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Selecting hazelnut (*Corylus avellana* L.) rootstocks for different climatic conditions of Iran

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ABSTRACT

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Hazelnut is grown in limited areas of Iran with high rainfall and high relative humidity. New cultivars and rootstocks that are tolerant of drought and low humidity are needed to expand hazelnut cultivation. To this end, seeds of 14 local *Corylus avellana* L. genotypes (A1, A2, Gerche, Gerde Eshkevarat, Gerde Shok, Mahalli Karaj, Nakhon Rood, Navan1, Navan7, Navan9, Navan10, Pashmineh, Shirvani and Sivri) with relative tolerance to environmental stresses were collected in different areas and sown with seedlings of controls Negret and Daviana. Seed germination, growth characteristics, graft success and chlorophylls of all rootstocks were evaluated. Based on the results, first-year seedlings of six genotypes with high germination and growth vigor were selected and transplanted for field evaluation. Results of the first year showed that seeds of Greche had the highest (69%) germination, and that genotypes Nakhon Rood and Sivri had the longest stems (18.22 and 12.44 cm, respectively), while Pashmineh and Nakhon Rood seedlings had the thickest shoot diameter (2.45 and 2.42 mm, respectively). In the second year, Mahalli Karaj and Nakhon Rood seedlings showed the greatest shoot length and diameter. The highest A and B chlorophyll levels were detected in Nakhon Rood seedlings (3.44 and 1.39 mg/per gram of leaf fresh weight, respectively). Grafts on Shirvani and Sivri rootstocks were the most successful, with 42.18 and 41.07%, respectively. Graft success was not different among different scions.

Keywords: chlorophyll, growth vigor, hazelnut, rootstock, shoot length

INTRODUCTION

Iran with an annual production of 14,299 tons, is the seventh hazelnut producer in the world. It also has a mean hazelnut yield of 698.7 kg ha^{-1}, which is lower than the mean yield at the global level (1271.3 kg ha^{-1}) (Soleimani *et al.*, 2011). Hazelnut growing areas of Iran are limited to Guilan, Aredbil, Mazandaran, Golestan and Qazvin Provinces, which are on the shores of the Caspian Sea (Hoseinova *et al.*, 2006) and have high rainfall and high relative humidity (RH). The genetic structure of the hazelnut population was formed by natural selection and is a valuable source of genetic diversity (Pop *et al.*, 2010). High relative humidity is necessary for successful hazelnut production. Low relative humidity in arid and semi-arid areas with high temperatures, frequent droughts and fairly low rainfall is considered unsuitable for hazelnut production. These conditions lead to leaf burn of hazelnut trees (Miletic *et al.*, 2009).

Hazelnut prefers subtropical climates with high relative humidity (75-80%) and with a cumulative temperature of 3800-4250 ºC. It grows better in areas where the annual average temperature is 13-14 ºC, the average temperature in the coldest month is not more than 3.5-5.5 ºC, the average temperature in the warmest month reaches 22-23 ºC, and annual rainfall is 1500-2000 mm (900-1200 mm during the growth period) (Mirotadze *et al.*, 2009). Baldwin (2009) reported that drought, particularly during kernel development, resulted in a higher proportion of poorly filled or shriveled kernels and that cultivars' response to drought differed. He also found that cultivar Enis was highly susceptible to drought, with 15-23% of poorly filled or shriveled kernels as compared with cultivars Barcelona and Tbc, which had 10-15% and 8-12% of poorly filled or shriveled kernels, respectively.

Leaf buds of the hazelnut require as much chilling as apple buds, but flower buds have lower chilling requirements. Thus, cold winters with 1500 hours of chilling and only a few degrees below freezing are suitable for hazelnuts (Aziz *et al.*, 2007).

Germination of hazelnut (*Corylus colurna* L.) seeds takes a long time under natural conditions. Rapid and uniform germination is desirable. Aygun *et al.* (2009) used different treatments for germinating hazelnut seeds. In the control treatment, seeds were planted directly in the field. Other treatments included acid scarification for 2 hours, shell splitting, and stratification in moist peat at 4 ºC for 100, 110 and 120 days. Results showed acid scarification, shell splitting, and 100 and 110 days of stratification did not improve germination. Seeds sown in the field or exposed to 120 days of stratification had 64% and 14.2% germination, respectively. All acid stratification treatments resulted in higher germination than the control, and with the 75 ppm GA_3 treatment, 100% of seed germinated (Aygun *et al.,* 2009).

The growth vigor of hazelnut seedlings is different in different varieties (Blagoeva and Nikolova, 2010; Miletic *et al.,* 2009; Ninic-Todorovic *et al.,* 2009; Tous *et al.,* 2009). Ninic-Todorovic *et al*. (2009) conducted a study on six Turkish hazelnut genotypes and showed that under the same conditions, seedlings of some hazelnut genotypes grew rapidly and could be used as rootstocks for grafting in the first year (Ninic-Todorovic *et al.,* 2009). Recently, Miletic *et al.* (2009) found that *C. colurna* is suitable as a rootstock. Grafting on this rootstock has some advantages over conventional breeding. The main advantages of using this rootstock are absence of suckering (which reduces maintenance costs), improved pest and disease resistance, higher drought tolerance and better fruit and kernel quality (Blagoeva and Nikolova, 2010; Miletic *et al.,* 2009). However, local hazelnut populations in Iran originated from the European hazelnut (*C. avellana* L.) and are adapted to the country's climatic conditions (Pop *et al.,* 2010).

During the last decade, Iran's horticultural community has become more interested in collecting and using local hazelnut germplasm, and a hazelnut breeding program was recently initiated. In this study, we evaluated and selected local hazelnut genotypes as suitable rootstocks in Karaj, Iran, which has lower rainfall and lower relative humidity.

MATERIALS AND METHODS

This study was carried out in 2007-2012 at the Seed and Plant Improvement Institute, Karaj, Iran. In the first phase, 14 superior hazelnut (*C. avellana*) genotypes (A_1, A_2, Gerche, Gerde Eshkevarat, Gerde Shok, Mahalli Karaj, Nakhon Rood, $Navan_1$, $Navan_7$, $Navan_9$, $Navan_{10}$, Pashmineh, Shirvani and Sivri) were selected from hazelnut growing areas including Alamot, Qazvin Province; Talesh and Eshkevarat, Guilan Province; and Fandoghloo in Ardebil and Alborz Provinces). Seeds of the selected genotypes along with fruits of cultivars Daviana and Negret (as controls) were collected after fruit ripening and soaked in water for 24 hours. For stratification, seeds were placed in a moist substrate of 50% sand and 50% soil for three months. The seeds were then potted using a randomized complete block design with three replications and 100 seeds in each experimental unit. Potted seed were kept in the greenhouse for germination (two months) and germination of each genotype was evaluated and recorded.

By the end of the growing season, growth factors including seedling height, growth of main and secondary shoots, rootstock diameter, mean of internode length, leaf size (leaf length and leaf width) and petiole length were recorded for all seedlings. Genotypes exhibiting high vigor were selected and transferred to the field. At this stage, seedlings of only seven genotypes (Gerche, Negret,

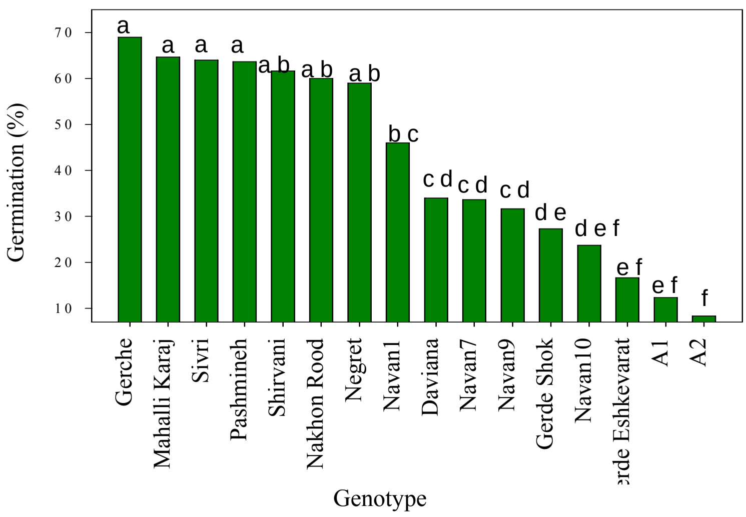

Fig. 1. Seed germination (%) of different hazelnut genotypes. Bars with at least one letter in common are not significantly different at the 1% probability level using Duncan's Multiple Range Test.

Table 1. Analysis of variance for growth characteristics of different hazelnut genotypes in the first year un

Source of variation	df	Mean squares				
		Seedling length	Main shoot growth	Secondary shoot growth	Rootstock diameter	Internode length
Rootstock	13	99.56 **	29.13 **	9.94 **	10.85 **	0.029**
Replication	2	60.20 ns	13.62 ns	22.28**	2.22 ns	0.017 ns
Error	26	22.90	9.35	2.03	1.92	0.008
% CV		10.89	13.58	20.22	18.59	4.43

****: significant at the 1% level of probability.**
ns: Not significant.

Table 2. Mean comparison of growth characteristics in different hazelnut genotypes in first year of growth unde

Genotype	Seedling height (cm)	Main shoot growth (cm)	Secondary shoot growth (cm)	Rootstock diameter (mm)	Internode length (cm)	Leaf (c
Nakhon Rood	30.2 a	18.2 a	11.2 a	10.5 ab	2.2 a	12
Sivri	21.9 b	12.4 b	7.4 cd	6.5 cdef	2.1 ab	13
Gerche	19.2 bc	9.6 de	5.1 de	7.5 cdef	1.8 de	12
$Navan_7$	18.6 c	10.5 de	7.3 cd	5.7 def	1.9 cde	10
Negret	17.2 cd	10.4 de	7.4 cd	8.7 bc	1.9 cde	9
Shirvani	16.8 cd	9.0 de	5.2 de	6.6 cdef	2.2 a	9
Pashmineh	15.5 de	11.7 bc	9.3 ab	11.3 a	2 abc	12
Mahalli Karaj	14.6 de	11 cd	7.7 cd	7.9 cde	2.1 ab	12
$Navan_{10}$	12.5 ef	9.8 de	6.9 cd	8.8 bc	1.9 cde	11
Gerde Shok	12.5 ef	10.2 de	8.2 bc	6.2 cdef	2 abc	12
Gerde Eshkevarat	11.5 fg	6.8 fg	6.9 cd	8.3 bcd	2 abc	10
Daviana	11.1 fg	7.9 ef	5.9 de	5.5 ef	2.1 ab	11
A_2	9.0 g	7.1 fg	6.0 de	5.1 f	1.9 cde	10
A_1	8.5 g	5.1 g	4.0 e	5.7 def	1.7 e	8

Means in each column followed by at least one letter in common are not significantly different at the 1% probability level using Dunc

Table 3. Analysis of variance for growth characteristics of different hazelnut genotypes in the second y

Source of variance	df	Mean squares				
		Seedling length	Main shoot growth	Secondary shoot growth	Rootstock diameter	Internode length
Rootstock	6	573.78 **	112.13 **	61.6 **	0.140 **	0.041 **
Replication	2	31.11 ns	35.67 ns	14.5 ns	0.006 ns	0.026 ns
Error	12	56.79	20.35	9.2	0.008	0.004
CV (%)		11.92	7.67	8.13	4.010	2.690

****: significant at the 1% level of probability.**
ns: Not significant.

Table 4. Mean comparison of growth characteristics, graft success and leaf scorch severity in different hazelnut genotyp

Genotype	Seedling height (cm)	Main shoot growth (cm)	Secondary shoot growth (cm)	Rootstock diameter (cm)	Internode length (cm)
Mahalli Karaj	82.4 a	36.6 a	28.5 a	2.4 a	2.4 a
Pashmineh	74.5 ab	32.9 a	24.2 bc	2.2 bc	2.3 b
Nakhon Rood	68.9 ab	36.2 a	25.1 b	2.4 a	2.2 bc
Shirvani	67.5 b	33 a	21.7 cd	2.3 ab	2.2 bc
Sivri	53.1 c	28.1 b	20.9 cd	2.3 ab	2.2 bc
Gerche	52.7 c	26.8 b	19.3 d	2.0 c	2.1 c
Negret	43.5 c	19.4 c	14.4 e	1.8 d	2.0 d

Means in each column followed by at least one letter in common are not significantly different at the 1% probability level-using Duncan's

Sivri, Mahalli Karaj, Nakhon Rood, Pashmineh and Shirvani) that showed high germination and high growth vigor were transplanted to the field using a randomized complete block design with three replications.

In July, scions of three hazelnut cultivars (Fertile, Segrob and Rond) were grafted onto two-year-old rootstocks at a height of about 20 cm by the chip budding method. Growth characteristics of each seedling were recorded before grafting. Also, six levels of leaf scorch on rootstocks and scions were recorded: no scorch, and very low, low, medium, high and very high scorch. The amounts of chlorophylls A and B in the rootstocks and scions were also recorded by the spectrophotometry method (mg/g of leaf). Chlorophyll content (SPAD value) was measured by the chlorophyll meter model (Minolta Co., Osko, Japan, SPAD 502). At the end of the growing season, graft success of each combination was evaluated.

RESULTS AND DISCUSSION

Seed germination

Seed germination (%) was significantly different ($P \leq 0.01$) among genotypes. Genotypes Gerche, Mahalli Karaj, Siviri and Pashmineh had 69%, 64.7%, 64% and 63.7% seed germination, while A_2 had the lowest, with only 8.3% (Fig. 1). Aygun *et al.* (2009) showed that seed germination of Turkish hazelnut varied with genotype.

Greenhouse growth

There was a significant difference ($P \leq 0.01$) between seedlings of hazelnut genotypes for seedling length, main and secondary shoot growth, rootstock diameter, leaf length, leaf width and petiole length under greenhouse conditions (Tables 1 and 2).

Genotype Nakhon Rood showed the highest seedling length and the greatest main and secondary shoot growth (30.2, 18.2 and 11.2 cm, respectively). A_1 genotypes had the lowest seedling length with 8.5 cm. A_1 seedlings also had the lowest main shoot length (5.1 cm) and secondary shoot growth (4 cm). Nakhon Rood and Shirvani seedlings had the highest internode length (2.2 cm). Seedlings of genotype Pashmineh with 11.3 mm rootstock diameter had the thickest diameter, followed by Nakhon Rood with 10.5 mm. A_2 genotypes had the lowest rootstock diameter (5.1 mm). Seedlings of Nakhon Rood, Sivri, Gerche, Pashmineh, Mahalli Karaj and Gerde Shok had the largest leaf size (length and width). Seedlings of Negret showed moderate vegetative growth, while Daviana showed poor growth (Table 2). Tous *et al.*

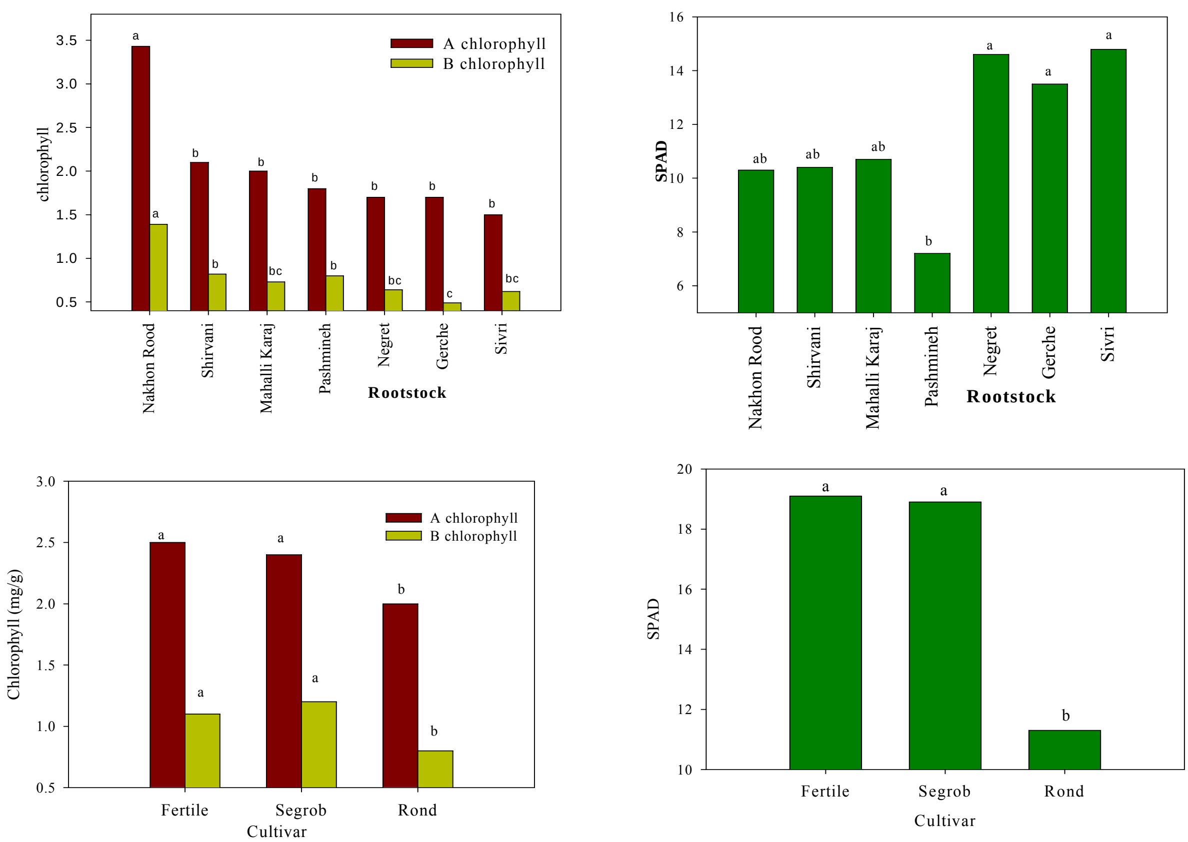

Fig. 2. Chlorophylls a and b and SPAD values of hazelnut cultivars and rootstocks. Bars with similar letters are not significantly different at the 1% probability level using Duncan's Multiple Range Test.

(2009) found that seedlings of cultivar Daviana had moderate growth and could be a suitable rootstock for grafting cultivar Negret. In our study, seedlings of Daviana showed poor germination and vegetative growth; therefore this genotype was eliminated from the second trial.

Chlorophyll

Chlorophyll a and b contents and SPAD values were significantly different ($P \leq 0.01$) in rootstocks and cultivars. Genotype Nakhon Rood had the highest amount of chlorophylls a and b (3.44 and 1.39 mg/g, respectively). Sivri, Negret and Gerche, with means of 14.8, 14.62 and 13.48, respectively, had higher SPAD values (Fig. 2). Fertile and Segrob had 2.45 and 2.44 mg/g of chlorophyll a, 1.15 and 1.07 mg/g of chlorophyll b, and 19.12 and 18.78 SPAD values, respectively (Fig. 2).

Field growth and graft success

Results of growth characteristics of seedlings in the second year in the field showed a significant difference ($P \leq 0.01$) among genotypes for seedling length, main and secondary shoot growth, rootstock diameter and internode length; however, leaf size (length and width) and petiole length were not different (Table 3).

Genotype Mahalli Karaj showed the highest seedling length, secondary shoot growth and internode length, with means of 82.4, 28.5 and 2.4 cm, respectively. Seedlings of genotypes Mahalli Karaj, Nakhon Rood, Shirvani and Pashmineh had higher main shoot growth, with means of 36.6, 36.2, 33 and 32.9 cm, respectively. Genotypes Mahalli Karaj and Nakhon Rood also had the thickest rootstock diameter (2.4 cm). Seedlings of Negret (the control) had low growth vigor for most evaluated characteristics (Table 4) and would not be a suitable rootstock for grafting hazelnut. On the other hand, seedlings of native genotypes are adapted to climatic conditions. Ninic-Todorovic *et al*. (2009) found that growth indicators of one-year-old seedlings of Turkish hazelnut (*C. colurna*) differed significantly in different genotypes; when adequate irrigation and nutrients were applied, seedlings of some genotypes were suitable for use as a rootstock for grafting in the first year (Ninic-Todorovic *et al*., 2009). Results of our study are in agreement with the findings of Ninic-Todorovic *et al*. (2009). However, we could not graft on seedlings of any genotype in the first year.

Differences in the growth indices of different genotypes over two years showed that growth characteristics of seedlings were strongly dependent on genotype. Miletic and *et al.* (2009) showed that in warm regions with frequent drought and low rainfall, grafting hazelnut genotypes onto rootstocks with vigorous root systems resulted in larger fruit and kernel size, as well as increased weight.

Graft success was significantly different $P \leq 0.01$) among rootstocks (but not among scions of cultivars Fertile, Segrob and Rond) and the reciprocal effects of cultivars and rootstocks was not statistically different. Higher graft success of Fertile, Segrob and Rond scions was observed on Shirvani, Siviri, Gerche and Pashmineh rootstocks, with means of 42.2%, 41.1%, 36.7% and 35.7%, respectively. The lowest graft success (16%) was shown by cultivar Negret (Table 4). Cerovich *et al*. (2009) showed that graft success of different cultivars on *C. colurna* rootstocks differed by 55-98.7%. Ninic-Todorovic *et al*. (2009) also reported that graft success on different rootstocks ranged from 82% to 92.79%. The mean graft success in our trial was lower than that reported by other researchers, likely due to the higher temperature and lower relative humidity in Karaj.

Cluster analysis using data for all characteristics

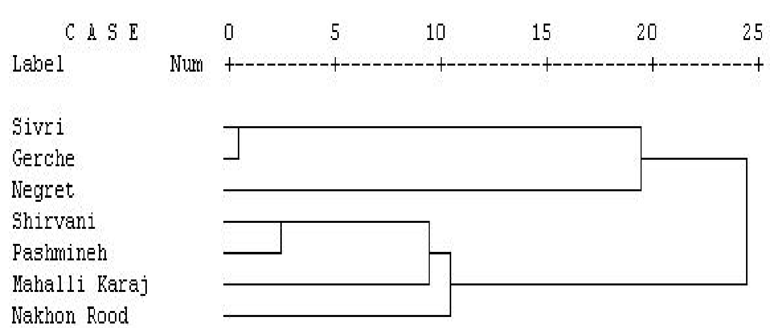

Fig. 3. Dendogram of hazelnut rootstocks based on all characteristics using the UPGMA method.

of seedlings of different genotypes showed that Sivri and Gerche had high similarity and formed one group. Shirvani and Pashmineh were also were similar and, together with genotypes Mahalli Karaj and Nakhon Rood, formed another group.

Negret, an exotic cultivar, had no similarity with native genotypes and stood alone in one group (Fig. 3). Results showed that due to their good germination (%), high growth vigor, leaf scorch tolerance and high graft success, genotypes Mahalli Karaj, Pashmineh, Nakhon Rood and Shirvani are suitable for use as seedling rootstocks in hazelnut growing areas with climatic conditions similar to those of Karaj.

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