastic bags in a cold chamber at 5 ± 0.5°C and darkness for 8 weeks.

Seed germination was observed every three days and was defined as the emergence of the radicle. The appearance of root tip was considered as germination. Germination percentage, index vigor and root initiation factors were calculated in both experiments.

Germination percentage (G%) = (number of germinated seeds / total number of seeds) × 100

Index vigor (IV) = G1 + G2 +... + Gn / D1 + D2 +… + Dn

Where, G is the number of germinated seeds in each day, D, number of days from the previous counting and n, number of days of the experiment. Root initiation time has been defined as the days needed for germination of 10% of seeds.

In the last experiment, seeds of 14 ecotypes of eight almond species (Table 1), were soaked in water for 48 h and disinfected as done earlier. The seeds were stratified in moist perlite in zip lock plastic bags in cold room (5 ± 0.5°C) in March. After stratification, twenty germinated seeds were sown in early April in 1 kg black plastic bags in outdoor condition. They were replanted in 5 kg black plastic bags filled with a 1:1:1 (V/V) mixture of fine sand, leaf compost mould and loamy soil one month later. The pots were in a nursery in Karaj (30 km north of Tehran), irrigated weekly and did not receive any fertilizer, hormones or pesticides.

The experiment was arranged in a complete randomized design with four replicates. The percentages of establishment and seedling growth parameters (stem length and diameter) were recorded during growing season. Stem diameter was recorded from 5 cm above soil surface.

The data were statistically analyzed by the Statistically Analysis System (SAS) software (SAS Institute Inc, 1990) according to a complete randomized design and means were compared using Duncan's multiple range test (DMRT). Data were subjected to appropriate statistical transformation as needed.

RESULTS AND DISCUSSION

In the first experiment, application of GA_3 did not affected germination characteristics (Table 2). Rouhi et al. (2003) did not observe any effect of GA_3 on *P. scoparia* seed germination at 7°C. They believed that endogenous GA_3 levels increased in seeds exposed to low temperature, therefore application of exogenous GA_3 had no clear effects. However, Khalil and Al-Eisawi (2000) applied GA_3 (750 ppm) successfully for increasing *P. arabica* seed germination.

Seed germination percentage, index vigor and root initiation time were significantly different among accessions at $p < 0.01$ (Tables 2 and 3). Germination percentage and index vigor were higher in *Prunus eburnea*

Table 1. List of wild almond species used in the experiments*, their accession numbers and place of origin.

Experiment	Numbers	Accession number	Scientific name	Province of origin	Area
First	1	42	*P. eburnea*	Kordestan	Kamyaran
	2	64	*P. elaegnifolia*	Fars	Darab
	3	65	*P. scoparia*	Fars	Darab
	4	96	*P. elaegnifolia*	Fars	Firoozabad
Second	1	24	*P. korshinski*	West Azarbaijan	Urmieh
	2	31	*P. brauhica*	West Azarbaijan	Mohabad
	3	53	*P. fenzliana*	West Azarbaijan	Makoo
	4	58	*P. eburnea*	Kerman	Sirjan
	5	60	*P. elaegnifolia*	Kerman	Sirjan
	6	97	*P. spartioides*	Fars	Firoozabad
Third	1	53	*P. fenzliana*	West Azarbaidjan	Makoo
	2	54	*P.* spp	Fars	Shiraz
	3	55	*P. lycioides*	Fars	Niriz
	4	56	*P. lycioides (horrida)*	Fars	Darab
	5	57	*P. scoparia*	Kerman	Orzoeieh
	6	58	*P. eburnea*	Kerman	Sirjan
	7	59	*P.* spp	Fars	Shiraz
	8	60	*P. elaeagnifolia*	Kerman	Sirjan
	9	61	*P. elaeagnifolia*	Kerman	Sirjan
	10	62	*P. haussknechtii*	Kordestan	Marivan
	11	63	*P. haussknechtii*	Kordestan	Marivan
	12	64	*P. elaeagnifolia*	Fars	Darab
	13	65	*P. scoparia*	Fars	Darab
	14	66	*P. dulcis*	West Azarbaidjan	Soufian

*Due to limited number of collected seeds, the experiment was not run on all accessions.

Table 2. Analysis of variance of germination percentage, index vigor and root initiation time of almond accessions in the first and second experiments.

Experiment	Variables	DF	Mean Squares		
			G (%)	IV	RIT
First	Accessions	3	3275.0**	0.84**	650.3**
	GA_3	3	247.2	0.15	148.7
	Error	41	327.4	0.07	75.8
	cv		66.8	72.1	32.9
Second	Accessions	5	756.6**	0.14**	203.4*
	Error	12	105.5	0.01	51.6
	cv		56.0	60.1	21

GA_3, Gibberellic acid; DF, degree of freedom; G%, germination percentage; IV, index vigor; RIT, root initiation time; cv, coefficient of variance; ** means are significantly different at $p < 0.01$; * means are significantly different at $p < 0.05$.

Table 3. Mean comparison (Duncan's multiple range test) of germination percentage, index vigor and root initiation time of wild almond accessions in the first and second experiments.

Experiment	Accessions number	Species	G (%)	IV (seed/day)	RIT (day)
First	42	*P. eburnea*	35.8 a	0.66 a	16.9 c
	64	*P. elaegnifolia*	10.8 b	0.16 b	27.4 b
	65	*P. scoparia*	15.8 b	0.15 b	36.7 a
	96	*P. elaegnifolia*	45.8 a	0.54 a	27.0 b
Second	24	*P. korshinski*	36.7 a	0.47 a	26 bc
	31	*P. brahuica*	6.7 b	0.09 b	34 abc
	53	*P. fenzeliana*	13.3 b	0.13 b	40 abc
	58	*P. eburnea*	3.3 b	0.007 b	51 a
	60	*P. elaegnifolia*	40.0 a	0.50 a	24 c
	97	*P. spartioides*	10.0 b	0.09 b	42 ab

G %, Germination percentage; IV, index vigor; RIT, root initiation time. Different letter in the column are significantly different by Duncan's Multiple Range Test.

and *P. elaegnifolia* (accessions no. 42 and 96, respectively) than other accessions. Different accessions of one species showed variable germination percentage. Germination percentage of *P. elaegnifolia* varied from 10.8% in accession 64 to 45% in accession 96.

Khalil and Al-Eisawi (2000) stated that germination percentage of stratified *P. arabica* seeds increased steadily with increasing stratification period. However, germination percentages of 45 days stratified seeds were not significantly different from those of 60 and 30 days. They also observed 15% germination in *P. arabica* seeds stratified for 30 days, which is close to germination percentage of *P. scoparia* in this research.

Root initiation time was significantly different among accessions. It was 36 days for *P. scoparia* (no. 65) and 27 days for *P. elaegnifolia* (both accessions no. 64 and 96) and 16 days for *P. eburnea* (no. 42). Therefore, root emergence was faster in *P. eburnea* (no. 42) than other accessions.

In the second experiment (Table 2), the species were significantly different in their germination percentage, index vigor ($p < 0.01$) and root initiation time ($p < 0.05$). Germinations percentage and index vigor of *Prunus korshinski* and *P. elaegnifolia* (no. 24 and 60) were significantly higher (Table 3) than other accessions. Root initiation time changed between 24 to 51 days.

The results of the two experiments showed that, different accessions of a species had different seed germination percentage. It changed from 3.3% in *P. eburnea* (no. 58) to 35% in accession no. 42. Different germination percentage of accessions could be due to different environmental conditions (temperature, humidity and altitude) they are exposed to during formation and development of the embryo (Madani et al., 2006).

Seed germination percentage in the studied wild almond species collected from Iran was very different (from 3 to 45%), and this can be affected by seed characteristics and quality. The low germination percentage can be due to hard shell which can induce seed coat dormancy. Evidences showed that stratification and gibberelline application could not overcome dormancy completely (REF). Scarification can be another possibility which can increase seed germination. Stony endocarps exist in all members of *Prunus* species and seeds often have seed coat dormancy. Endocarp may also offer some resistance to germination and removal of the endocarp may hasten or increase germination in stone species (Garcia-Gusano et al., 2004; Heidari et al., 2008).

In the third experiment, *Prunus haussknechtii* (no. 62) and *P. scoparia* (no. 65) showed the most and the least seedling establishment (Table 4). *P. scoparia* (no. 65) died after one month of planting, maybe because of sensitivity to soil burn disease. *Prunus dulcis* (no. 66), *Prunus* spp. (no. 54), *P. haussknechtii* (no. 63) and *Prunus fenzliana* (no. 53), had the highest establishment percentage and were not statistically different from *P. haussknechtii* (no. 62). Different accessions of the same species from different provinces had different establishment percentage.

Prunus spp. (no. 59 and 54), *P. dulcis* (no. 66) and *P. fenzliana* (no. 53) produced the tallest seedlings (Figure 1), while *P. elaeagnifolia* (no. 64) had the shortest one (14.4 cm). These results are in agreement with that of Khatamsaz (1992) who showed different sizes of wild almond species. Wild species with fast seedling growth are required in the nursery for rootstock production (Baninasab and Rahemi, 2006) and short species can be used as dwarf rootstock for almond cultivation. The seedlings of some almond species can made 30-50% less growth than those of *P. communis*, therefore almond cultivars grafted on them showed a similar reduction in

Table 4. Establishment percentage of wild almond accessions in the nursery.

Accession no.	Species	Establishment (%)
53	*P. fenzliana*	55 abcd*
54	*P.* spp	70 abc
55	*P. lycioides*	50 bcd
56	*P. lycioides (horrida)*	40 cde
57	*P. scoparia*	10 ef
58	*P. eburnea*	40 cde
59	*Prunus* spp.	40 cde
60	*P. elaeagnifolia*	40 cde
61	*P. elaeagnifolia*	40 cde
62	*P. haussknechtii*	90 a
63	*P. haussknechtii*	70 abc
64	*P. elaeagnifolia*	20 def
65	*P. scoparia*	0 f
66	*P. dulcis*	85 ab

*Different letter in the column are significantly different at 5% by Duncan's multiple range test.

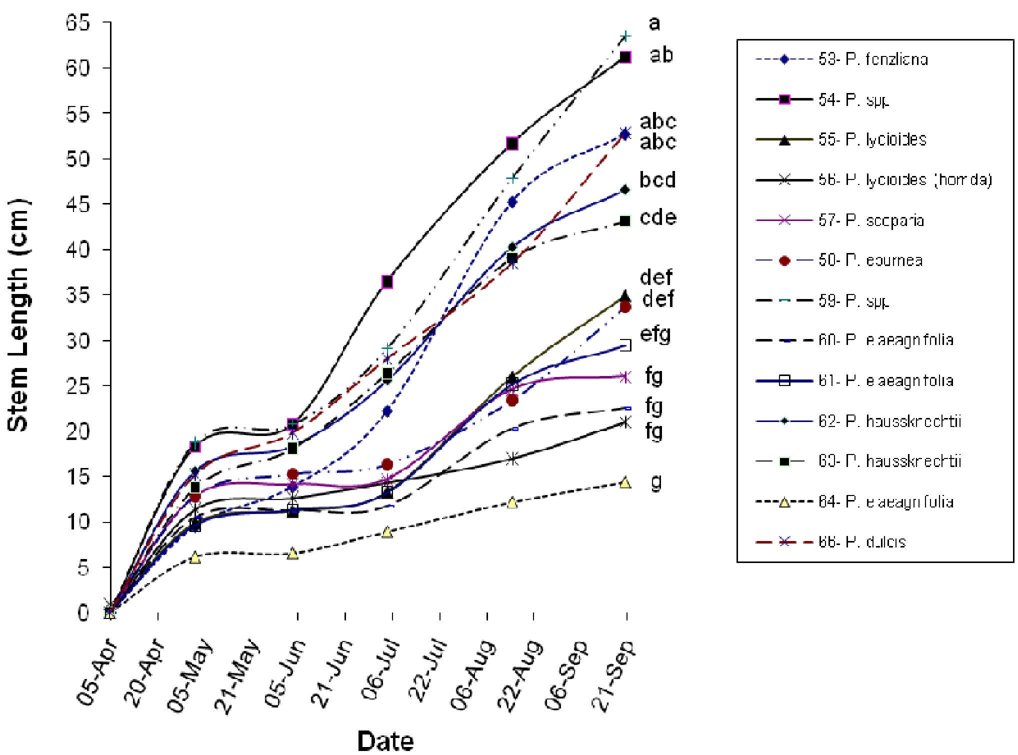

Figure 1. Seedling growth of wild almond accessions during the first growing season. Means were separated at 5% by Duncan's multiple range test.

vigor (AK et al., 2001). Evaluation of three wild species of almond (*P. scoparia*, *P. webbii* and *P. orientalis*) in Iran showed a significant difference between species. *P. scoparia* had highest (20.58 cm) and *P. webbii* lowest stem length (14.88 cm) four months after sowing (Baninasab and Rahemi, 2006). The study on characteristics of stem length in three wild almonds (*P. scoparia*, *P. elaeagnifolia* and *P. eburnea*) showed that *P. scoparia* and *P. eburnea* had the highest and lowest stem length, respectively (Madani et al., 2006).

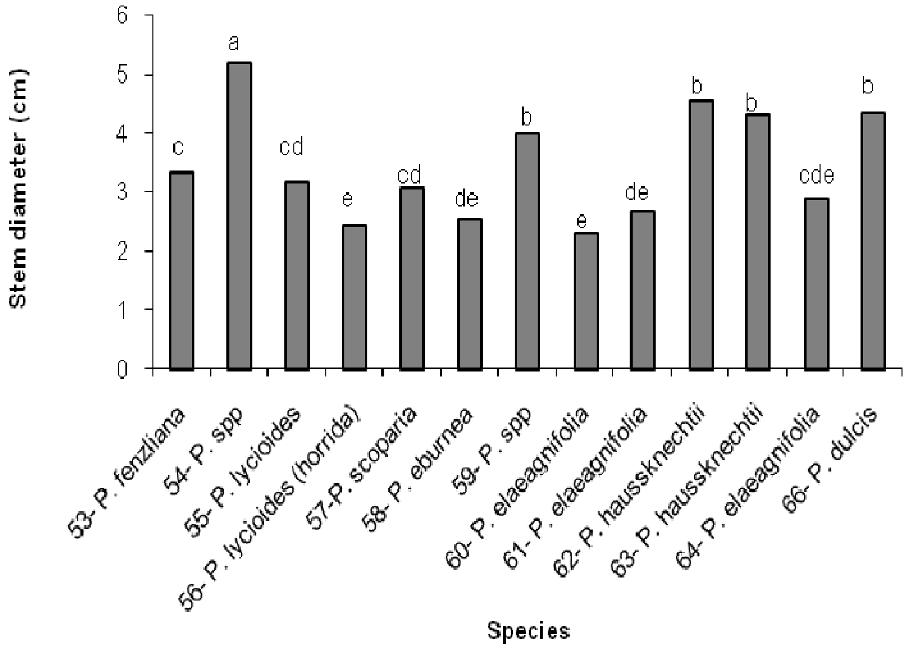

Figure 2. Stem diameter of almond accessions at 5 cm over the soil surface. Means separation at 5% level by Duncan's multiple range test; means with the same letter are not significantly different.

Prunus spp. (no. 59 and 54) had the highest growth rate (stem length growth/time, data not presented), specially at the first month of germination, while the dwarf ecotypes like *P. elaeagnifolia* (no. 64) and *P. lycioides* (horrida, no. 56) had gradual growth during growing season. *Prunus* spp. (no. 54) had the highest stem diameter, while ecotypes *Prunus lycioides* (horrida), (no. 56) and *P. elaeagnifolia* (no. 60) had the thinnest stem among other ecotypes (Figure 2). Growth capacity can be determined by stem diameter. Higher stem diameter allow early budding and transplanting in the nursery (Baninasab and Rahemi, 2006). The seedlings of wild species used in the last experiment have been planted in the research orchard of university of Tehran for more survey.

Conclusions

Germination percentage and seedling establishment of different accessions from a species, which was collected from different locations, were very different, maybe due to the environmental condition they undergo during growth and development. Wild species had different germination percentage ranging from 3 to 45% in *P. eburnea* and from 10.8 to 45.8% in *P. elaegnifolia*.

P. hauskonetchii, P. dulcis and *Prunus* spp. had higher vegetative growth and establishment percentage than other species. They can be fast growing candidates for use as a rootstock.

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