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Phenotypic variation in native walnut populations of Northern Albania

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Abstract

Juglans regia L. (Juglandaceae) germplasm from the region of Dibra, located in the northeastern part of Albania, was evaluated to determine the variability in walnut germplasm and to identify promising material for preservation. Considerable genetic variation in pomological and phenological characteristics was found in native trees which were of seedling origin. Variability found in nut weight was between 3.8 and 21.1 g, in kernel weight between 1.85 and 9.8 g, in weight kernel/weight nut ratio between 32.6 and 63.8% and in fat content between 42.0 and 71.5%. Nut size, bud breaking time, nut maturity time and phenological characteristics, were also evaluated. The obtained data indicate that the walnut trees studied in this region fall into seven botanical varieties.

Keywords: Genetic diversity; Germplasm; *Juglans regia*; Juglandaceae; Nut

1. Introduction

English walnut (*Juglans regia* L.) is an ancient species (Fjellstrom and Parfitt, 1995) originating in Central Asia, the West Himalayan chain and Kyrgyzstan (Fernández-López et al., 2000), and was being cultivated in southern Europe by 1000 BC (Ducci et al., 1997). Being perennial, monoecious, wind pollinated and growing in a wide range of different

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soils and climatic conditions, walnut shows high variability in both pomological and phenological traits. A high variability in the shape and size of fruit, color and thickness of shell and kernel, shape and size of crown, stem and leaves has been reported for walnut trees from different regions (Solar, 1990; Frutos Tomás, 1990; Malvolti et al., 1994, 1996; Draganescu et al., 2001; Balci et al., 2001; Rouskas and Zakynthinos, 2001; Çağlarirmak, 2003). The most promising genotypes are being maintained in various national germplasm collections (Aletá and Ninot, 1997; Rouskas and Zakynthinos, 2001; Solar et al., 2002).

Walnut is an important crop species in Albania used for both timber and fruit production (MAFA, 2001). Individuals of *J. regia* L. (Juglandaceae) show different characteristics in different areas of the country. It grows in all types of soils, avoiding gypseous, compact, or dry conditions and the optimum soil pH ranges from 6.5 to 7.5 (Çiçi, 1973). English walnut achieves its best growth in the phytoclimatic zone *Castanetum* and in particular cases, it can grow in the cold subzone of *Laurietum* or warm subzone of *Fagetum* (Mitrushi, 1955). In these phytoclimatic zones, it can withstands both high summer temperatures and low winter temperatures.

However, due to its high value, the species has been aggressively harvested, creating a limited supply of quality walnut trees. The number of walnut trees growing today is approximately 30% of that growing in 1970, when there were more than 721,000 *J. regia* trees in the country (Çiçi, 1973). The trend of decreasing numbers of walnut trees necessitates that steps be undertaken to preserve the existing germplasm. The first step is the characterization of the variability. This genetic variability might be useful to breeding programs desiring high yield, late bud breaking, resistance to late spring frost or resistance to diseases.

We focused our study to the region of Dibra, which has the largest plantings of walnut trees in Albania and represent a heterogeneous and interesting genetic source for breeders. These data from investigations of local populations can be essential for planning the genetic breeding process.

The goal of this study was to characterize the variability in walnut in this region of Albania and to classify the *J. regia* studied trees as botanical varieties. This evaluation may help for identifying valuable selections that can serve as sources for germplasm collections.

2. Materials and methods

2.1. Collection site and sampling method

Dibra lies in northeastern part of the country between 41°15′N and 41°52′N longitude and 20°08′E and 20°32′E latitude. It has a mild continental climate with the mean annual temperature 12.9 °C, amplitude of temperature 18.6 °C and the annual rainfall 1765 mm (Anonymous, 1972). A total of 253 trees were initially labeled based on the interviews with local people and on the data from Directorate of Agriculture of Dibra. All trees were of seedlings origin and growing naturally in the countryside. After first observations, many of these trees were excluded because they either showed symptoms of walnut blight caused

by the bacteria *Xanthomonas arboricola* pv. *juglandis* or symptoms of anthracnose or the average nut weight was lower than 6.5 g. Ultimately, 65 of them were selected to be studied further. Data were recorded for three successive years according to the relevant season for each trait, unless otherwise specified.

2.2. Analyzed traits

Recorded data included quantitative as well as qualitative traits.

(a) *General features*: each controlled tree was geographically localized and diameter at 1.30 m and total height of tree and yield were taken. Total height and diameter at breast height were measured at the end of the secondary growth (at the end of August) of the first year. Annual yield was estimated after asking local people or the owners.
(b) *Phenological traits*: bud break, male and female flowering time, their coincidence on time (dicogamy) and maturity time. Data were recorded for three successive years.
(c) *Nut traits*: nut weight, kernel weight, kernel weight/nut weight ratio, length and width of nut and fat content. Data are from a sample of 100 randomly selected nuts per tree and are reported as an average of 3 years. Fat content was measured by the method given by Folch et al. (1957).

Features included under (b) and (c) paragraphs were recorded considering UPOV descriptors (1989), but an own scale was made because not all reference cultivars considered in the descriptor were available.

The bud breaking time was recorded in early spring and the trees were grouped, from the earliest (bud breaking before April 10) to the latest (bud breaking after May 30) in intervals of 10 days. The appearance of the first male and female inflorescences was recorded at the beginning of flowering and according to UPOV descriptors (1989) trees were classified as protandrous, homogamous or protogenous. Time of nut maturity was recorded when at least 50% of nuts had ripened, and then trees were classified as early (fruits ripened before August 30), early to medium (fruits ripened between September 1 and 10), medium (fruits ripened between September 11 and 20), medium to late (fruits ripened between September 21 and 30) and late (fruits ripened in October).

According to UPOV descriptors (1989), pericarp was excluded and all the measurements were made on physiologically ripe nuts, immediately after harvest. Based on the size, nuts were classified as very small (nut weight below 7.5 g), small (nut weight between 7.6 and 9.0 g), medium (nut weight between 9.1 and 10.5 g), large (nut weight between 10.6 and 12.0 g) and very large (nut weight more than 12 g). Measurements of kernel were performed 1 month after harvest, during which time nuts were kept at room temperature. Ease of kernel removal was categorized as very easily, easily, medium or difficult. All the measurements were repeated for three successive years.

All the above-mentioned traits were used to classify walnut trees of Dibra as different botanical varieties, var. *macrocarpa* (De Candolle, 1864), var. *tenera* (Shcepotev, 1978), var. *elongata* (Hegi, 1926), var. *racemosa* (De Candolle, 1864), var. *Dura* (De Candolle, 1864), var. *semidura* (Shcepotev, 1978) and var. *serotina* (De Candolle, 1864).

2.3. Statistical procedures

The Statistical Package for Social Sciences (SPSS) for Windows version 10.1 (SPSS Inc., Chicago) was used for the statistical analysis. One-way analysis of variance (one-way ANOVA) was used to test the hypothesis of equality for all individual means.

Traits included in statistical analysis were the following, fruit weight, fruit length and width, kernel weight, kernel weight/nut weight ratio and fat content.

3. Results

In the region of Dibra, as in most of Albania, walnuts do not exist in large monoculture stands, but are usually found as isolated trees, or as small groups of trees with a dense canopy cover level. These native trees are often of most interest to plant breeders because of the large genetic variability found among them. The altitude of the individual trees studied varied from 480 m in Zdojan, to 1250 m in Klenje. The majority of trees (57.8%) were found between 650 and 900 m. The smallest tree diameter at breast height was 18 cm and the largest was 110 cm. Most trunks were medium-sized, being between 50 and 75 cm. The smallest tree was 9 m high while the tallest was 27 m. Most were within the range of 12–20 m. The differences were largely due to tree age, varying from 25 to 150 years of age.

3.1. Botanical variability

Individual trees differed in numerous morphological and physiological characteristics. Based on the recorded data, the 65 walnut trees were classified under the seven botanical variety groups considered. Relevant data are summarized in Table 1.

1. Var. *macrocarpa* DC (De Candolle, 1864): This variety is widely distributed in the Dibra region because its good fruit size and productivity have been attractive to farmers. Fruit weight is over 10 g, and 80% of the nuts are not full of kernel. In this study, 20 of the 65 trees were considered to belong to this variety.
2. Var. *tenera* DC (Shcepotev, 1978): The nut shell is very thin and almost naked at the tip. The membrane is transparent and fruits can be cracked easily. The weight ratio of kernel to fruit is very high (more than 52%). It is found frequently in Dibra with 12 individual trees from different locations belonged to this variety.
3. Var. *elongata* (Hegi, 1926) or var. *bartheriana* (De Candolle, 1864): The fruit is narrow approximately 1.5 times longer than wide. The ratio kernel/fruit is low. The maximal fruit diameter was 2.5–3 cm with fruit length often greater than 6.5 cm. It is relatively rare, with five individual trees being identified from different locations.
4. Var. *racemosa* (De Candolle, 1864): The stem was relatively short and the crown was small but the trees were still considered very productive because in each raceme there were more fruits than in other varieties (usually between 6 and 20 small fruits). This variety was relatively rare and only three individual trees were examined in the study.
5. Var. *dura* (De Candolle, 1864): This occurs naturally in the Dibra region mainly in dry and shallow soils. Fruit have a very strong shell resulting in the difficult removal of the

Table 1
Variability in phenological and pomological traits among studied walnut varieties

J. regia variety	Number of individuals	Flowering period	Kind of dicogamy[a]	Maturity time	Nut characteristics					
					Nut weight (g) (±S.D.)	Nut length (mm) (±S.D.)	Nut width (mm) (±S.D.)	Kernel weight (g) (±S.D.)	Weight kernel/ weight fruit ratio (%) (±S.D.)	Fat content (%) (±S.D.)
macrocarpa	20	15.04–10.05	PR	01.09–20.09	16.65 ± 2.85*	45.20 ± 5.01*	36.58 ± 3.96	7.20 ± 1.44*	43.64 ± 7.40	57.10 ± 5.70
tenera	12	15.04–10.05	PG	20.08–10.09	8.43 ± 1.80	32.60 ± 3.75	27.26 ± 3.23	4.70 ± 0.80	56.28 ± 4.18*	61.60 ± 6.67
elongata	5	15.04–10.05	PR	01.09–20.09	9.80 ± 1.32	46.40 ± 3.91*	26.60 ± 5.22	4.40 ± 0.82	44.60 ± 3.65	59.60 ± 6.68
racemosa	3	15.04–30.04	PG	01.09–20.09	7.53 ± 2.15	30.33 ± 1.53	27.0 ± 1.73	3.67 ± 1.33	48.00 ± 2.61	68.33 ± 1.53*
dura	10	15.04–10.05	PR	01.09–10.09	7.76 ± 1.71	33.0 ± 4.97	25.90 ± 1.91	3.53 ± 0.91	45.10 ± 4.10	66.10 ± 4.65
semidura	14	15.04–30.05	PG	01.09–20.09	9.46 ± 1.95	36.79 ± 4.96	30.0 ± 4.3	4.73 ± 0.92	50.14 ± 3.10	61.11 ± 8.85
serotina	1	01.07–15.07	HG	01.10–10.10	5.00	28.00	25.00	2.30	46.00	58.00

[a] PR: protandrous; PG: protogenous; HG: homogamous.
* Significant differences among varieties at $P = 0.05$.

kernel from the shell, and so low market value. The advantage of this variety is its tolerance and its use as rootstock where high fruit production is desired. Ten individual trees from different locations were considered to belong to this variety.

6. Var. *semidura* (Shcepotev, 1978): Ratio kernel-to-fruit is higher than 50% with very strong but thin nut shell. This is the most widely cultivated variety in Dibra. Generally, all fruit characteristics lie between the varieties *macrocarpa* and *dura*. Fourteen individual trees from different locations were placed in this variety.
7. Var. *serotina* (De Candolle, 1864): Trees of this variety usually have flowering times 2–3 weeks later than other varieties growing at the same altitude. Flowering late also means it misses the pollen shed by other varieties. The most interesting selection, in terms of wood characteristics, pomological traits and phenology, was an individual tree grown in Radovesh, called by the local people ‘‘the crazy walnut’’. This tree flowers 2 months later than trees of other varieties growing in the vicinity.

Very early or very early to early bud breaking and flowering phenotypes (before mid April) have been noted but were not recorded in this study. Most of the observed trees had their bud breaking time between 20 and 30 April (62%) while 30% of them between May 1 and 10. Late bud breaking (after May 10) was rare (5.5%). There is no clear-cut boundary among most of varieties concerning bud breaking and flowering time, but within each variety, there is a little variation depending on the weather climatic conditions of the year. The exception was var. *serotina* which during our observation flowered between July 5th and 15th regardless of annual weather conditions.

Flowering habit was different for different varieties. *J. regia* var. *serotima* was the only one which had homogamous flowering habit (Table 1). Although the majority of the trees of var. *macrocarpa* were protandrous, three individual trees showed protogenous flowering habit.

Fruit ripened between September 1 and 10 (early to medium) in most trees (73.6%). The majority of individuals from varieties *macrocarpa*, *tenera*, *semidura*, *dura* and *elongata* belonged to this group. Fruit ripening between September 11 and 20 (medium) occurred in 18.4% of the trees. Early phenotypes (fruit ripening before September 1) were rare (3.2%) and these were only from var. *tenera*. Medium to late ripening individuals were also rare (3.2%) and were only from var. *semidura*. The individual found in Radovesh (var. *serotina*) was also an exception and had fruit ripening in the earlier days of October (Table 1).

Good inshell fruit and kernel quality are important properties for walnut varieties. Fruit size (length and width) varied among varieties and so did the fat content, but no correlation was found between them. Kernel removal was difficult for var. *dura*, and very easy for var. *tenera*. Varieties *macrocarpa, serotima* and *elongata* can be grouped as easily while varieties *racemosa* and *semidura* as medium concerning the kernel removal. Differences were also found in kernel weight. In all trees, usually the kernel weight varies between 40 and 63.8% of the fruit weight. There were significant differences among varieties for several fruit characteristics; *J. regia* var. *tenera* had the highest weight kernel/weight nut ratio (51.4–63.8%) while *J. regia* var. *racemosa* and *dura* had significantly higher fat content (61.2–71.5%). Fat content also varied among varieties. There was no correlation

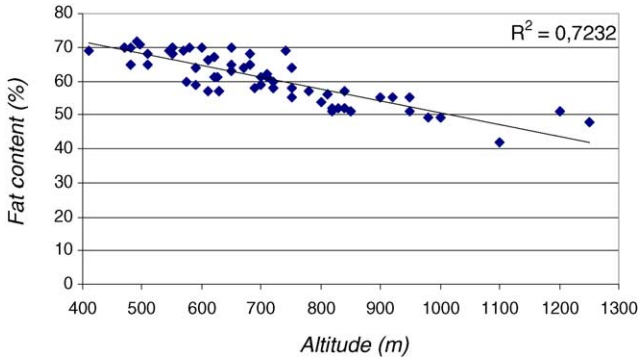

Fig. 1. Relationship between altitude and fat content of walnut fruits. Every individual tree is represented by a single point in the graph.

between walnut variety and its fat content, but there was good correlation between the fat content of the nut and the altitude of the tree ($R^2 = 0.723$) suggesting that fat content is strongly affected by altitude (Fig. 1).

J. regia var. *macrocarpa* differs significantly from other varieties as far as fruit weight and length and kernel weight are considered, being two to three times larger than *J. regia* var. *serotima* which had the lowest values for most of pomological traits. There were also significant differences in fruit length among *J. regia* var. *macrocarpa* and *J. regia* var. *elongata* and other varieties (Table 1).

Var. *semidura* has the largest altitude range growing from 650 to 1250 m above sea level (a.s.l.). Var. *racemosa,* had the most limited amplitude, growing from 510 to 620 m a.s.l. There is no clear-cut boundary as far as altitude is concerned where one or another walnut variety can or cannot grow, with the exception of var. *racemosa* which cannot tolerate frost and is limited to lower altitude (Fig. 2).

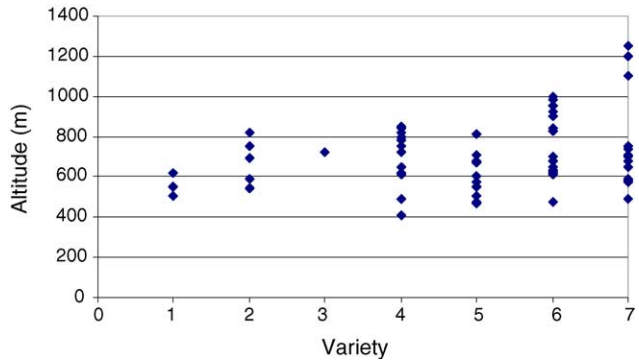

Fig. 2. Altitudinal distribution of walnut varieties. Numbers represent, respectively, *J. regia* (1) var. *racemosa*; (2) var. *elongata*; (3) var *serotima*; (4) var. *tenera*; (5) var. *dura*; (6) var. *macrocarpa*; (7) var. *semidura*.

4. Discussion

English walnut is a species with a very wide distribution range. Populations and individuals located in different ecological niches have differentiated adaptive traits, by natural selection, with limited gene flow and limited phenotypic plasticity (Eriksson, 1998). In the present study, a wide range of variation was observed in nut and kernel characteristics as well as in other traits. Using the standard UPOV guidelines for distinguishing walnut varieties, the variability in the characteristics studied, indicate that they are seven botanical varieties of the walnut in the Dibra region. The investigated varieties differ in their bud breaking time. These very early to early walnuts are characteristic of genotypes common to California, the Mediterranean, and Iberian Peninsula (Germain et al., 1998). The time of walnut bud breaking did not depend only on the general climatic conditions of the area, but also on specific weather conditions in different years. The phenomenon of variation in bud breaking time in early genotypes is reported also from Solar et al. (2002). The proportion between early and late genotypes found in this study is in agreement with these reported from Aletá and Ninot (1993); Atefi (1997); Rouskas and Zakynthinos (2001) and Solar et al. (2002). In the Dibra region, where late spring frosts are sometimes a problem, trees that leaf-out late enough to escape the frosts (for example, the so called ''crazy tree''), should be selected (Shreve, 1999).

Natural and human selection have been strong forces in shaping walnut populations in the Dibra region, leading to a high phenotypic variability. Kernel weight for some varieties (var. *macrocarpa* and var. *elongata*) and kernel percentage for some other varieties (var. *tenera*) are even higher than those reported for some of the Californian commercial cultivars (Hendricks, 1997). The variability found in the present study is in agreement with that reported for the Eurasian walnut distribution range, from India (Sharma and Sharma, 2001); Kyrgyzstan (Hemery, 1998), Turkey (Akccedila and Sen, 2001; Yarilgac et al., 2001), Romania (Draganescu et al., 2001; Botu et al., 2001), Serbia and Sllovenia (Paunovic, 1990; Solar, 1990), to Italy and western Europe (Malvolti et al., 1993; Malvolti et al., 1996). Similar variability is also reported for phenology (Solar, 1990; Barone and Zappia, 1993; Atefi, 1997; Rouskas and Zakynthinos, 2001; Solar et al., 2002), fat content (Çağlarirmak, 2003), tolerance to diseases (Botu et al., 2001; Kondratenko et al., 2001; Olson and Buchner, 2002) or in other nut species (Grauke and Thompson, 1996; Rink et al., 1997).

Little information is presently available concerning the amount of genetic diversity or the existence of founder effects in the Albanian walnuts. Part of the variability found in the present study may be attributed to the abrupt changes in climatic conditions prevailing on the mainland of Albania.

Native walnut genotypes from this study were evaluated as promising with regard to fruit characteristics. Similar evaluations have been reported from other selection studies conducted in Albania (Çiçi, 1973; Dano and Zeneli, 2000; Kola, 2001). These trees can serve as the core of future germplasm collections due to their high variability in nut production and forestry traits. Further information on the nature and the degree of genetic diversity present in walnuts could help to identify elite trees for genetic improvement through hybridization. Guidelines for walnut nut and timber production in Albania should

include recommendations on profitable management of native walnut forest, forest plantations and walnut orchards, as proposed by Shreve (1999).

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