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Evaluation of an isolated Persian walnut (*Juglans regia* L.) population from Eastern Transylvania, Romania

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Abstract

An isolated walnut population was investigated in Eastern Transylvania, Romania, in order to collect information for improving the present assortment of scion cultivars for the National and International Breeding Program. A total of 147 walnut individuals were investigated to determine 1) the main biological characteristics of the trees; and 2) to ascertain the main physical and qualitative characteristics of the nuts. The walnut population we studied can be considered as a genetically valuable one. Of the individuals 11% flowered between 29th of April and 10th of May, thus avoiding late spring frost damage. Such types bear abundantly in cold climate on lateral shoots and also have high quality fruit. In the present evaluation, 89% of the individuals had a mean flowering time between 18th and 28th of April. Although injuries from late spring frost are frequent in this area; the spring frost damage was low, and high quality fruit was obtained. Among the 147 individuals, 10 provided the best proportion of kernel mass compared with endocarp. Sensory characteristics of the nuts (size, colour, strength and flavour) were tested. The most valuable individuals, which were considered as prospective elites, were tested and compared with internationally recognised cultivars ('Franquette', 'Hartley', 'Milotai 10' and 'Alsószentiváni 117'). Comparing the total quality scores of the 14 elites and cultivars in the study, we can observe that only one had a low score (Ozsd-37); however, its strength and flavour were similar to other cultivars. According to our observations, many individuals from this isolated population showed the same quality as some internationally recognized cultivars.

Key words: Flowering periods, size, colour, strength, flavour of kernel, prospective elites.

Introduction

Persian walnut (*Juglans regia* L.) is commercially cultivated in many regions around the world for its timber, as well as for its nuts. European production of Persian walnut still largely depends on trees originated from seedlings. During the last 20 years, important work on seedling selection has been carried out in local populations of walnut throughout Europe. Also, the characteristics of wild walnut trees have been described in Bulgaria, Germany, Greece, Hungary, Italy, Poland, Portugal, Romania, Slovenia, Spain and the Ukraine [1-10].

In Romania over 85% of the yield is obtained on isolated walnut trees which can be found around houses or on private properties. Local populations present great genetic variability due to the method of seed reproduction, and also because of an intensive exchange of seminal material with Hungary, Austria and Serbia [3]. Phenotypes of the cultivars in Romania are described by their physical characteristics and named after the localities where they can be found, e.g., Sibişel 2, 3, 21, 22, 32, 34, 35, 39, 44, 45, 50 ; Geoagiu 1, 2, 53, 66, 67; Homorod [1]; Gelmal [1]; Romoşel 1; Apold 163; Crăciunel 1, 2, 3, 4 Meza 1, 3, 4, 6, 7, 8, 9, 10; Măgureni 3, 6; Rotunda de Satu Mare, Gubău 22; Gurbău 24; Sărmas 16; Cluj 8; Selistea 26, Cãtina 20; Bistriţa 3; Dumitra 2, 9; and Viisoara 10.

Valuable edible nuts produced by walnut trees are well appreciated because they are rich in unsaturated fat (linoleic, oleic acid). They also contain other beneficial components like plant protein (e.g., arginine, leucine), carbohydrates (e.g., dietary fibre), vitamins (e.g., vitamin A, E), pectic substances, minerals (magnesium, potassium, phosphorus, sulphur, copper and iron), plant sterols and phytochemicals (phenolic acids, flavonoids etc.). Improved walnut cultivars require a well-sealed shell with a light-coloured kernel, free of flavours and comprising about 50% of the nut weight. Oil quality may be a concern in the future [11, 12]. From the consumer's point of view (satisfaction and further consumption), the sensory properties of nuts are important, especially their visual and taste properties, which means sensory characterization is quite advisable [13]. Due to the variety of locations where the Persian walnut (*J. regia*) is cultivated in Eastern-Europe and Western-Asia, it is essential to know the biological characteristics and nut quality of the various prospective elites and cultivars. Some studies have shown that nuts obtained from the high-altitude locations were lighter-coloured and required more force to fracture the shells than those harvested at lower altitudes [14, 15].

The region of Eastern Transylvania where the investigation was carried out may be one of the coldest both in Romania and in UE (- 10 to -25^{o}C in winter and 25 to 30^{o}C in summer). Therefore, this isolated population, growing by seedlings, may be very resistant to a number of abiotic and biotic factors. We concentrated on walnuts growing in the countryside as possible seminal material for the National and International Breeding Programme. The survey continued from 2005 to 2007, and the flowering synchronization was surveyed each year from March to May. The main physical characteristics of fruits were observed by collecting 100 fruits from each individual each year, and the following characteristics were measured under laboratory conditions: the thickness of the endocarp and the proportions of endocarp and nutmeat.

Sensory characteristics of nuts of the most valuable individuals, considered as prospective elites, were tested and compared with internationally recognised control cultivars (‘Franquette’, ‘Hartley’, ‘Milotai 10,’ and ‘Alsószentiváni 117’). These measurements were carried out in the laboratory of the National Institute for Agricultural Quality Control with a group of 16 independent assessors. Visual properties of the kernel (size and colour) were evaluated, and then the kernel was tasted to assess its flavour. Nuts were harvested in September 2006 and 2007.

Ten prospective elite codes were used which contained the first three or four characters of the respective locality and its GPS code number (Table 1). From each elite and cultivar 14 fruits were used for the quality test. Each was coded from 1 to 14. Sixty minutes before the evaluation, the shell of each nut was cracked and the kernel divided into four quarters. Then each assessor was allotted four quarters derived from the 14 different nuts. Testing was performed at room temperature. Between the assessment of different samples, water and bread were available for taste neutralization. Sensory evaluation comprised estimation of external and internal properties of the nuts. The following properties were evaluated on a range of 1 to 10 for data analysis purposes: the size, the colour, the strength and the flavour of the kernel.

The one-way analysis of variance (ANOVA) was used for testing differences in the data of endocarp and kernel mass proportion; and sensory evaluation between elites and cultivars. ANOVA may be used to examine the effects of two or more categorical variables (factors), both individually and together, on an experimental response. Back transferred means and $P < 0.05$ were considered as statistically significant differences [16].

Results

Altogether 147 individuals were found in 19 localities. The population can be considered one of the most isolated in Romania; individuals have been growing by seedling for hundreds of years in local communities. One of the main objectives of the study was to select late leafing and late flowering walnut types with high lateral yield within a seedling population, walnut types which would not suffer injuries from late spring frost. Of the individuals 11% flowered between 29th of April and 10th of May, thus avoiding late spring frost damage. Such types also bear abundantly in cold climate on lateral shoots and have high quality fruit. As a result of the expeditionary observations, forms were selected, according to characteristics of late leafing, lateral yield and blight resistance. Of the individuals 89% had mean flowering time between 18th and 28th of April. They may suffer injuries from frequent late spring frost in this area; however, the spring frost damage was low and trees produced high quality fruit (Fig. 1). For many individuals in the population, the period of time during which pollen is shed does not coincide with the period of female flower receptivity to pollen. These individuals require another cultivar for pollination since the timing of the male and female functions on a given tree is different. This is referred to as dichogamy. Three possible forms were identified: protogyny (first female) where the female flower becomes receptive before pollen shedding begins. These genotypes represented only 1.7% of total genotypes in the area.

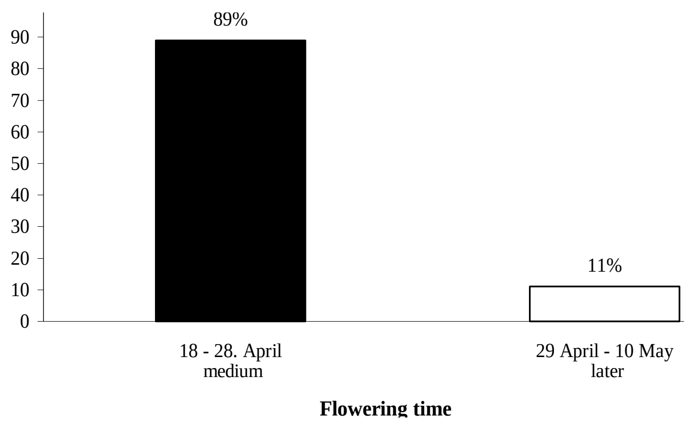

Figure 1. The flowering time of the individuals in population.

Table 1. The mean mass of 100 fruits, endocarp and kernel, and mass proportions between endocarp and kernel.

Cod	Elites and cultivars	Total mass (g)	Endocarp mass (g)	Kernel mass (g)	Kernel mass proportion (%)	Endocarp mass proportion (%)
1	Cser-78	262.95	175.58	87.37	0.33	0.67
2	Alb-23	270.98	174.93	96.05	0.35	0,65
3	Szen-10	225.37	141.69	83.68	0.37	0.63
4	Alb-22	194.73	135.64	59.09	0.30	0.70
5	Ozsd-37	250.22	154.56	95.66	0.38	0.62
6	Fut-11	244.82	153.85	90.97	0.37	0.63
7	Mak-29	245.63	160.95	84.68	0.34	0.66
8	Ozsd-36	277.00	174.41	102.59	0.37	0.63
9	Fut-79	275.79	190.27	85.52	0.35	0.65
10	Poi-75	291.30	197.90	93.4	0.32	0.68
11	Franquette	163.17	126.20	36.97	0.31	0.69
12	Hartley	62.15	38.86	23.29	0.23	0.77
13	Milotai 10	201.45	121.46	79.99	0.37	0.63
14	Alsószentiváni 117	283.48	166.72	116.76	0.40	0.60

The second form is protandry (first male), where pollen shed begins before the female flower becomes receptive, represented by 65.5% of the total genotypes. This was also present for the most valuable genotypes (Table 2).

In some cases (29.3%), there was overlap in the functional periods, allowing for some degree of self-pollination (Fig. 2). There was a significant tendency for protandrous cultivars to have greater overlap or incomplete dichogamy, than for protogynous, resulting in more self-pollination in protandrous cultivars. Some cultivars have no overlap of pollen shed and female receptivity, and these are sometimes termed functionally dioecious.

Adding to the complexity of the issue, the high climatic variation in the area can influence the degree of dichogamy expressed by a given cultivar, and, less commonly, the type of dichogamy.

Nut characteristics: Considering nut phenotypes, the 147 individuals can be classified into four different groups:
a) Roundish, with 26.35% in the cumulative sample and dominant in nine localities and 40 genotypes.
b) Elliptic, with 35.13% in the cumulative sample and dominant in six localities and 51 genotypes
c) Egg-shaped, with 35.13% in the cumulative sample and dominant in two localities and 51 genotypes
d) Asymmetric egg-shaped 3.37% in the cumulative sample and also dominant in two localities and 5 genotypes.

The thickness of the endocarp varied between 1.29 mm for roundish forms and 1.75 mm for elliptic forms. Considering the mean mass proportion of kernel and endocarp of the 147 individuals, the differences were statistically significant ($F = 22.4$, $P < 0.001$) (Fig. 3). Among the 147 individuals, 10 had the best mass proportion of kernel. These genotypes also possessed late flowering properties, and they could be considered in breeding programs as prospective elites to develop new cultivars with consistently high fruit quality (Table 1).

Sensory evaluation: The 16 assessors evaluated four properties of the 14 coded cultivars using a scale from 1 to 10. These properties were the size, colour, strength and flavour of the kernel. The size and colour were estimated visually by appearance, which is a vital attribute and involves analysis of both geometric attributes and colour attributes [11]. There were marginally significant differences among the 14 cultivars in terms of the size of kernel, and many prospective elites proved to have the same or even greater kernel size as the recognized cultivars, e.g., 'Hartley' (Fig. 4.).

The colour of kernel was evaluated with shelled walnuts. The colour of the elites Szen-10 and Alb-22 and the colour of 'Franquette', 'Hartley' and 'Alsószentiváni 117' were evaluated as the lightest by assessors and received the highest marks. On the other hand, the nuts of local cultivars – except Ozsd-36 – had the darkest shell among all cultivars. The cumulative score accorded by assessors differed significantly between the two groups ($F = 7.76$, $P < 0.01$) (Fig. 5). There were no statistically significant differences among the cultivars in the strength of the kernels (Fig. 6).

Perceived taste (internal properties of kernel) of assessors was estimated very similarly; however, the external or visual properties of walnuts for many elites and cultivars were different (Fig. 7). Taste and flavour are two different attributes that are frequently

Table 2. The flowering characteristics of the most valuable individuals.

Cod	Local genotypes	Leafing and flowering	Protogyny	Protandry	Simultaneous
1	Cser-78	medium	-	+	-
2	Alb-23	late	-	+	-
3	Szen-10	medium	-	+	-
4	Alb-22	late	-	+	-
5	Ozsd-37	medium	-	+	-
6	Fut-11	medium	-	+	-
7	Mak-29	medium	-	+	-
8	Ozsd-36	medium	-	+	-
9	Fut-79	medium	-	+	-
10	Poi-75	medium	-	+	-

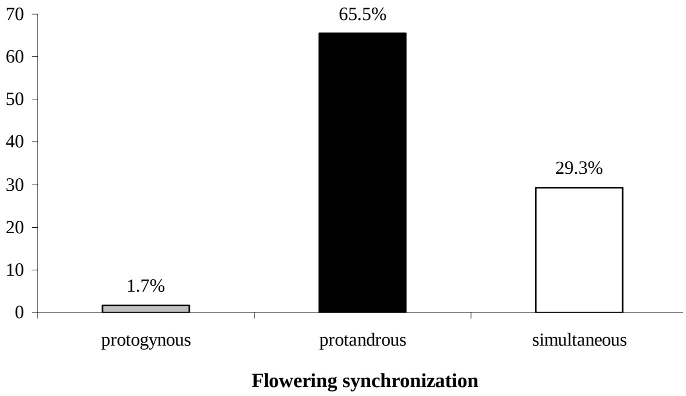

Figure 2. The flowering synchronization of the individuals in population.

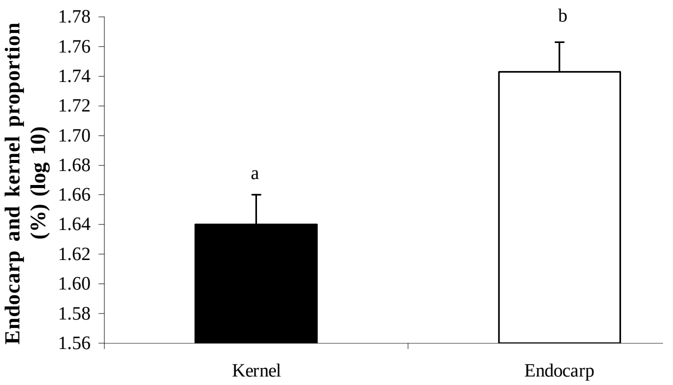

Figure 3. Similarity between the mean mass proportion of endocarp and kernel of the 147 individuals. a and b mean statistically significant differences ($P < 0.01$) between columns (ANOVA).

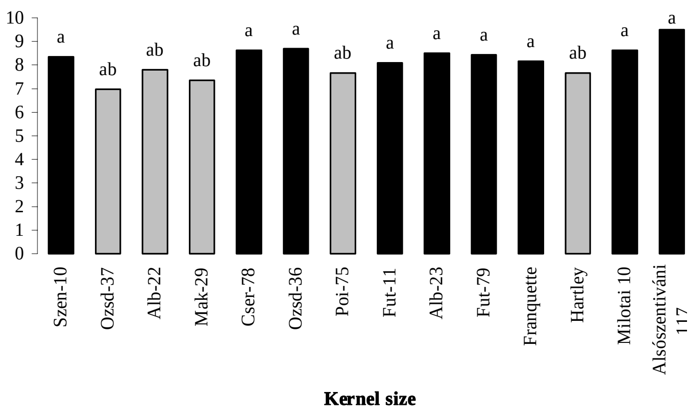

Figure 4. The mean proportion of kernel size of the ten prospective elites and four cultivars.

wrongly equated in the scientific literature. Human peripheral taste and smell systems are completely separate and distinct. Water-soluble compounds contribute to taste when they interact with specialized cells in the oral cavity. Certain volatile compounds contribute to flavour via the olfactory organ situated in the nasal cavity [11]. Comparing the total quality scores of the 14 cultivars investigated, we can observe that only one had a lower score (Ozsd-37), however, its strength and flavour were similar to other cultivars and displayed the highest kernel mass proportion. According to our studies, many individuals from this isolated population presented the same quality properties as some internationally recognized cultivars (Fig. 8).

Discussion

The walnut population presented in this study can be considered as a genetically valuable population. Ten prospective elites were selected with above average quality fruits and high mass proportion of kernel. These could play one important role in a possible breeding programme of walnut aimed at improving the present assortment of scion cultivars from Transylvania. Due to the high variability (kernel/endocarp proportion, colour, strength, flavour) of the nuts and the diversity of the local prospective elites, seedling methods for mass production may not be commercially useful.

The trend in walnut cultivation and consequently in selection and breeding, is to separate the production of wood from the production of fruit. However, all genetic information issued from walnut breeding programmes is also being used in current programs in Western Europe which are focused on wood production (FAIR III-CT-96-1887). The clear separation that exists among breeding programs implies the necessity of including the two objectives in the genetic conservation of the species, which is also recommended by other authors [17].

Our investigation also provides useful data regarding sensory properties of the local prospective elites which were evaluated, compared with those of recognized cultivars. Based on this study it can be suggested that consumer preferences should be considered for the genetic selection. The sensory characteristics of nuts depend not only on the cultivar, but also on the growing conditions of the production area; further sensory analyses need to be conducted [11, 17].

At present, most of the nut supply in Romania comes from walnuts growing in the countryside. As this species is very sensitive to a number of abiotic and biotic factors such as climate, fungus attacks and bacterial diseases, it is possible that natural selection is a very strong force in walnut populations [5]. Due to the large scale climatic variations in the investigated region (- 10 to - 20°C during the winter and + 20 to + 25°C during summer) these populations may be relatively resistant and well adapted to abiotic conditions. Accordingly, a long-term national breeding programme would be necessary for conserving and improving these individuals.

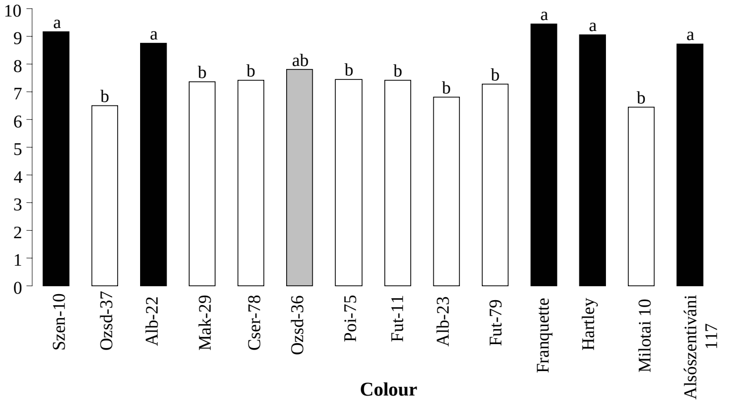

Figure 5. The mean proportion of kernel colour of the ten prospective elites and four cultivars. a and b mean statistically significant differences (P < 0.01) between columns (ANOVA).

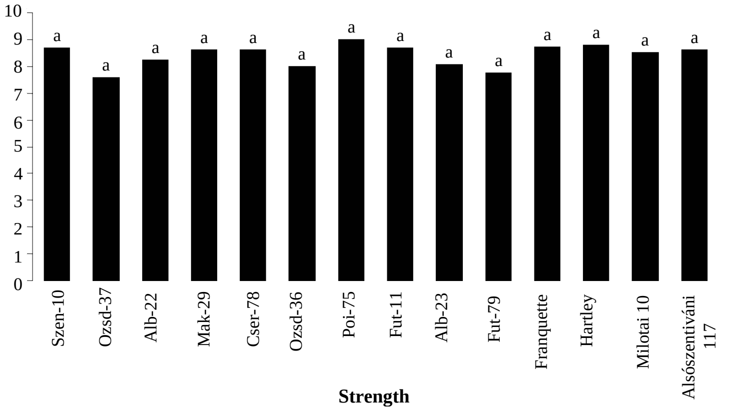

Figure 6. The mean proportion of the estimated kernel strength of ten prospective elites and four cultivars.

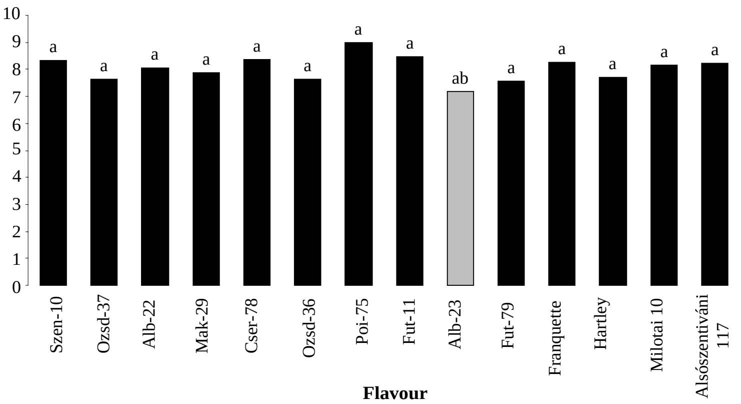

Figure 7. The mean proportion of the estimated kernel flavour of ten prospective elites and four cultivars.

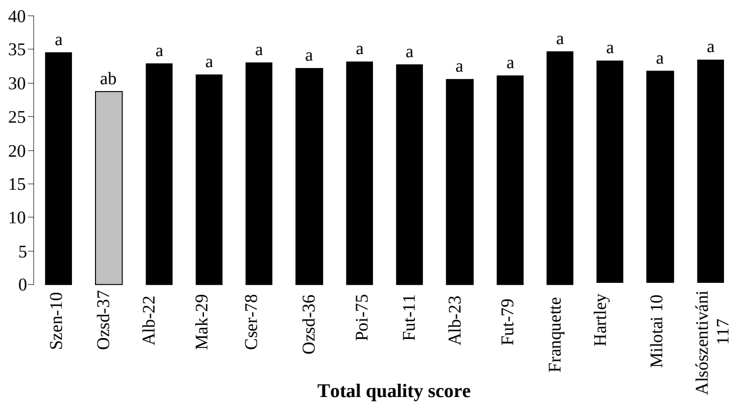

Figure 8. The mean proportion of the estimated total quality score of kernel quality.

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