

## STUDIES OF SOME MOLDOVAN WALNUT (*Juglans regia* L.) LOCAL GENOFOND CHARACTERISTICS

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**ABSTRACT.** *Juglans regia* L., Persian or English walnut, is an economically fruit crop species of edible kernels and widely planted in the temperate zones. Moldova has ancient walnut culture tradition. In the centuries out-spreaded by seed occurred. Orchards with Moldovan registered or foreign varieties are established. Searching and selection of walnut biotypes for establishing new varieties with high nut qualities and productive potential are important for future. Study focused on evaluation of local trees from natural population through biometric, sensory and biochemical traits is the aim of the present research. Surveys in the northern (N), North West (NW) and central (C) agro ecological zones of the Republic of Moldova were done and nuts were harvested from seventy trees of walnut from natural populations, for two consecutive years. Fruits analyses showed high variability: the whitest shell color in N zone, the highest flavor and sweetness intensity in C zone, the highest oil % content in N zone. Furthermore, correlation for some specific traits were found, protein content versus nut weight or versus unsaturated fatty acid in oil). On the basis of these results, the genetically valued native trees can be assessed.

**KEY WORDS:** *walnut, pre breeding, seed populations, local biotypes, fruit characteristics.*

### INTRODUCTION

Common or Persian walnut (*Juglans regia* L.) is an important fruit tree that has been grown since ancient times in Europe and Asia. Nowadays, walnut is commercially cultivated throughout southern Europe, northern Africa,

eastern Asia, USA and western South America. The walnut is one of the oldest fruit species present in the Republic of Moldova and it always had special economic and social significance. Significant research efforts have been done to make the walnut more profitable in terms of nut production, oil quality, wood production for furniture and leaves phytotherapy applications (Balan et al. 2001; Cociu 2007; Gajim 2005; Pinteau et al. 2014; Cosmulescu et al. 2014). It is generally accepted that *J. regia* L. is native to the mountain ranges of west-central Asia, extending from Xinjiang Province of Western China, to Iran, Caucasus and Eastern Turkey (McGranahan and Leslie 1991). In Europe walnut was brought by the Greeks in the 5<sup>th</sup>-8<sup>th</sup> century B.C. and the archeological excavation in Southern part of Moldova indicated that the walnut entered to Moldova during this period (Cociu 2007, Gajim 2005). During the last centuries over 90% of the walnut trees have been grown in vineyards, small orchards, gardens, courtyards, forest belts and along roadsides, all of which make difficult to obtain uniform high yield and uniform quality of fruits (Fulga 2005). A substantial collection of fruits for walnut germplasm has been done and as results of breeding programs new varieties or elite trees were selected in prospects to improve walnut cultivation and yield quality (Pinteau 2004; Tsurcanu and Comanici 2004). The most important premises for development of walnut culture in the Republic of Moldova are based on the "law of walnut", adopted in 1999 and more recently the National Program for the Development of Nuts Crops until 2020. To reach those goals searching and evaluation of the walnut germplasm biodiversity occurred in nature and wild growing is a sure premise.

Walnut biodiversity evaluation has been conducted in many countries throughout the world, high variations in nut traits have been observed, local walnuts with high variation are important in breeding programs (Sharma and Sharma 2001; Solar and Stampar 2003; Zeneli et al. 2005; Aslantas 2006; Martínez and Maestri 2008; Malvolti et al. 2010; Golzari et al. 2012; Cosmulescu and Botu 2012; Cosmulescu 2013; Yuemei et al. 2014).

In the present study surveys, in the local walnut pomological zones (north, north-west and central), were focused on selected trees from different natural populations for morphological, biochemical evaluation of nuts in the perspective to select and valorize walnut biotypes for future breeding programs.

## MATERIAL AND METHODS

**Plant material.** Seventy walnut trees were chosen during several yearly surveys around walnut growing areas in the northern and central agro ecological zones of the Republic of Moldova. Eleven trees from north west (NW), 16 trees from north (N) and 43 trees from central (C) were the material for this research. For two consecutive years (2014 and 2015) from each trees, nuts were harvested at full ripe in September. The nut traits were measured according the Union Internationale pour la Protection des Obtentions Végétales (UPOV) and the United Nations Economic Commission for Europe (UNECE) guidelines (UPOV 1999; UNECE 2010). Fruit descriptive sensory analysis were done by trained panelists, six kernels from each tree nut sample were presented to each panelist. Sensory evaluation was done mainly as described (Warmund et al. 2009; Mosivand et al. 2013).

**Proximate analyses.** Kernels from each genotypes were analyzed for the main biological compounds. The mature walnut fruits were deshelled and the kernel dehydrated by freeze-dry. The kernels were powdered in a porcelain mortar and mixed with hexane for oil extraction (Malvolti et al. 2010). Fatty acid (FA) analysis was conducted after hydrolysis of extracted oils from each kernel. Essentially, an aliquot of oil (5  $\mu$ l) was hydrolyzed in 1 ml of 1 % NaOH in MeOH, at 80°C for 30 min. The solution was dried under vacuum and the residue dissolved in 2 ml of H<sub>2</sub>O plus 0.3 ml of 1 M H<sub>2</sub>SO<sub>4</sub>, and then shaken energetically. The FAs, recovered from water sulfuric acid solution with 1 ml of hexane, were qualitatively and quantitatively analyzed on a High Performance Liquid Chromatography-Evaporative Light Scattering Detector (HPLC-ELSD) as described by Bravi et al. (2006) and Guo et al. (2012). Briefly, an HPLC system pump and degasser PU2089 (Jasco, Tokyo, Japan) equipped with Alltech 3300 ELSD (Grace, Deerfield, USA) was used. The FAs were separated on Luna 5  $\mu$ m C8 column (150  $\times$  4.6 mm) (Phenomenex, Bologna, Italy), using acetonitrile: isopropanol: water (50:30:20, v/v/v) at a flow rate of 1 ml/min for 15 min. Data from the detector were recorded, integrated and elaborated by the Borwin software program (JMBS Dev., Le Fontanil, France). The FAs were identified and quantified in comparison with FA standards (Fig 1A) (Sigma-Aldrich, St. Louis, USA), composition was determined in triplicate and the quantities of individual fatty acids were expressed as percentage of total identified FAs (w/w).

Tocopherols were analyzed in HPLC by direct injection of oil (Gimeno et al. 2000). The tocopherol isomers were separated using an HPLC Jasco Tritotar III and Jasco MD910 Diode Array Detector (DAD). An amount of 5-10  $\mu$ l of pure oil was loaded into a Kinetex 2.6  $\mu$ m C18 100A (Phenomenex) column (100  $\times$  4.6 mm) and eluted with 1.5 ml/min MeOH 95 %. The DAD spectra gave information on the purity of tocopherols. Absorbance at 280 nm was elaborated by the Borwin software system to determine tocopherol amounts in comparison with standards (Sigma-Aldrich, St. Louis, USA).

Protein was extracted from defatted flours using a Plant Total Protein Extraction Kit (Sigma-Aldrich, St. Louis, USA). Protein content was determined via a Quantum Protein Kit (Euroclone, Milan, Italy), using Bovine Serum Albumin (BSA) as standard.

Determination of starch content was by the colorimetric technique based on the method of Magel (1991), using potato starch (Sigma- Aldrich, St. Louis, USA) as standard. Total fiber was determined according to Association of Official Analytical Chemistry (AOAC) 2009.01 method with K-INDTF kit (Megazyme, Wicklow, Ireland).

Data obtained were subjected to descriptive statistical analyses (mean, standard error, elaborated with Microsoft Excel 2010 - Statistical Analysis and Graphpad Prism 6 software.

## RESULTS AND DISCUSSION

During the surveys conducted in the last years valued and interested walnut trees have been identified in all the typical walnut growing areas: north (N), north-west (NW), and central (C). The areas are characterized by annual average temperature of 7.5°C in N and NW and 10°C in C as well as different rainfall that only partially satisfies water requirement for tree species. The presence of variable complexity of soil creates many specific pedo-climatic condition also inside the three areas of surveys. Each selected tree was characterized by its GPS localization, ecophysiological area and tree phenotypic characteristics. It was highlighted that pistillate and staminate flowering period was about 5-7 days later in the north and north-west zones in comparison to the central zone. During the years, the lateral flowering and nut-bearing biotypes in all zones, and biotype with inflorescences/fruits clusters in central zone was also appreciated. Among hundreds trees considered, from 70 trees the nuts were harvested at full ripe during September 2014 and 2015 and completely characterized for morpho-biochemical traits.

Tables 1 and 2 show the main size, morphological and sensory traits from the three harvesting areas. It is evident that nuts weight were different in the examined areas. Normally the nut with higher weight showed lower percentage of edible kernel, well evident in samples 4 NW, 5 C and 22 N. Large part of nut samples showed kernel/nut ratio over, or around, 50 %, with maximum value over 56 % for nuts 2 NW and 17 C, both had also gained maximum points in the overall fruit evaluation. In general the shell

Table 1. Nut and kernel weights and percentage of edible material of nuts from some selected walnut trees.

Tree Code	Nut weight (g)	Kernel weight (g)	Kernel/Nut (%)	Overall fruit evaluation (points, 1-9) <sup>Z</sup>	Index of roundness <sup>Y</sup>
1 NW	16.46±0.027	7.99±0.05	48.70±0.42	7	122.20
2 NW	13.59±0.13	7.74±0.06	56.96±1.01	9	154.67
3 NW	13.47±0.27	6.81±0.04	50.23±0.78	9	127.19
4 NW	21.72±0.19	8.88±0.23	43.92±0.77	9	126.76
6 NW	13.12±0.22	6.30±0.32	47.33±1.55	7	179.35
9 NW	11.93±0.03	6.04±0.01	50.55±0.16	7	132.82
10 NW	10.72±0.09	5.14±0.15	48.01±1.20	7	151.51
11 NW	12.51±0.02	6.56±0.08	52.22±0.77	5	156.68
12 NW	16.05±0.10	7.96±0.17	49.57±0.80	5	137.86
13 NW	14.82±0.13	7.26±0.24	48.79±1.10	5	147.08
1 C	11.68±0.35	5.64±0.22	48.28±0.59	7	115.27
3 C	16.08±0.13	8.03±0.057	49.89±0.052	7	152.50
4 C	17.82±0.01	8.43±0.11	47.33±0.63	7	131.43
5 C	24.29±0.35	5.64±0.22	22.76±0.63	5	120.55
7 C	14.36±0.09	6.93±0.39	48.11±2.39	7	150.86
8 C	14.68±0.07	6.41±0.06	43.95±0.66	9	104.65
12 C	11.93±0.02	5.92±0.03	49.34±0.19	7	141.37
13 C	13.37±0.01	5.63±0.02	42.32±0.15	7	134.54
14 C	15.35±0.02	6.27±0.07	40.78±0.42	7	151.26
16 C	12.81±0.09	6.72±0.15	55.52±0.87	7	127.06
17 C	16.13±0.03	8.92±0.39	56.17±2.87	9	115.39
18 C	15.91±0.07	8.36±0.13	52.48±1.027	9	139.14
13 N	15.69±0.11	8.33±0.44	53.06±3.63	7	131.14
15 N	11.79±0.03	5.41±0.11	45.91±1.09	9	147.64
16 N	14.60±0.18	6.99±0.19	47.88±0.86	9	149.52
18 N	10.29±0.14	5.68±0.09	55.14±0.18	9	113.45
19 N	12.93±0.15	5.81±0.20	44.52±1.19	9	156.94
20 N	12.96±0.14	6.06±0.06	46.76±1.00	7	132.85
22 N	25.53±0.71	9.88±0.37	38.71±0.45	9	144.71
23 N	14.19±0.23	7.12±0.12	50.25±0.06	9	124.10
25 N	15.05±0.007	7.56±0.28	50.25±1.89	5	141.29
26 N	15.18±0.13	7.13±0.33	46.97±1.77		
27 N	11.70±0.37	6.29±0.27	53.54±1.26	7	147.38
28 N	14.23±0.54	7.24±0.28	50.87±0.09		

<sup>Z</sup> Overall fruit evaluation from 1 (low) to 9 (maximum)

<sup>Y</sup> Calculated by formula:  $R = (E + L)/2H$  (E = thickness; L = width; H = height)

Table 2. Main morphological and sensory traits of nuts from some selected walnut trees.

Tree Code	Shell appearance (points 1-8) <sup>X</sup>	Shell thickness (points 1-5) <sup>W</sup>	Adherence of shell (points 1-5) <sup>V</sup>	Kernel color (points 1-5) <sup>U</sup>	Overall nutty aroma (points 1-5) <sup>T</sup>	Overall sweetness (points 1-5) <sup>S</sup>
1 NW	6.4±0.4	2.8±0.16	3.8±0.3	2.8±0.16	4.2±0.3	3.4±0.41
2 NW	5.8±0.4	2.4±0.2	3.6±0.32	2.4±0.2	3.4±0.2	3.6±0.32
3 NW	7.0±0.26	2.6±0.2	4.8±0.16	4.0±0.0	4.0±0.0	1.8±0.4
4 NW	4.6±0.49	2.2±0.16	4.2±0.16	2.8±0.16	3.8±0.16	3.2±0.16
6 NW	5.4±0.32	3.0±0.0	4.0±0.0	2.4±0.2	3.4±0.2	2.8±0.3
9 NW	5.2±0.30	3.0±0.0	4.6±0.2	2.8±0.16	3.8±0.16	2.8±0.47
10 NW	5.4±0.41	3.0±0.0	5.0±0.0	3.0±0.0	4.4±0.2	3.4±0.49
11 NW	6.0±0.26	2.6±0.2	4.8±0.16	2.8±0.16	4.4±0.2	3.2±0.47
12 NW	5.8±0.16	3.0±0.0	4.4±0.2	3.0±0.0	4.2±0.16	1.8±0.6
13 NW	5.6±0.32	2.4±0.2	4.2±0.16	2.8±0.16	5.0±0.0	1.8±0.6
1 C	3.0±0.26	2.8±0.16	4.8±0.16	2.2±0.16	4.0±0.0	4.8±0.16
3 C	5.0±0.26	2.4±0.2	4.8±0.16	2.2±0.16	4.0±0.0	4.0±0.25
4 C	4.0±0.25	2.8±0.16	5.0±0.0	3.0±0.0	2.4±0.2	0.6±0.32
5 C	7.0±0.26	2.2±0.16	4.8±0.16	2.8±0.16	2.4±0.2	1.6±0.32
7 C	1.6±0.32	2.4±0.2	4.8±0.16	2.4±0.2	4.2±0.16	2.6±0.32
8 C	3.0±0.26	3.0±0.0	5.0±0.0	2.2±0.16	3.8±0.16	2.4±0.2
12 C	3.2±0.16	2.0±2.0	5.0±0.0	2.4±0.2	4.8±0.16	2.4±0.32
13 C	3.2±0.16	2.0±0.0	4.8±0.16	2.0±0.0	5.0±0.0	2.4±0.2
14 C	4.0±0.25	2.4±0.2	4.2±0.16	2.2±0.16	5.0±0.0	2.6±0.32
16 C	6.2±0.47	3.0±0.0	4.4±0.2	2.0±0.0	4.4±0.2	2.4±0.2
17 C	3.6±0.32	2.2±0.16	4.4±0.2	2.2±0.16	4.8±0.16	1.4±0.2
18 C	3.2±0.16	1.6±0.32	4.8±0.16	2.2±0.16	4.8±0.16	1.4±0.2
13 N	5.4±0.2	4.6±0.32	4.0±0.0	2.2±0.16	4.0±0.0	3.0±0.0
15 N	4.4±0.2	1.2±0.16	4.8±0.16	2.2±0.16	4.4±0.2	4.2±0.16
16 N	5.4±0.2	4.6±0.32	5.0±0.0	2.4±0.2	5.0±0.0	4.8±0.16
18 N	3.4±0.2	1.4±0.2	4.0±0.0	2.0±0.0	3.6±0.32	3.4±0.2
19 N	6.8±0.4	4.4±0.2	5.0±0.0	2.4±0.2	4.2±0.16	4.4±0.2
20 N	3.0±0.36	3.4±0.2	5.0±0.0	1.8±0.4	4.2±0.16	3.6±0.2
22 N	5.0±0.36	4.2±0.16	4.2±0.16	3.0±0.0	3.4±0.2	3.0±0.26
23 N	5.2±0.16	3.4±0.32	3.6±0.2	2.4±0.2	3.6±0.32	2.4±0.2
25 N	4.0±0.36	2.6±0.32	4.6±0.2	2.8±0.16	4.0±0.26	2.6±0.32
26 N	7.2±0.16	4.2±0.16	4.8±0.16	2.8±0.16	3.6±0.32	3.4±0.2
27 N	6.6±0.2	3.8±0.47	4.4±0.2	2.2±0.16	4.0±0.0	2.4±0.2
28 N	2.2±0.16	1.6±0.32	1.2±0.16	2.2±0.16	4.2±0.16	2.4±0.2

<sup>X</sup> 1, smooth; 2, slightly grooved; 3, grooved; 4, very grooved; 5, slightly wrinkled; 6, wrinkled; 7, very wrinkled; 8, embossed

<sup>W</sup> 1, very thin; 2, thin; 3, medium; 5, thick

<sup>V</sup> 1, very weak; 2, weak; 3, medium; 4, strong; 5, very strong

<sup>U</sup> 1, very light; 2, light; 3, medium; 4, dark; 5, very dark

<sup>T</sup> Intensity of all nutty characteristic from 1 (low) to 5 (maximum)

<sup>S</sup> Taste associated with sucrose or other sweet substances from 1 (low) to 5 (maximum)

appearance (Table 2) seemed more grooved in C area than in NW area, where shells were more wrinkled. Those differences, however, did not give rise to evident differences in shell thickness and adherence. The basic proximate composition of nuts was shown in Table 3. The percentage of oil in kernels ranged from 50 % to 60 % and more, with nuts from north area reaching values of 63 % and 66 % in samples 27 N and 18 N respectively. Higher values in oil content was normally associated to lower protein content (Table 3). Observing the oil fatty acid composition (Table 4 and Fig 1 B, C) it was clearly evident that over 90 % were represented by mono- and poly-unsaturated C:18 acids (oleic, linoleic and linolenic acids). The saturated acids, palmitic and stearic, represented a small fraction, that it is higher in nuts harvested in central area in comparison to the north and north-west areas. Furthermore, from Table 4 and comparing chromatograms (Fig 1 B, C) it was evident that some samples in all the agro ecological zones are characterized by high oleic acid content (see as example: 1 NW, 13 C, 18N, 27 N), this high oleic acid is always related to decrease in linoleic acid. The oleic acid content was reported to vary among walnut kernels, the typical percentage is around 15% of total fatty acids but it can reach values over 30% (Golzari et al. 2012; Yuemei et al. 2014; Ünver et al. 2016). It is well documented for many plant species (Canvin 1965; Izquierdo et al. 2006) and also for walnut nuts (Malvolti et al. 2010; Yuemei et al. 2014) that saturated acids are higher in warmer areas, and the present results were in agreement with the reported data. The results on nuts proximate composition, oil content, and fatty acid composition were comparable to those reported for two Moldavian walnut varieties (Bernic et al. 2007). The tocopherols showed a relative high values but seemed very stable in all genotypes analyzed (Table 3). From a preliminary statistical analysis the Pearson correlation ( $r$ ) coefficient showed a positive correlation of nut weight versus protein content and, more interesting, correlations between protein content and unsaturated fatty acids.

Evaluation of morphological and biochemical characteristics have been reported as one of the tools for studying walnut genotypes and biodiversity

Table 3. Distribution of the main components of nuts from some selected walnut trees.

Tree Code	Water (%)	Proteins (%)	Starch fiber (%)	Vitamin E $\mu\text{g} / \mu\text{l}$ of oil	Oil (%)	Oil density
1 NW	2.77±0.18	17.38±0.36	24.07±0.68	2.44±0.02	55.78±0.14	0.912±0.001
2 NW	2.29±0.11	15.09±0.079	22.80±1.69	2.83±0.08	59.82±1.73	0.920±0.002
3 NW	3.11±0.01	15.65±0.54	26.10±1.98	1.83±0.16	55.14±1.44	0.925±0.001
4 NW	3.85±0.62	18.12±0.81	25.48±2.75	2.37±0.05	52.55±1.32	0.907±0.001
6 NW	2.22±0.19	17.81±0.94	24.21±0.49	1.72±0.06	55.76±0.26	0.908±0.001
9 NW	3.45±0.28	18.18±0.58	26.38±0.71	2.67±0.09	51.98±1.57	0.920±0.001
10 NW	2.96±0.26	14.44±0.41	24.90±0.72	3.25±0.07	57.71±1.39	0.924±0.001
11 NW	2.76±0.05	18.02±0.48	24.09±1.56	2.74±0.04	55.14±1.13	0.922±0.001
12 NW	2.17±0.16	16.71±0.16	25.71±1.11	2.06±0.15	55.41±0.79	0.916±0.001
13 NW	3.34±0.22	14.64±0.14	22.96±1.11	2.93±0.37	59.05±0.763	0.937±0.001
1 C	3.27±0.86	16.38±0.75	21.19±0.35	1.73±0.04	59.16±0.24	0.927±0.001
3 C	3.34±0.29	19.86±0.003	24.11±0.29	2.81±0.04	52.69±0.59	0.930±0.002
4 C	3.61±0.44	17.15±1.59	27.09±1.64	2.51±0.17	52.15±0.38	0.924±0.001
5 C	2.69±0.39	21.92±0.72	24.48±1.61	2.03±0.05	50.91±1.28	0.935±0.002
7 C	2.47±0.06	17.24±1.76	22.93±2.09	1.79±0.02	57.36±0.38	0.919±0.011
8 C	2.98±0.07	17.22±0.11	22.11±0.79	2.54±0.07	57.69±0.62	0.939±0.005
12 C	3.35±0.19	16.28±0.70	26.54±2.67	2.35±0.027	53.83±1.77	0.932±0.001
13 C	2.62±0.02	16.78±0.27	26.67±0.81	2.06±0.05	53.93±1.11	0.915±0.002
14 C	3.31±0.46	19.74±1.08	25.88±1.72	2.51±0.05	51.07±0.19	0.933±0.001
16 C	3.54±0.53	12.00±0.75	26.73±1.62	2.42±0.22	57.72±0.34	0.925±0.001
17 C	2.99±0.06	20.22±0.68	23.05±0.17	2.52±0.12	53.74±0.91	0.935±0.001
18 C	2.58±0.11	18.86±1.45	22.85±0.63	2.47±0.22	55.71±0.92	0.922±0.005
13 N	2.83±0.15	17.88±0.35	18.71±0.77	2.45±0.17	60.58±1.27	0.921±0.003
15 N	2.59±0.18	14.07±1.19	22.91±1.51	2.96±0.37	60.42±2.87	0.926±0.006
16 N	3.01±0.26	12.05±1.46	24.03±1.83	1.83±0.11	60.92±3.56	0.935±0.001
18 N	2.24±0.01	11.82±0.17	19.85±0.96	2.77±0.13	66.09±1.15	0.922±0.002
19 N	3.04±0.06	17.66±0.962	20.25±1.45	2.28±0.08	59.09±2.47	0.928±0.014
20 N	2.49±0.01	17.63±0.74	20.38±1.09	2.49±0.05	59.49±0.35	0.925±0.008
22 N	2.51±0.09	17.45±0.21	22.02±0.29	1.86±0.03	58.02±0.41	0.925±0.001
23 N	3.16±0.12	15.00±0.31	22.48±1.19	2.95±0.13	59.36±1.38	0.918±0.007
25 N	2.84±0.19	19.48±0.65	19.46±1.89	2.29±0.01	58.22±1.06	0.932±0.008
26 N	3.01±0.08	16.99±0.39	21.77±0.84	3.05±0.10	58.22±0.53	0.931±0.002
27 N	2.87±0.31	10.74±1.39	23.07±2.35	2.22±0.03	63.31±4.06	0.911±0.005
28 N	2.55±0.045	17.91±0.55	18.45±1.37	2.22±0.01	61.1±1.97	0.920±0.003

(Ercisli et al. 2011; Malvolti et al. 2010; Ünver et al. 2016; Yuemei et al. 2014; Cosmulescu et al. 2010), the survey and result presented can be a valid contribution in the Moldovian walnut biodiversity study. In our re-

search, genetic analysis with appropriate ISSR markers designated for walnut (Pollegioni et al. 2009) are in progress, with the aim to ascribe the

Table 4. Fatty acids percentage composition in oil of selected walnut trees

Tree Code	Linolenic acid ( $\omega$ 3)	Linoleic acid ( $\omega$ 6)	Palmitic acid	Oleic acid	Stearic acid
1 NW	1.35±0.28	71.53±1.78	1.46±0.02	24.70±1.3	0.97±0.16
2 NW	2.97±0.06	76.89±1.09	1.69±0.10	16.79±0.55	1.66±0.50
3 NW	1.99±0.22	78.38±0.13	2.26±0.06	16.21±0.18	1.14±0.10
4 NW	5.56±0.94	81.53±0.33	2.12±0.17	6.83±0.37	3.96±0.40
6 NW	2.07±0.03	79.92±1.57	1.53±0.14	15.07±1.48	1.42±0.02
9 NW	1.36±0.19	81.00±0.10	1.57±0.01	14.65±0.05	1.42±0.06
10 NW	3.74±0.16	81.45±0.99	1.55±0.043	11.95±0.89	1.31±0.01
11 NW	2.04±0.05	74.13±1.82	1.77±0.06	20.64±1.87	1.42±0.07
12 NW	2.21±0.09	74.92±1.97	1.62±0.03	19.42±1.96	1.83±0.06
13 NW	2.01±0.14	86.46±0.57	1.52±0.03	8.55±0.58	1.45±0.18
1 C	3.37±0.29	83.08±2.06	2.41±0.42	9.50±1.02	1.64±0.32
3 C	2.37±0.17	82.14±0.25	2.29±0.01	10.38±0.08	2.81±0.15
4 C	2.37±0.09	76.40±0.95	2.30±0.13	16.68±0.73	2.25±0.19
5 C	2.75±0.40	83.56±0.07	1.64±0.10	9.25±0.30	2.81±0.06
7 C	1.73±0.16	82.02±0.68	2.28±0.14	11.56±0.42	2.41±0.24
8 C	2.31±0.48	80.93±1.05	2.45±0.032	12.21±1.42	2.09±0.09
12 C	3.12±0.14	84.13±0.69	2.64±0.29	7.90±0.03	2.22±0.29
13 C	3.02±0.14	71.61±2.84	1.91±0.12	21.75±2.98	1.72±0.16
14 C	2.54±0.03	72.12±1.37	1.93±0.28	21.13±1.09	2.28±0.03
16 C	2.58±0.39	79.63±0.36	2.15±0.04	13.46±0.69	2.19±0.08
17 C	2.17±0.05	83.42±0.37	2.11±0.17	9.87±0.56	2.43±0.03
18 C	2.96±0.14	80.67±0.21	2.34±0.04	11.82±0.23	2.21±0.08
13 N	2.18±0.11	81.30±0.26	3.43±0.23	11.37±0.23	1.72±0.15
15 N	2.65±0.42	81.02±2.88	2.27±0.13	11.76±2.79	2.30±0.21
16 N	3.49±0.05	65.47±5.97	2.09±0.15	26.46±5.87	2.49±0.29
18 N	3.52±0.35	56.86±0.63	2.85±0.31	35.17±0.16	1.59±0.13
19 N	3.33±0.14	57.30±2.96	2.22±0.15	35.02±3.18	2.13±0.07
20 N	2.42±0.05	79.66±0.71	2.66±0.10	12.68±0.89	2.58±0.13
22 N	1.91±0.06	79.30±0.65	2.11±0.02	14.69±0.42	1.99±0.31
23 N	2.62±0.18	83.79±0.95	2.02±0.34	9.56±0.33	2.01±0.11
25 N	2.85±0.19	77.83±0.65	2.33±0.10	14.61±0.30	2.38±0.06
26 N	3.77±0.24	69.80±0.33	2.97±0.34	20.85±0.11	2.61±0.14
27 N	4.77±0.053	58.50±0.07	1.64±0.10	33.05±0.40	2.05±0.26
28 N	2.77±0.007	74.20±0.53	2.46±0.11	17.85±0.65	2.71±0.24

differences observed to genotype biodiversity or to the interaction of phenotype with environmental condition.

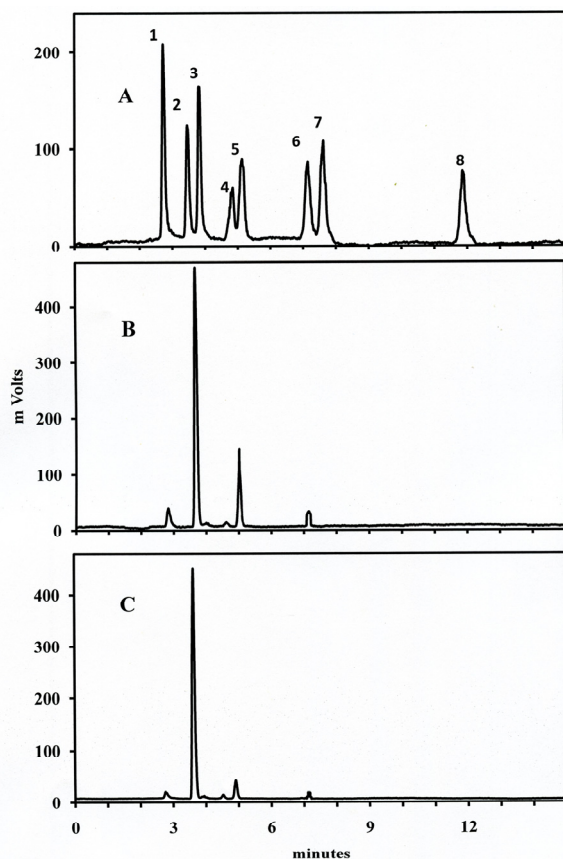


Figure 1. Fatty acid analysis on HPLC-ELS detector: A, standard fatty acid chromatogram; B, fatty acid chromatogram of 27 N nut sample rich in oleic acid; C, chromatogram 3 NW nut sample with low oleic acid content (bottom). Peak 1, linolenic acid (C18:3); peak 2, palmitoleic acid (C16:1); peak 3, linoleic acid (C18:2); peak 4, palmitic acid (C16:0); peak 5, oleic acid (C18:0); peak 6, stearic acid (C18:0); peak 7, eicosatrienoic acid (C20:3); peak 8, erucic acid (C22:1).

## CONCLUSIONS

The studies on genotype/phenotype walnut resources are of interest both for genofond conservation and quality nut food production. Those studies will be of importance for actions to support and enhance sustainable agriculture and fruit food industry processing as well as the social and economic development. From the surveys and fruits analyses high variability were found: the whitest shell color in N zone, the highest flavor and sweetness intensity in C zone, the highest oil % content in N zone. Furthermore, correlation for some specific traits were also found (protein content versus nut weight or versus unsaturated fatty acids component of oil). All confirm that through Moldovan walnut nuts some biotypes can have high commer-

cial and nutritional importance and are in agreement with the UNECE standards (UNECE 2010). At present, a restriction or change of the walnut distribution areas 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. Thus, on the basis of obtained data, it is clearly inferred the existence of genetically important native trees (biotypes) all around the natural wild walnut growing and investigated areas in the Republic of Moldova.

**ACKNOWLEDGEMENT.** The research was done in the frame of cooperation agreement between National Research Council (CNR), Italy and Academy of Sciences of Moldova (ASM), Rep. Moldova.

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