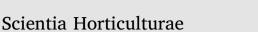
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Morphological variation among Persian walnut (*Juglans regia*) genotypes within the population and depending on climatic year



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A R T I C L E I N F O 	A B S T R A C T				
	The main goals of this study were to compare walnut genotypes of spontaneous flora of Romania, based on main sources of phenotypic variability, using both univariate (ANOVA and Tukey tests) and multivariate (PCA) sta- tistical approaches. Six main sources of phenotypic variability (nut length, nut diameter I, nut diameter II, nut weight, kernel weight, kernel ratio) were used for the study. Based on this analysis, the aim is to provide a recommendation for culture, and to find out which of the analyzed walnut genotypes have the optimal values for all the observed traits. The results of the study indicate that the conclusions based on the use of univariate and multivariate statistical methods can be used successfully in identifying genotypes with superior characteristics in genetic diversity assessment studies. Six walnut genotypes (B29, B40, B8, B5, B6, B11) were determined to possess optimal values for all six main sources of phenotypic variability. These results show large phenotypic				

diversity among the selected walnut genotypes.

1. Introduction

Local walnut populations in different areas offer the possibility of selecting higher genotypes with the most appropriate features of ecological and commercial conditions of each region to improve the current walnut assortment or create a new assortment. Studies on biodiversity of the Juglans regia, based on morphological characteristics of fruits, have been conducted in many countries (Arzani et al., 2008; Gunn et al., 2010; Ahandani et al., 2014; Vahdati et al., 2015; Vahdati and Mohseniazar, 2016; Giovanardi et al., 2016; Ebrahimi et al., 2017). Solar and Stampar (2004) evaluated walnut genotypes in Slovenia and identified genotypes based on specific traits. A large phenotypic variability in weight, shell thickness, kernel skin color, appearance and taste was found in nuts collected from wild accessions of Persian walnut in the Friuli Venezia Giulia region (North-Eastern Italian Alps) by Poggetti et al. (2017). Superior walnut genotypes were selected from the north-eastern Anatolia to be used for further breeding purposes in terms of yield capacities, nut characteristics, and cold hardiness (Aslantas, 2006). Based on phenological and pomological characteristics evaluation, the five promising walnut genotypes in Karaj, Iran showed very good performance in comparison with the commercial walnut cultivars (Mahmoodi et al., 2016). There is a high genetic variability in walnut populations exist in Romania, due to seed propagation, high heterozygosity and dichogamy (Cosmulescu and Botu,

2012; Cosmulescu et al., 2010, 2012, 2017; Tsampas and Botu, 2013; Botu et al., 2014). Eighty walnut genotypes native to Oltenia region of south-western Romania were evaluated by Cosmulescu (2013) to identify superior genotypes. Moreover, Botu et al. (2001) assessed genetic variability of the Juglans regia L. natural populations from Oltenia region of Romania based on morphological characters. In another study, Godeanu et al. (1997) aimed to identify valuable walnut selections for intensive growth in Romania. Draganescu et al. (2001) studied the genetic diversity of native walnut populations existing in Banat region of Romania. The high variability in local populations in different regions is important for the breeding program, indicating a high return on the selection of new individuals with superior characteristics. The objectives of this study were therefore to assess the morphological variation of walnut genotypes growing naturally in Oltenia region and to identify promising genotypes with superior tree and fruit quality traits. The results of this evaluation will be used in future breeding programmes.

2. Materials and methods

2.1. Experimental sites and selection procedure

The study was carried out in areas of Oltenia region in Bechet sites, Romania. Oltenia is located in the south-west of Romania, north of the

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lower Danube river, crossed by the 45 °N parallel; one third of Oltenia (southern) territory having an altitude below 200 m. Bechet is located in the southern part of the region (43°47′N/23°57′E), where the average temperature is 11 °C and the annual average rainfall is 500 mm. Bechet's altitude is 23 m in the meadow (near the Danube) and 40 m (in the sandy area). A total of 98 mature trees were initially labelled in situ based on interviews with orchards owners, local people, and on the tree and nut characteristic information. Following primary observations, many of the trees were excluded because they showed either visual symptoms of bacterial blight (*Xanthomonas arboricola* pv. *juglandis*), either the production and the fruit did not meet the selection criteria. In the end, 40 genotypes were deemed valuable and adequate for further study.

2.2. Nut and kernel traits

Phenotypic and genetic variability, between genotypes within the same year and between different years, were studied by morphological variables of fruit during three successive years (2015, 2016, 2017). Data are collected from a sample of 100 randomly selected nuts per tree. Nut dimensions (length and diameter) were measured using a digital micrometer (accuracy of 0.01 mm) and the nut weight was measured using electronic balance (accuracy of 0.001 g) and kernel ratio using the formula: kernel weight / nut weight x100.

2.3. Statistical analysis

For statistical analysis, Microsoft Excel, XLSTAT Pearson Edition version 2014.5.03, Addinsoft and R Core Team (2013) were used. Data were analyzed statistically using univariate (ANOVA and Tukey tests) and multivariate (PCA) analysis for determining genetic diversity based on morphological parameters.

3. Results and discussions

3.1. Characteristics of genotypes

Minimum, maximum, mean, standard deviation (SD) and coefficient of variation (CV) for the nut and kernel characteristics in the selected genotypes are provided in Table 1. According to these data, nut length variability ranged between 24.34 (B10) and 58.72 mm (B29); nut diameter between 20.24 (B35) and 55.03 mm (B9); nut weight between 5.00 (B15) and 27.93 g (B40); kernel weight between 2.20 (B15) and 13.87 g (B29); and kernel ratio between 18.66 (B20) and 62.93% (B29).

Table 1

 $\mathsf{Descriptive\ statistics}^*$ for the nut and kernel characters in the selected genotypes.

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Characters	Year	minimum	maximum	mean	SD	CV (%)
Nut length (mm)	2015	29.67	58.17	38.22	4.63	12.12
	2016	24.34	56.22	37.07	4.40	11.87
	2017	26.29	58.72	38.22	4.62	12.10
Nut diameter I (mm)	2015	23.48	49.09	32.28	2.88	8.92
	2016	21.20	55.03	32.40	3.46	10.67
	2017	20.24	45.20	32.41	2.96	9.15
Nut diameter II (mm)	2015	21.34	45.21	32.46	2.93	9.03
	2016	21.91	51.78	31.18	3.06	9.82
	2017	20.53	44.24	32.45	2.84	8.74
Nut weight (g)	2015	5.00	27.93	12.73	2.86	22.50
	2016	7.14	26.65	12.45	2.73	21.91
	2017	6.93	27.90	13.00	2.93	22.57
Kernel weight (g)	2015	2.20	13.11	5.81	1.55	26.70
	2016	2.25	12.96	5.56	1.42	25.64
	2017	3.03	13.87	5.91	1.71	28.91
Kernel ratio (%)	2015	20.91	62.34	45.69	6.45	14.12
	2016	18.65	60.24	44.74	6.04	13.50
	2017	23.52	62.93	45.32	6.14	13.55

The size of the nut is an important feature for improvement and market. Previous research (Khadivi-Khub et al., 2015) in 540 seedling accessions selected in Neiriz region, Iran, showed that the length of nuts varied from 25 to 47 mm, while the nut diameter varied from 24 to 47 mm. Nut weight is an important parameter influencing the quality. Nut weight varied from 3.60 to 20.28 g. The highest value of nut weight between genotypes studied was found in the genotype B40 (27.93 g). The highest value for nut weight among the evaluated genotypes was higher than the corresponding data reported by Hussain et al. (2016) for Gilgit-Baltistan region of Pakistan (18.12 g) and Khadivi-Khub et al. (2015) for Neiriz region, Iran (20.28 g). Kernel ratio is a highly important feature in determining the quality selections and an important character for improvement. This feature is influenced by walnut weight and kernel. Among all of the studied genotypes, 11 of them proved to be promising for new selections owing to higher kernel ratio i.e. > 50%. A high percentage of kernel ratio (3-year average) at the selections studied was found at B29 (54.02%), B5 (52.28%) and B16 (51.6%). Also, for kernel ratio, the data obtained in this study are higher than those obtained by Akca et al. (2015) to selections from Turkey (58.57%). The genotypes studied also had a high coefficient of variation (CV) for traits of nut weight (21.91-22.57%) and kernel weight (26.38-28.91%). Among characters measured, nut diameter date showed the lowest coefficient of variation (CV = 8.74-10.67%).

3.2. Nut and kernel traits variability among genotypes within the population

Quantitative phenotypic characters were subject to a two-way multivariate analysis of variance (MANOVA) to determine the relative percentage of variance explained in the population depending on climatic conditions and among individuals within the population surces of variation. The tests indicated the existence of highly significant differences between the 40 walnut genotypes in multivariate environments (group centroids), for the 2 analyzed years (2015 and 2017). Multivariate average values, in the year 2017, do vary significantly (P < 0.0001) depending on genoptypes, following tests are clearly conclusive: Wilks' lambda = 0.014, $F_{186,4514} = 25.733;$ Pillai'trace = 2.399, $F_{186,4608}$ = 16.505; Hotelling-Lawley trace = 10.128, $F_{186,4568}$ = 41.455. The same significant differences (P < 0.0001) between the 40 walnut genotypes in multivariate environments were also recorded for the year 2015 (Wilks' lambda = 0.003; $F_{210,5092} = 41.725$; Pillai'trace = 3.322, $F_{210,5178} = 30.58$; Hotelling-Lawley trace = 15.187, $F_{210.5138} = 61.929$. The MANOVA analysis of morphological data in 190 walnut genotypes from the two Italian regions (Campania and Abruzzo) was also used by Malvolti et al. (2010).

The same was achieved by simple ANOVA variance analysis for each variable (all differences were highly significant). ANOVA indicates whether the variables are different or not among the 40 genotypes, without indicating the genotypes between which the differences in size or weight are significant, which is why we applied the Tukey test that shows exactly the genotypes with significant differences. For the data obtained in 2017, within genotypes of the Bechet population, the Tukey test indicated the following significant differences (P < 0.0001): for the variable nut diameter (ANOVA $F_{31.768} = 3.854$) the genotype B29 was significantly superior to the other genotypes; for the variable nut length (ANOVA $F_{31,768} = 80.697$) the genotypes B29 and B8 were significantly superior to the other genotypes; for the variable nut weight (ANOVA $F_{31,768} = 99.331$) the genotypes B29 and B8 were significantly superior to the other genotypes; for the variable kernel weight (ANOVA $F_{31,768} = 19.166$) the genotype B29 was significantly superior to the other genotypes; and for the variable kernel ratio (ANOVA $F_{31,768} = 4.829$) the genotypes B29 and B6 were significantly superior to the other genotypes. Data obtained through ANOVA for the year 2015 have made the difference or not between genotype pairs. It is, however, noted that the same genotypes as group leader are generally retained for the studied characteristics. Individuals within populations

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