DOI: 10.5281/zenodo.5042962
CZU: 634.51:576.37

MOLDOVAN WALNUT (*JUGLANS REGIA* L.) BIOTYPES STUDIES FROM DIFFERENT POMOLOGICAL ZONES

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Abstract. The studies were focused on evaluation of perspective local walnut biotypes, selected from different natural populations of local pomological zones (North, North-West, Central and partially South) of the Republic of Moldova. The whitest shell color and high index of kernel/endocarp relation: 45-59% of kernel were noticed at 13 biotypes of north zone. Most of the biotypes had a moderate kernel fill and shriveling score. In the same time the highest score of aroma, flavor and sweetness intensity was noted in central pomological zone (1Cmd, 2Cmd, 3Cmd, 17Cmd). In general bitterness, puckeriness and sweetness assessments greatly change among local varieties and biotypes. Crispness rating of the biotypes was almost the same in all zones. Thus, we suppose the existence of genetically important walnut (*Juglans regia* L.) genetical resources within Moldovan investigated area.

Key words: *Juglans regia*; Biotypes; Fruit; Organoleptic properties; Fatty acids; Republic of Moldova.

INTRODUCTION

Walnuts in Republic of Moldova are traditional local domestic trees with highest socio economic impacts. Republic of Moldova is a country with ancient walnut culture tradition (Pintea, M. 2004; Pintea, M., Balan, V., Cimpoies, Gh. 2014). During a lot of centuries walnut trees are intensively outspreaded by seed multiplication. Main selected and used for establishing orchard biotypes was local bearing trees (also originated from seed) has good qualities and high productive potential. Actually, it is indispensable to grow adequate varieties in concordance with the specific arpo-ecological conditions and especially for modern market demands. Local varieties and selected biotypes are characterized by high resistance to biotic and abiotic factors. The presented researches are directed to valorization and conservation of important Moldovan biotypes from different pomological zones. The items of the cooperation project are the creation and organization of a data-base on genetic resources for either gene conservation and fruit production. Data-base include information on geographical location, ecophysiological, phenotypic characteristics, molecular genetics, biochemical and nutritional analysis and descriptions. This approach is presented within walnut researches effectuated in different regions of walnut culture (Mc Granaham, G., Leslie C.A. 1991; Warmund, M.R. 2009; Ercisli, S. et al. 2011; Yuemei, C.,et al. 2014; Unver, H et al. 2016; Bujdosó, G., Izsépi, F., Szügyiné Bartha, K., Varjas, V. and Szentiványi, P. 2020; Bujdosó, G., Cseke K. 2021).

MATERIALS AND METHODS

The material of this research consists of 70 walnut trees chosen during surveys around walnut growing areas in Moldova Republic: 11 trees from north west, 43 trees from central and 16 trees from north. From each tree, nuts were harvested at full ripe in September. From the nut samples traits which describe the morphological size and characteristic were measured according international UPOV guidelines (UPOV, 1999). The assessment enclosed walnut main descriptors, and sensory attributes including nut form, weight, structure (including index of shape form, total kernel weight, index of kernel/endocarp relation), kernel structure, color, flavor, texture, specific eating qualities, etc. Fruit descriptive sensory analysis were done by trained panelists, six kernels from each tree nut sample were presented to each panelist. Sensory evaluation were done mainly as described (Warmund, M. R. et al. 2009; Mosivand, M. et al. 2013). Oil and fatty acids content were defined by direct extraction with hexane, total protein determined by Lowry method. Fatty acids composition as well as vitamin E (tocopherols) were determined by high performance liquid chromatography (HPLC) using suitable methods and quantified by ELSD and UV detectors respectively (Malvolti, V. et al. 2010).

The data were subjected to statistical evaluation with GraphPad Prism 6. Pearson's correlation coefficients among nut traits were calculated to establish significant differences ($P \leq 0.05$ and $P < 0.01$). Valued and interested walnut trees has been present in all the typical walnut growing areas (N, NW, C). The selected trees have been individual characterized by its GPS localization, ecophysiological area and phenotypic characteristics. Samples of nuts and kernel from about 100 selected trees were collected in 2014 and 2015 for biochemical and molecular marker analyses. Kernels of nuts were analyzed for proximate contents and oil composition and vitamin E level were determined by HPLC. Tables show summary of preliminary results grouped by harvest area: north, north-west, central, south. For molecular marker analysis genomic DNA was extracted and purified from leaf tissue or defatted kernels. All samples were genotyped using 10 unlinked nSSR loci already used for characterization of (*Juglans regia* L.) walnut. PCR amplification fragments were collected and genotype profiles were assigned with Gene Mapper v. 4. Statistical analysis was carried out by the GenAlEx version 6 software. From the preliminary data presented in the Figure of the Principal Coordinates, is calculated that the first three axes expressed the 30.95 % of the total genetic variation. This value is low and demonstrates no genetic structure among populations. Data for each biotype's trees are and will be available and used for full evaluation.

RESULTS AND DISCUSSIONS

The primary characteristics of the selected biotypes of walnut were fructification and organoleptical proprieties of kernel. On the basis of comparative analysis of the most important biologic and agronomic characters of more than 100 biotypes we could consider relevant the following results. The nut shape of the biotypes, selected as important ones was mainly rated oblate to ovate. The biotypes of north zone were recorded to have the whitest shell color and high index of kernel/endocarp relation: 45-59% of kernel were noticed at 13 biotypes. Most of the biotypes had a moderate kernel fill and shriveling score. The highest score of aroma, flavor and sweetness intensity was noted in central pomological zone (1Cmd, 2Cmd, 3Cmd, 17Cmd). In general bitterness, puckeriness and sweetness assessments greatly change among local varieties and biotypes. Crispness rating of the biotypes was almost the same in all zones.

It should be noticed that the period of pistillate and staminate flowering period there are later around of 5-7 days in the North and North- West zones for Moldovan walnut in general. During the research years we could appreciate lateral flowering and bearing biotypes (fig. 1) in all zones, as well as cluster of inflorescences/fruits in central zone. Thus, on the basis of obtained data we suppose the existence of genetically important native trees (biotypes) all around the Moldovan investigated areas.

According the data of table 1 the most affected by environmental conditions are the characters: nut weight and kernel weight. In the frame of pomological zones, the genotypes of North are more changeable, being followed by North-West and Center. There is medium influence regarding correlation between kernel and nut weights, more expressed being in Center zone, followed by North and North-West zones.

Table 1. *Nut and kernel weights and % of some nutritional substances of nuts from North-West, Center and North pomological zones after selection of biotypes according organoleptical proprieties*

Cod of populations	Nut weight, g	Kernel weight, g	Kernel/nut %	Oil, %	Water, %	Proteins, %
Media NW±ES*	13,85±0,87	6,99±0,47	50,44±1,69	55,83±0,79	2,89±0,18	16,60±0,48
CV** NW	19.93%	21.48%	10.62%	4.49%	19,52	9.11%
Media C±ES*	14,56±0,72	6,61±0,34	45,91±1,87	54,66±0,80	3,06±0,11	17,80±0,74
CV** C	17.24%	17.91%	14.14%	5.13%	12,93	14.37%
Media N±ES*	14,07±1,10	6,26±0,41	44,96±1,66	60,40±0,67	2,76±0,08	15,72±0,83
CV** N	27.29%	22.80%	12.82%	3.88%	10,07	18,43%
Media total±ES*	14,18±0,52	6,60±0,23	46,91±1,06	57,03±0,61	2,914±0,07	16,72±043
CV**	21.37%	20.61%	13.22%	6.26%	14,67	15.13%

Table 2. *Fatty acids percentage composition in oil of selected biotypes (media of zones North, North-West and Center*

Cod of zones	Linolenic acid (ω3)	Linolic acid (ω6)	Palmitic acid	Oleic acid	Stearic acid	Vitamin E mg/g of kernel
Media NW±SE*	2,53±0,40	78,62±1,384	1,71±0,086	15,48±1,72	1,66±0,27	2,482±0,157
CV** NW	50.56%	5.57%	15.91%	35.09%	35.09%	20,03%
Media C±SE*	2,61±0,133	79,98±1,25	2,21±0,079	12,96±1,32	2,26±0,10	2,310±0,096
CV** C	17.65%	5.42%	12.43%	35.25%	15.63%	14,33%
Media N±SE*	3,02±0,23	72,09±2,93	2,42±0,14	20,26±2,80	2,21±0,10	2,446±0,119
CV** N	26.13%	14.08%	20.13%	47.88%	16.15%	16,84%
Media total±SE*	**2,7±0,15**	**76,79±1,31**	**2,14±0,08**	**16,28±1,29**	**2,06±0,10**	2,409±0,069
CV**	32.21%	9.96%	21.51%	46.10%	28.63%	16,92%

The obtained data show a slightly influence of environmental conditions on oil percent content and its density (tab.2). In our case the oil content is higher-up in Central zone, followed by North-West and North. The oil density is more expressed in North-West zone, followed by Center and North zones (tab. 2.). The obtained data of 2014 - 2018 years show the following composition of poly unsaturated oil acids: Linolenic Acid have maximal cotes in the probes of North zone-3,02%, small quantity in Center zone- 2,61%, and minimal for probes of North-West zone - 2,53%. Linolic Acid is present in maximal cotes in Central zone-79,98%, smaller – North-West-78,62% and minimum in North zone -76,92%.

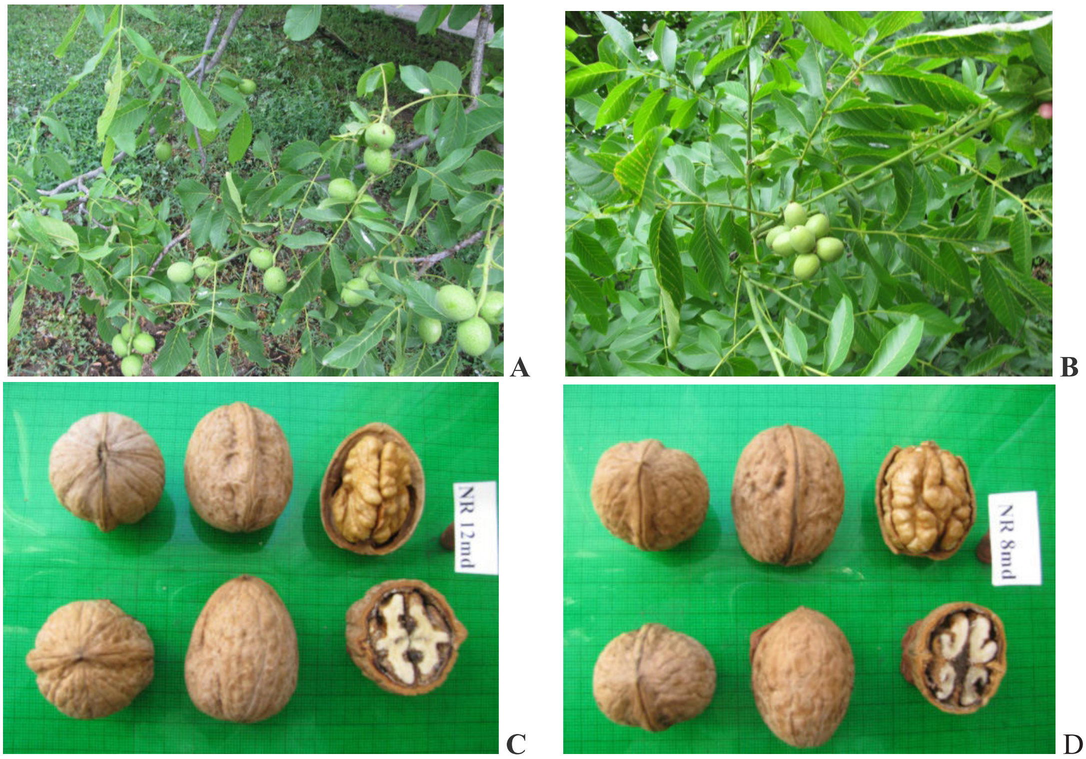

Figure 1. *A - lateral flowering and bearing biotype; - B- cluster of nuts, Central zone. C, D- nuts of biotypes of Central and North pomological zones*

For molecular marker analysis genomic DNA was extracted and purified from leaf tissue or defatted kernels. All samples were genotyped using 10 unlinked nSSR loci already used for characterization of Persian walnut. PCR amplification fragments were collected and the genotype profiles were assigned with Gene Mapper v. 4. Statistical analysis carried out by the GenAlEx version 6 software. From the preliminary data of the Principal Coordinates, is calculated that the first three axes expressed the 30.95 % of the total genetic variation. This value is low and don't demonstrate genetic structure among populations. The data for each tree are and will be available and used for full evaluation. The organization of a data-base on genotype/phenotype walnut resources including information on the geographical location, environmental characteristics, phenotypic, biochemical, nutritional, and genetic characteristics is of interest both for genfond conservation and quality nut food production. All these traits will be of importance for actions to support and enhance agriculture with manufacturing processes and social and economic development. From the survey and fruits analyses high variety together correlation for some specific traits were found, as example protein content versus nut weight or protein content and unsaturated fatty acids component of oil that make walnuts of high commercial and nutritional importance and to be in line to the UNECE standards (2010). At present, a restriction or change of the walnut distribution area can be the occurring, owing to climate and land-use changes. These occurrences are causing a considerable erosion of plant genetic resources; collection, characterization, propagation and sustainable use of walnut genetic resources, assessment of the adaptive potential and phenotypic plasticity, are therefore items of considerable importance both for the preservation in situ and ex situ biodiversity and basis for the improvement of new varieties and the prevention of the extinction of genetic sources.

Preliminary obtained results could be considered and examined with other European populations in the framework of a research devoted to investigate on the origin of walnut in Europe.

CONCLUSIONS

We suppose that on the basis of diversity of spreading biotypes in different pomological zones of country Rep. of Moldova could be considered as a "pan-population", the walnut genotypes are able to exchange genes by pollen cloud.

It should be accentuated that the trees with lateral fruit bearing potential, founded also in natural population, makes the country interesting for walnut improvement since this trait is very important to enhance the productivity aimed by breeders collection, characterization, propagation and sustainable use of walnut genetic resources, assessment of the adaptive potential and phenotypic plasticity, are therefore items of considerable importance both for the preservation *in situ* and *ex situ* biodiversity and basis for the improvement of new varieties and the prevention of the extinction of genetic sources. Two biotypes with lateral fructification and perspective indicators of fruits and fructification, selected from north and central pomological zones are transmitted to State Commission for testation.

REFERENCES

1. ERCISLI, S., KARA, M., OZTURK, I., SAYINCI, B., KALKAN, F. (2011). Comparison of some physico-mechanical properties of nut and kernel of two walnut (*Juglans regia* L.) cultivars. In: Notulae Botanicae Horti Agrobotanici, vol. 39 (2), pp. 227-231. DOI: 10.15835/ nbha3926045.
2. BUJDOSÓ, G., IZSÉPI, F., SZÜGYINÉ BARTHA, K., VARJAS, V., SZENTIVÁNYI, P. (2020). Persian walnut breeding program at Naric Fruticulture Research Institute in Hungary. In: Acta Horticulturae. vol. 1280, pp. 89-94. DOI: 10.17660/ActaHortic.2020.1280.13
3. BUJDOSÓ, G., CSEKE, K. (2021). The Persian (English) walnut (Juglans regia L.) assortment of Hungary: nut characteristics and origin. In: Scientia Horticultuae. vol. 283(39). DOI: https://doi.org/10.1016/j.scienta.2021.110035.
4. MALVOLTI, M.E., POLLEGIONI, P., BERTANI, A., MAPELLI, S., CANNATA, F. (2010). *Juglans regia* provenance research by molecular, morphological and biochemical markers: a case study in Italy. In: Bioremediation, Biodiversity and Bioavailability, vol. 4, pp. 84-92.
5. MC GRANAHAN, G. and LESLIE, C.A. (1991). Walnuts (*Juglans*). In: J.N. Moore and J.R.Jr. Ballington, eds. Genetic resources of temperate fruit and nut crops. International Society for Horticultural Science, Wageningen, pp. 907-951.
6. MOSIVAND, M., HASSANI, D., PAYAMNOUR, V., JAFAR AGHAEI, M. (2013). Comparison of tree, nut, and kernel characteristics in several walnut species and inter-specific hybrids. In: Crop Breeding Journal, vol. 3, no. 1, pp. 25-30.
7. PINTEA, M. (2004). Walnut: reproductive biology. Chisinau: F.E.-P.Central Printing. 366 p. (in Romanian).
8. PINTEA, M., BALAN, V., CIMPOIES, G. (2014). Following walnut footprints in Republic of Moldova. In: D. Avanzato et al., eds. Following walnut footprints (*Juglans regia* L.) cultivation and culture, folklore and history, traditions and uses. Leuven, International Society for Horticultural, Scripta horticulturae, vol. 17, pp. 203-211. ISBN 978-94-6261-003-3.
9. UPOV (1999). Guidelines for the conduct of tests for distinctness, uniformity and stability. Walnut (*Juglans regia* L.). Geneva, 34 p. Available: http://www.upov.org/en/publications/tg-rom/tg125/tg_125_6.pdf
10. ÜNVER, H., SAKAR, E., SÜLÜŞOĞLU, M. (2016). Determination of pomological and morphological characteristics with fatty acid composition of high kernel ratio walnut genotypes. In: Erwerbs-Obstbau, vol. 58(1), pp. 11–18. ISSN 0014-0309.
11. WARMUND, M.R., ELMORE, J.R., ADHIKARI, K., MCGRAW, S. (2009). Descriptive sensory analysis of light, medium, and dark colored kernels of black walnut cultivars. In: Journal of the Science of Food and Agriculture, vol. 89, pp. 1969–1972. ISSN:1097-0010.
12. YUEMEI, C., JUNMIN, D., CAIHONG, Z. (2014). The analysis on fat characteristics of walnut varieties in different production areas of Shanxi Province. In: Journal of Plant Studies, vol. 3, no. 1, pp. 28-34.

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Received: 22.04.2021
Accepted: 10.06.2021