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Provenance Variation and Seed Zone Delimitation of *Acer velutinum* (Boiss.) Seedlings in the Hyrcanian Forest

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ABSTRACT

Persian Maple, *Acer velutinum,* has a wide distribution in Hyrcanian forests. In this investigation, a provenance test (two years) was conducted with the open-pollinated seeds of seven populations collected from different altitudinal gradients. In the first year, all populations' seedling height and diameter/height ratio decreased significantly. However, no significant difference in collar diameter was detected in the populations. Seedlings from the populations of the lower altitudes (<700m) tended to grow taller compared to those from the higher altitudes (>1200m). The productivity in the first year indicated that some populations originating from the middle altitudes had higher production. In contrast, during the first growing season, there were no significant differences in survival rate among the various origins. In the second year, the seedlings originated from the middle altitudes as well as the local province (cite the elevation) had the highest survival rate. To minimize the mortality rate, we categorized four altitudinal groups and recommended restoring the genetic type of coverage of the original forest, using the seed from the same zone, because seeds from other zones have increased mortality.

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Introduction

Susceptibility to cold is the most important factor, limiting both crop quality and productivity. Frost damage is more crucial than other physiological damages caused by insect defoliation, grazing etc. In addition, frost restricts geographic distribution of temperate species (Muffler *et al.*, 2016) and may lead to injury, reduced growth and death of seedlings (saplings) (Lenz *et al.*, 2013; Montwé *et al.*, 2018; Marcante *et al.*, 2012) as well as mature individuals in forest species (Duffield, 1956). Plant cells contain a lot of water during frost periods, so that its conversion to ice may lead to the destruction of the cell components. The amount of water in the stems differs according to the phonological stage of the plant. Moreover, frost damage depends on the phonological stage (Vitasse *et al.*, 2014; Kollas *et al.*, 2014). Successful over-wintering depends mainly on two factors: seedling stress resistance and environment. The frost hardiness of different tissues and their resistance to winter-drying are the factors that, in over-wintering, influence the survival of tissues (i.e. foliage, buds, stem cambium and roots), (Gusta *et al.*, 2013; Hamilton *et al.*, 2016; Malmqvist *et al.*, 2018; Luoranen *et al.*, 2022). Knowledge about the hardiness to fall and spring seasons of the genetic stock is critical to the success of a tree improvement program, *i.e*., to know where the seed originates from affects the selection of the place for planting in frost zones. The Persian Maple (*Acer velutinum* Boiss.) is one of the tallest maple trees with broad leaves, growing up to 25m tall. It can be propagated and spread easily via its seeds. It has broad distribution in the Caucasus and in the mountains of Talysh (Gelderen *et al.*, 1994). The Hyrcanian forests extend from the flat littoral along the sea to 2200 m above sea level in the Alborz Mountain Range (Yosefzadeh *et al.*, 2009). This region is listed as a ecoregion (WWF 2000) and comprises 15% of the Iranian forest and 1.1% of the country's land area (Khosroshahi and Ghavvami, 2006). A main feature of this deciduous broadleaved forest is the presence of many Arcto-tertiary relict elements such as *Parrotia persica*, *Geldishia caspica*, *Alnus subcordata*, *Quercus castaneifolia*, *Petrocaria fraxinifolia* (Zohary,1973; Leestmans, 2005; Yosefzadeh *et al.*, 2010) and widespread presence of Fagus orientalis (32.7 percent of volume of Hyrcanian forest). *Acer velutinum* usually appears as individual or occasionally as a group in these forests (Tabari *et al.* 2008; Sabeti 1993). *Acer velutinum* is an important forest tree species in the North of Iran used extensively in planting and restoration in deforested areas. Since this species is sensitive to the frost phenomena, it is subject to massive rates of mortality during planting and in nurseries. Damage is especially serious in *Acer velutinum* seedlings, when they are at the stage of shoot elongation.

Gholizadeh *et al.*, 2020 showed that altitudes and mean annual temperature are the most important environmental variables affecting the compositions and distribution of the Hyrcanian forest vegetation types. Furthermore, Morgenstern (2011) noted that the species that grow in a wide range of environmental conditions are different at the population level in both cold hardiness and growth traits. Since temperature decreases with increasing the altitude in temperate forests, plants originating from lower elevation are often more frost-resistant compared to the plants in higher altitudes (Benowicz *et al.*, 2003). Therefore, to reduce the potential risks, restricting the plant translocation to certain regions or "seed zones" is a useful approach (Vander Mijnsbrugge *et al.*, 2010).

A long distance transfer of seed may cause undesirable consequences such as cold injury and growth loss or mortality (Ying and Yanchuk, 2006). Seed zones represent an area in which natural genetic exchange occurs, so that within the zones, transfer of plant material should have no negative impact. In many countries, seed zone is defined for restoration. For example, In Flanders, four main seed zones are delineated for woody species based on the distribution ranges of different plant species, climate and soil (Vander Mijnsbrugge *et al.*, 2004). Also, In the UK, 24 local seed zones are defined based on major geological and climatic regions modified by altitude (Herbert *et al.*, 1999). Therefore, it is important to provide information about the frost hardiness and delineate seed transfer zones to support nursery management and planting. In this study, we (1) measure and assess the relative hardiness to cold in 2-year-old seedlings of seven provenances of the Hyrcanian forest in relation to the altitudinal source, (2) identify the superior provenance in terms of growth, form and frost tolerance, (3) delineate seed zones of suitability, and (4) develop seed transfer guidelines for matching the genotypes with their appropriate planting sites.

Material and Methods

Nursery experiment

The experiment was carried out at the Orimelk nursery located in Sangedeh Area, Mazandaran Province, North of Iran. The annual rainfall of the area is about 860 mm and its altitude is 1550 meter above the sea level. In this research, seeds were collected from different geographical populations of *Acer velutinum* in the Hyrcanian forests (Table 1). The characteristics of the collection sites of various sources of *A. velutinum* seed are given in Table 1.

Table 1. Locations of seven *Acer velutinum* populations sampled along an altitudinal transect in the west of Mazandaran Province.

Provenance	Latitude	Longitude	Altitude	Mean Temerature(°c)	Mean Precipitation
Park Nour	36º30'52"	52º3'11"	20	16.4	1097
Ladjim	36º19 '33"	52º 3'22"	400	14	1600
Jourband	36º27' 8"	52º8'2"	700	13	1450
Lamzer	36º22' 2"	52º3'35"	1000	11	1300
Shahnazar	36º22'26"	52º3'45"	1200	9	1500
Sangedeh (Test site)	36º3'36"	53º 5' 5"	1550	9	821
Ashack	36º8'36"	53º 5' 25"	2200	6.5	937

Seeds were gathered from the middle part of the crown (Merwin *et al.*, 1995) from 10 phenotypically superior trees, depending on the availability of the ideotypes (healthy individuals with a straight-bole lower branch number and a smaller crown), (Cornelius *et al.*, 1995), at a distance of at least 50 m from each other so as to avoid the potential effects of family relationship. The seeds were sown in a mountain nursery (Orimelk, 1550 m above sea level) in 1 m^2 plots in a randomized complete-block design with 3 blocks. In each plot, 50 seeds were sown. The distance between the plots was 50 cm, while the blocks were separated by 100 cm. After one year of seed germination, the height and collar diameter of the seedlings were measured. Survival, seedling production efficiency, performance, and annual mortality rate for two growth seasons were calculated as follows:

Survival = (number of residual seedling / number of seed germinated) × 100

Seedling production efficiency = (number of residual seedling/ number of seed sown) × 100

Performance = height × survival (Lindgren and Ying, 2000).

Annual mortality rate $= \frac{n1 - n2}{n1} \times 100$;

Where: n1 and n2 are the number of residual seedlings at the end of the first and second year of growth, respectively. We applied the Sáenz-Romero *et al.* (2006) method to determine the seed zone with a little difference: performance was substituted with height for determining the seed zone.

Data Analysis

The means of survival, height, collar diameter, and seedling production efficiency were subjected to ANOVA (analysis of variance). The data of percentage survival was transformed to remove bias (Sokal and Rohlf, 1995). Assumptions of normality were assessed for all variables and checked using Shapiro-Wilk's test. Homogeneity of the variances was tested in all levels with the Bartlett test (Sokal and Rohlf, 1995). ANOVA analysis was done using the following formula:

$$Y_{ijk} = \mu + B_i + P_J + T_k(P_j) + e_{ijk}$$

Where Yijk is the observation on the lth tree of the jth provenance in the ith block, μ is the overall mean, B_i represents the effect of the ith block, P_j represents the effect of jth provenance, $T_k(P_j)$ indicates the effect of the kth tree nested in the jth provenance, and eijk is the error term. For significantly different treatments, the Duncan's Multiple Range Test was used to compare the means of different treatments (Steel and Torrie, 1980). Regression analyses were conducted to examine the relationship between the 1st year understudy parameters and altitude. Also, the relationship between the genetic variation among the provenances and the altitude was assessed using a quadratic model (Sáenz-Romero *et al.*, 2006). The model was as follows:

$$Y_{ij} = \beta_0 + \beta_1 X_i + \beta_2 X^2_i + e_{ij}$$

Where Y_{ij} is the population mean seedling height, β_0 is the intercept, β_1 and β_1 are the regression parameters, X_i indicates the altitude (m) of i^{th} provenance origin, and e_{ij} represents the error. All analyses were conducted in SPSS Software, Ver. 16 (SPSS, Chicago, IL, USA).

Results

In the first year, the seedling height and the ratio of diameter to height both declined significantly in the elevated planting sites (Fig. 1). There was a negative relationship between the plant height

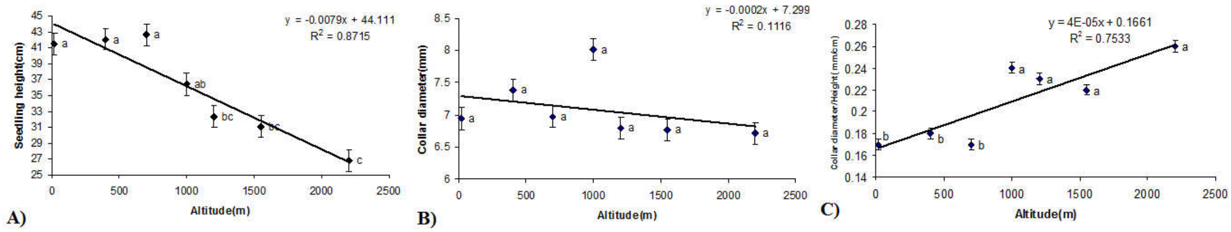

Fig. 1. The relationship between seedling height per provenance (a) and collar diameter (b) and collar diameter to height ratio (c) in the first year, against the altitude of source of seven *Acer velutinum* provenances. The vertical bars represent the standard error and different letters indicate significantly different means according to Duncan's test ($\alpha = 0.05$).

and the altitude of the site of origin (Fig. 1). The largest population discrepency was between the lowest altitude (Park Nour- 20m, average seedling height = 41.4 cm) and the highest altitude populations (Asheck - 2200 m, average seedling height = 26.8 cm).

A positive relationship was found between the diameter to height ratio (D/H) and the altitude, so that with increasing the site elevation, the D/H ratio also increased. Moreover, the seedlings of the populations from the altitude nearest to the investigation site (Asheck, Lamzer and Shahnazer) were superior to those of the populations from the lower altitudes, because they had the greatest diameter to height ratios in comparison to other populations. There was no significant difference in the collar diameter between the populations of the first-year seedlings.

Survival

There was no significant difference in survival between the seedlings from various origins within the first year (= growth season). However, during the second year, the highest and lowest survival rates were at Sangedeh (the local site) and Lamzer (the nearest place to the local site) stations and Asheck (the highest elevation) and Park Nour (the maximum elevation difference with the local site) provenance**s**, respectively (Fig. 2).

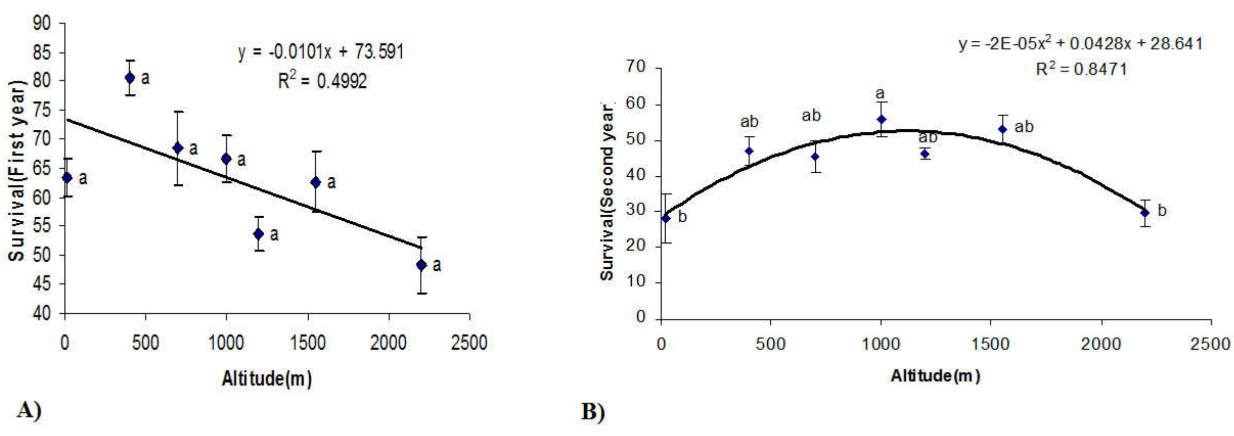

Fig. 2. The relationship between the means of plant survival per population at (a) first and (b) second years, against the altitude of origin of seven *Acer velutinum* populations: The vertical bars represent the standard error and different letters indicate different means according to Duncan's (α=0.05) test.

Seedling production

The results of the first-year productivity showed that some seeds from the middle-altitude origin had high production. However, during the second year, seeds from Lamzer, Lajim and the native origin (Sangedeh) had the highest production rate. Also, Asheck (highest elevation) and Park Nour (lowest elevation) with 7.3 and 18 percent seedling production, respectively, had the lowest production rates. In addition, the annual comparison of the production rates for each origin illustrated that the lower altitudes (Lajim, Park Nour and Jorband) had a significant reduction in the production rate (Fig. 3).

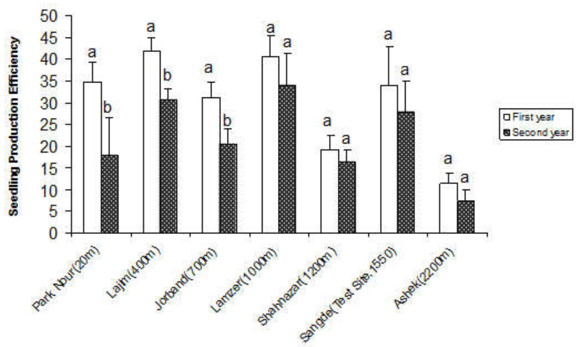

Fig. 3. Comparison of seedling production in first year and second year; the vertical bars represent the standard error and different letters indicate different means according to t-test (α=0.05).

In addition, the annual mortality rate from the end of the first-year growth season to that of the second year decreased with increasing the elevation of the provenance from th sea level to 1000 m, then the rate reached a steady level for the height of 1000 to 1550 m. For further increase in the elevation of the provenance from the sea level, the mortality increased. In fact, the middle-elevation provenances (Lamzer, Shahnazer, Sangedeh) had lower mortality rates (Fig. 4).

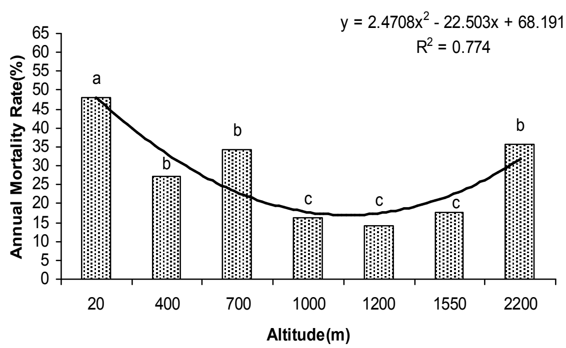

Fig. 4. Relationship between the percentage of Annual Mortality population and altitude of source of seven *Acer velutinum* provenances. Different letters indicate different mean grouping according to Duncan test (α=0.05).

Seed zone

Considering Sáenz -Romero *et al.* (2006), in this research, the seed zones should include populations differing by no more than 648 of average seedling performance. Also, the altitudinal limits could be defined from the regression of population/elevation on the seedling performance (Fig. 5).

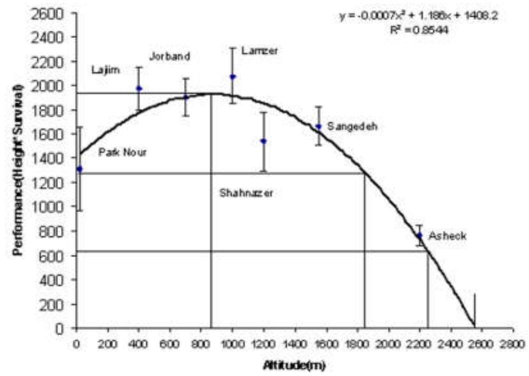

Fig. 5. Limits of four *Acer velutinum* seed zones, based on altitude. Vertical lines denote upper and lower limits for a seed zone in terms of altitude and horizontal lines denote the upper and lower interval limits (at 95% confidence level) for the 2nd year performance.

For example, in Fig. 5, the seed zones delimited in intervals starting from the maximum predicted performance of 1930. By using both of these limits on the expected response of *Acer velutinum* provenances, four seed zones were distinguished (Table 2).

Table 2. Limits and ranges of four *Acer velutinum* seed zones delimited under altitude critereia.

Altitudinal criteria (meter)			
	Limits		
Seed zone number	Lower	Upper	Range
1	0	880	880
2	880	1845	965
3	1845	2280	445
4	2280	2570	290

Discussion and Conclusion

Our findings indicate that there are considerable performance differences among the provenances. Phenotypic differentiation among the populations tends to follow an altitudinal cline. Therefore, populations from the lower altitudes generally have larger height growth potential than those from the higher altitudes. The pattern likely results from discrepancy in selection pressures along the altitudinal gradients: populations from the lower altitudes tend to be adapted to the milder climates, while populations from the higher altitudes display a lesser growth potential and greater tolerance of the cold (Rehfeldt 1989; Rehfeldt 1993). Moreover, in some researches, populations from the middle elevations grow taller than those from the lower or higher elevations (Ohsawa and Ide, 2007).

Variability in the annual mortality rate is related to the altitude of the seed origin, so that the provenances from the lower altitudes as well as that of Asheck provenance in the highest altitude had the highest annual mortality rate, whereas it was lower in provenances from the middle altitude. Park Nour provenance, located at a coastal origin with higher temperatures, had the most severe damage from high mortality. Furthermore, the high mortality rate of Asheck provenance may be caused by a summer drought in the growth season.

In the absence of available information about the frost hardiness of various provenances, it would seem advisable to select provenances with higher altitude for the areas that have early frost in autumn, but it may be possible also to utilize bud set or autumnal leaf yellowing (which is positively correlated) as the indices of autumnal physiological activity. Hence, susceptibility to the autumnal frost can be used in supporting the provenance selection. For *Acer velutinum*, a high growth potential may indeed be the characteristic of populations from the lower altitudes. However, this does not necessarily mean that the highest growth potential occurs at the lowest extreme of the species distribution. A similar patterning of variation was found for *Pinus brutia*, where provenances from the middle altitude zone (400 - 900 m) had greater growth than those from the periphery of the species' distribution (Isik *et al.* 2000). Similar trends have also been detected in *P. sylvestris* (Rehfeldt *et al*, 2003). The results are consistent with the view that there is an optimal climate for the growth of each population (Vander Mijnsbrugge *et al.*, 2010; Rehfeldt *et al.* 1999, 2003) and also deviation in either direction from the optimum result in a loss of growth.

For practical purposes, given the limited distribution and sparse reforestation of *Acer velutinum* from the altitudes above 2280 m (the fourth seed zone), we propose the delimitation of three seed zones, the first zone between 0 and 1000 m, the second zone between 1000 and 1900 m, and the third zone above the 1900 m altitude. The recognized four different seed zones are somewhat corresponding to the elevational communities reported by Gholizadeh *et al.* (2020). Thus, it may be suggested that for restoring the coverage of the original forest, the seed collected in a given seed zone should be used within the same zone. When seedlings from the lower sites are utilized to reforest the highest seed zones, the risk of mortality would be high due to the frost (Viveros-Viveros *et al.*, 2009, Sáenz-Romero *et al.*, 2009).

Also, for a commercial plantation, to have a better performance (more growth and less susceptible to cold) and minimize the risk of disrupting the local gene pool, using seed from local provenance for silviculture and reforestation in Hyrcanian forest is recommended.

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Conflicts of interest

The authors declared no conflicts of interest.

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