Variability of seedling vigour in Persian walnut as influenced by the vigour and bearing habit of the mother tree

By R. REZAAEI*, K. VAHDATI and M. VALIZADEH
1Department of Seed and Plant Improvement, Agricultural and Natural Resources Research Center, West Azerbaijan, P.O. Box 365, Uromich, Iran
2Department of Horticultural Science, College of Abooraihan, University of Tehran, P.C. 3391653755, Tehran, Iran
3Department of Crop Production and Breeding, Faculty of Agriculture, University of Tabriz, P.C. 5166614766, Tabriz, Iran
(e-mail: rezrezae@yahoo.com)
(Accepted 17 October 2008)

SUMMARY
Observations on dwarf and precocious (early maturing) seedlings of Persian walnut (Juglans regia L.) in nurseries, and the consistency of their growth under orchard conditions, encouraged us to study the genetic nature and variability of morphological traits related to seedling vigour. In the present study, two trials were conducted using open-pollinated offspring of six and 18 half-sib families in 2006 and in 2007, respectively. Significant variation was found both within and between families in terms of seedling height, stem diameter, the number of nodes, and internode length in both 45-d-old and 1-year-old seedlings. Moderate to high heritability (0.39 - 0.88) was estimated by between-family variance, implying that the majority of phenotypic variations were under additive genetic control. Seedling height was positively correlated with seedling diameter ($r = 0.89$), indicating that measuring only seedling height was adequate to identify families that produce dwarf seedlings. The shortest seedlings were generally observed in the offspring of low-vigour and cluster-bearing families. The relevance of early maturing seedlings in developing genetically dwarf walnut rootstocks and/or cultivars is discussed. In conclusion, we suggest a recurrent selection programme for the selection of dwarf and easy-to-root genotypes, in order to exploit their advantages in a high-density orchard system.

For many decades, high-vigour walnut (Juglans regia L.) trees suitable for both timber and nut production have been used as a seed source in many countries (McGranahan and Forde, 1985; Diaz and Fernandez-Lopez, 2005; Hemery et al., 2005). As a result, trees in present populations are usually tall, making them inconvenient to prune, spray, and harvest (Forde and McGranahan, 1996). Currently, interest in reduced-sized fruit trees and in high density orchards has increased worldwide (Cummins and Aldwinckle, 1983; Faust and Zagaja, 1984; Cousins, 2005). Dwarf trees have many advantages, including increased planting density, higher production and photosynthetic efficiency, more effective spraying, and easier harvesting (Faust, 1989).

Reductions in tree size by using genetically dwarf rootstocks is a key component for high density, ladder-free, mechanised orchard systems (Cousins, 2005). Size-controlling rootstocks or cultivars have been reported for many fruits such as apple, pear, cherry, apricot, plum, peach, and blueberry (Draper et al., 1984; Faust and Zagaja, 1984; Fideghelli et al., 1984; Faust and Zagaja, 1989). Several independent genetic characteristics, including internode length, branching angle, branching location (i.e. basitonic, mesotonic, and acrotonic), and rate of growth, determine tree size. These characteristics can easily be found among forest, fruit, and landscape tree species and it is only a matter of time and effort to discover them (Faust, 1989).

Despite progressive pressures of natural and/or artificial selection in favour of high-vigour trees, genetically dwarf and precocious walnut genotypes can be found among the seedlings of some Persian walnut genotypes. Rezaee et al. (2006) reported that seedling size and precocity in Persian walnut varied depending on the seed source used in nurseries, which provides an opportunity to select dwarf and precocious seedlings. They concluded that these genotypes maintained these characteristics 3 years after replanting under well-spaced orchard conditions. Germain et al. (1997) and Breton et al. (2004) also reported on a compact, basitonic growth and early maturing habit of walnut seedlings from central Asia. These dwarf seedlings exhibited a higher rooting ability in response to stool-layering (Vahdati et al., 2008), as well as a reduction in scion growth after grafting (Rezaee et al., 2008). Such genotypes may have potential for developing dwarf or semi-dwarf cultivars and rootstocks for further improvement of hedgerow planting systems in Persian walnut.

Currently, high density walnut plantings are based on mechanical hedging of lateral-bearing cultivars, such as ‘Chico’, spaced at approx. 7 m $\times$ 4 m (Olson et al., 2001). In addition, genetically dwarf seedlings have been considered to be important to reduce the juvenile period and, as a result, the costs of cultivar
development in apple, pear, peach, and olive (Santos-Antunes et al., 2005). However, virtually no information is available on the inheritance patterns of dwarf and precocity traits in Persian walnut. Therefore, the main objectives of this study were: (i) to evaluate the influence of the maternal parent on seedling vigour; (ii) to characterise the behaviour of 1-year-old seedlings derived from open-pollinated trees having different vigour and bearing habits; and (iii) to estimate the heritability of seedling height and its components, as well as correlations between traits, to determine an efficient strategy for the selection of dwarf and precocious walnut cultivars.

MATERIALS AND METHODS
All experiments were carried out at the Kahriz Agricultural Research Station, western Azerbaijan Province, northwestern Iran (45°10' E; 37º 53' N; 1,325 m a.s.l.). The mean temperature and relative humidity during the growing season (April – October) were 18.3°C and 48.2%, respectively. The soil texture at each collection site at two nurseries was a uniform sandy loam with a pH of 7.9.

First trial (2005 – 2006)
A half-sib family test was conducted using open-pollinated offspring of six parent trees, selected for their vigour and bearing habit, three with a high-vigour (tree height > 8 m) terminal bearing habit (fruiting mainly on the tips of annual shoots), and three with a low-vigour (height < 4 m) cluster-bearing habit (fruiting mainly on the modified male inflorescences (catkins) and/or lateral bearing (fruiting along the shoots)). All of the parent trees were 10-year-old local genotypes, and have been planted in the same collection. In October 2005, 75 fruits were collected from each tree and stored in a room with adequate air circulation to remove excess moisture from the husks. After 7 d, the husks were removed, and the seeds were kept under ambient temperature (24°C) until completely dry. In November 2005, seeds were planted directly in a nursery, in rows spaced at 200 cm × 15 cm. Seedlings were grown in a nursery until the end of the growing season (2006). Growing practices included furrow irrigation of seedlings once a week during late Spring and Summer, and manual weed control, as necessary, with no applications of herbicides, insecticides, or fertiliser. The germination rates of the seeds differed between families, so that the number of seedlings ranged from 9 – 24, with a total of 86 seedlings used for data collection.

Seedling stem diameter (SD) at ground level, seedling height (SH) from the base to the tip, the number of nodes (NN; counting leaves from the base to the tip of the seedling), and the mean length of the internodes (LI; dividing SH by NN) were measured at the 45-d-old stage and again at the end of the growing season. Mean values for all of the seedling families and the different traits were compared using Duncan’s multiple range test (DMRT) at P ≤ 0.01.

Second trial (2006 – 2007)
To investigate the nature of phenotypic variations in seedling vigour, the number of half-sib families examined was increased to 18. In early October 2006, at harvest time, tree height and stem (trunk) diameter (based on single trees), mean seasonal growth, shoot diameter, the number of nodes, and the length of the internodes were measured in ten 1-year-old shoots that were sampled at random from the mid-portion of the tree canopy in the selected families. Dioecy (i.e., overlapping male and female flowering) and the bearing status of the mother trees were determined in early Spring, as described by Zeneli et al. (2005) and by Solar and Stampar (2003), respectively. Appro. 20 – 30 seeds were harvested from each of these half-sib seedling families (SF) and sown in a nursery, as in the first trial, but with a smaller number of seeds provided for planting. To improve the germination rate, seeds were first soaked in water for approx. 48 h, with the water replaced every 12 h, and were then sand-stratified in an unheated room for approx. 2 months before planting in the nursery in late March 2007 (Vahdati and Hoseini, 2006). Using this method, the germination rate of seeds was improved compared to direct planting of seeds in early December, as in the previous trial, so that we obtained 8 – 11 seedlings (from 20 – 30 initially sown seeds) per family, with a total of 178 seedling from the 18 families. Data were collected as in the previous trial, but only when the seedlings had completed their growth in early October 2007. One-way analysis of variance was performed, and the mean values of families were separated by DMRT at P ≤ 0.01.

Data analysis
In the first trial, heritability (in the narrow-sense) was calculated according to the following equation (Hill et al., 1998).

\[ h^2_{BF} = \frac{4\sigma^2_{BF}}{\sigma^2_{BF} + \sigma^2_{WF}} \]

where \( \sigma^2_{BF} \) and \( \sigma^2_{WF} \) refer to between-family and within-family variances, respectively. The components of

| Traits related to seedling vigour among six half-sib families of walnut, evaluated at the 45-d-old stage and at the end of the growing season in 2003 – 2006 |
|---------------------------------|---|---|---|---|---|---|
| Seedling family (SF) | Seeding height (cm) | No. of nodes | Length of internodes (cm) | Seedling diameter (cm) |
| | 45 d | 1 year | 45 d | 1 year | 45 d | 1 year | 45 d | after 1 year |
| SF1 (LV)\(^1\) | 5.1 ± 1.7 a\(^1\) | 13.5 ± 2.4 a | 5.5 ± 0.3 b | 10.4 ± 1.6 b | 0.56 ± 0.10 a | 0.9 ± 0.03 a | 0.4 ± 0.05 a |
| SF2 (HV) | 11.2 ± 1.3 b | 28.0 ± 2.0 bc | 9.2 ± 0.6 a | 15.4 ± 1.8 a | 0.83 ± 0.06 abc | 1.4 ± 0.07 bc | 0.7 ± 0.04 b |
| SF3 (HV) | 7.4 ± 1.8 ab | 17.5 ± 2.3 ab | 6.2 ± 0.8 b | 12.0 ± 1.3 ab | 0.92 ± 0.15 bc | 1.6 ± 0.09 c | 0.5 ± 0.05 ab |
| SF4 (HV) | 10.6 ± 2.8 a | 30.2 ± 8.7 c | 6.8 ± 1.3 b | 15.4 ± 2.5 a | 1.15 ± 0.10 c | 1.4 ± 0.18 bc | 0.7 ± 0.10 b |
| SF5 (LV) | 4.4 ± 0.8 a | 15.0 ± 1.9 a | 4.7 ± 0.3 b | 11.1 ± 1.1 ab | 0.64 ± 0.12 ab | 0.9 ± 0.08 ab | 0.4 ± 0.05 a |
| SF6 (LV) | 4.8 ± 1.1 a | 14.2 ± 2.1 a | 5.2 ± 0.5 b | 11.4 ± 1.6 ab | 0.72 ± 0.11 ab | 1.0 ± 0.04 ab | 0.4 ± 0.05 a |

\(^1\)LV, low vigour; HV, high vigour walnut genotypes classified based on their height and fruiting type.

\(^2\)Mean values (± SD) followed by different lower-case letters are significantly different by DMRT at P ≤ 0.01.
variance were estimated by apportioning mean squares to the variance components. Since differences between families account for only 25% of the additive genetic variance in a half-sib progeny test, the nominator in the above equation was multiplied by 4 to give the additive genetic variance. The Pearson correlation test was used to determine phenotypic correlations between the measured traits, at \( P \leq 0.01 \). All statistical analyses were carried out using SPSS software (SPSS, 2002).

RESULTS AND DISCUSSION

In the first trial, significant variations in SH, NN, and LI were found among families at the 45-d-old stage and at the end of the growing season (Table I). Within each family, there were significant differences for all traits (± SD; Table I) between individual seedlings. The lowest SH values were recorded 45 d after germination in families SF5, SF6, and SF1 (4.4, 4.8, and 5.1 cm, respectively). These families also retained their ranking of the lowest SH at the end of the growing season, and their dissimilarity was not significant. Moreover, these families displayed the shortest internode lengths, as well as the lowest seedling diameters (Table I). The SS:SD ratio, which is an index of compactness, was also significantly different between families, with SF2 having the highest (39.89) and SF1 having the lowest (28.27) values (data not shown).

Significant positive correlations were observed between SH and all other traits at both sampling times, with the highest correlation value (\( r = 0.93 \)) being between SH and SD at the end of the growing season (Table II). The high correlation between SH and its components, in this study, suggests an efficient criterion for selecting dwarf and/or semi-dwarf breeding lines by only measuring seedling height. On the other hand, the positive correlations between traits measured at the 45-d-old stage with those measured at the end of the growing season, suggest the possibility of early selection for seedling vigour. Early selection of plant material is a necessity for improvement programmes of all tree species (Rweyungeza et al., 2004) in order to save time and costs, as well as to avoid the masking of genetic effects by environmental factors (Dierig et al., 2001).

Moderate-to-high heritability (0.39 – 0.87), that remained relatively consistent at the end of the growing season (0.39 – 0.88), was estimated for all traits (Table III). SH had the highest estimates of heritability in both sampling periods (0.87 and 0.88, respectively). When compared to the moderate estimates of heritability for seedling height (0.32 and 0.63) reported by Diaz and Fernandez-Lopez (2005), the present estimate of heritability (0.88) for SH seems to be high, which may be attributed to the different genetic material and/or environmental circumstances. Higher heritability estimates (approx. 1.00) were also reported for SH in 1-year-old black walnut (\( J. \) nigra L.; Jacobs et al., 2006). High heritability, especially for SH, indicates the importance of genetic factors in controlling this trait and the possibility of its improvement in future generations by appropriate selection methods.

In the 2006 – 2007 trial, significant differences (\( P \leq 0.01 \)) in SH, SD, and NN were observed between half-sib families (Table IV). The shortest (10.2 cm) and tallest (24.2 cm) offspring were produced by SF13 and SF18, a low-vigour and lateral-bearing tree, and a high-vigour and terminal-bearing tree, respectively. Within families, variations in SH were low in families SF8, SF13, and SF18, and they produced a higher proportion (> 80%) of offspring resembling each other, and their mother tree, in terms of vigour (Figure 1).

Knowledge of genetic variation and heritability is essential to understand the extent to which individual phenotypes are determined by their parental genotype, and for selecting appropriate breeding methods. Our results indicated that walnut seedlings display wide phenotypic variability for all of the traits evaluated and related to seedling vigour. This may be attributed to the heterozygous nature of Persian walnut species (Forde and McGranahan, 1996; Solar and Stampar, 2003; Zeneli et al., 2005), and also to the divergent classes of low- and high-vigour parents used in this study.

Previously, it was reported that SH, the number of nodes, and the length of the internodes were the main components contributing to seedling vigour (Faust and Zagaja, 1984). In our study, the above-mentioned traits varied significantly within and between seedling families, underlying the potential for selection. Dwarf seedlings with short internodes were more frequent in the low-vigour half-sib families, but the opposite type of seedlings (i.e., low-vigour seedlings with long internodes) were also present in the same population. These observations are consistent with similar results reported in other fruit tree species (Draper et al., 1984; Zagaja and Faust, 1983).

Based on the results of this study, it is difficult to draw conclusions on the exact mechanism of gene action

<table>
<thead>
<tr>
<th>Trait</th>
<th>VA</th>
<th>VP</th>
<th>h²</th>
<th>45-d-old seedlings</th>
<th>VA</th>
<th>VP</th>
<th>h²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling height (SH)</td>
<td>2.633</td>
<td>3.002</td>
<td>0.87</td>
<td>15.200</td>
<td>17.243</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Number of nodes (NN)</td>
<td>7.92</td>
<td>19.88</td>
<td>0.39</td>
<td>10.95</td>
<td>31.07</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Length of internodes (LI)</td>
<td>12.86</td>
<td>14.80</td>
<td>0.39</td>
<td>24.92</td>
<td>31.09</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Seedling diameter (SD)</td>
<td>–</td>
<td>–</td>
<td>0.47</td>
<td>4.27</td>
<td>6.44</td>
<td>0.66</td>
<td></td>
</tr>
</tbody>
</table>

**All correlation coefficients are significant (\( P \leq 0.01 \)).**
resulting in reduced growth rate; but, as a quantitative trait, it seems that complementary additive genes at different loci may be responsible for controlling vigour (Draper et al., 1984; Cook, 1994; Hill et al., 1998). Dwarf genotypes of walnut may have a higher proportion of homozygous loci that are responsible for reduced vigour of growth, which consequently produce a higher proportion of dwarf offspring. The single gene system may also be proportional for dwarf and precocious walnut trees as the most extreme dwarf offspring were obtained from dwarf and cluster-bearing parents. It has been reported that the action of a single gene designated "Co" or dwarf "Dw" leads to unprecedented growth reduction, accompanied by a significant reduction in internode length and an increase in spur formation in apple and peach (Faust and Zagaja, 1984).

We did not observe any fruit-set in 1-year-old walnut seedlings in either trial, which contrasts with Germain et al. (1997), who reported 15% flowering of 1-year-old dwarf Persian walnut seedlings. This could be due to different genetic material, or climatic differences between the experiments. Under our climatic conditions, dwarf genotypes usually started blooming in the second or third year of growth. Persian walnut has a long juvenility period, so that, under field conditions, first flowering usually occurs when trees are 5–9 years-old (Sabatier and Barthelemy, 2001) and occasionally even >12 years-old (personal observation).

In conclusion, the presence of genetically dwarf and precocious walnut trees provides an opportunity for breeding truly-dwarf walnut cultivars suitable for high-density plantings. Considering the improved rooting ability of dwarf seedlings in response to layering (Vahdati et al., 2008) and in vitro (unpublished data), we suggest a simultaneous recurrent selection programme to select dwarf and/or semi-dwarf, as well as easy-to-root clones to increase fruit yield in high-density orchard systems. Moreover, wide genetic variability in seedling vigour in Persian walnut collections could also be evaluated in terms of their rooting ability, to develop own-rooted cultivars, consistency of vigour, to induce vigour and/or precocity on scions, and/or to develop size-controlling rootstocks. It would then be possible to increase the frequency of favorable alleles by inter-crossing and/or selloing of selected clones for advanced genetic analysis and QTL mapping of vigour-controlling loci.

**REFERENCES**


SPSS (2002). SPSS for Windows. Release 11.5, Chicago, IL, USA.