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EVALUATION OF FOUR SEA BUCKTHORN BIOTYPES FROM THE SPONTANEOUS FLORA OF ARGEŞ COUNTY, ROMANIA

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Abstract

Hippophae rhamnoides L. subsp. carpatica Rousi is one of the nine subspecies of the genus Hippophae, which belongs to the family Elaeagnaceae and its native area is East - Central and South - East Europe (Austria, Germany, Hungary, Romania, Serbia). In Romania, sea buckthorn grows spontaneously in the Subcarpathian area of Moldova and Muntenia but also may be found down to Black Sea coast, where sometimes forming groves or even dense bushes. This study aims to identify new biotips for horticulture studies, in breeding programs, as well to ensure the preservation of germoplasm resources of interest to spontaneous species of Hippophae rhamnoides out of the natural enviroment from the center of Romania. For this purpose, four biotypes named 'Leordeni 3', 'Leordeni 4', 'Leordeni 5' and 'Leordeni 6', were selected during 2020-2021 in Leordeni area from Argeş County. All these native biotypes were subjected to study in order to evaluate the fruits quality traits and the results were compared with 'Piteşti 1' cultivar. These researches identified the selection 'Leordeni 4' with the highest values of Vitamin C: 142,56 (mg/100 g FW) and also in the total of polyphenols content: 18,97 (mg gallic acid equivalent/ 100 g FW) meanwhile 'Leordeni 6' had good results at fruit weigh 0.59 (g) and soluble solid 12.62 (°Brix).

Key words*: sea buckthorn fruits, biochemical constituents, biometrical measurements, wild flora.*

INTRODUCTION

Hippophae rhamnoides L. subsp. *carpatica* Rousi is one of the nine subspecies of the genus Hippophae , which belongs to the family Elaeagnaceae and its native area is East - Central and South - East Europe (Austria, Germany, Hungary, Romania, Serbia) (Yongshan et al., 2003; Ilhan G et al., 2021).

In Romania, sea buckthorn grows spontaneously in the Subcarpathian area of Moldova and Muntenia ranging from 0 to 1200 m. In the plate area, the sea buckthorn is found in riverbeds (Bistrita Valley, Siret Valley, Buzau Basin, Danube Delta), also in the hilly and submontane area exploring the sunny slopes. Sea buckthorn, being so widespread in wild flora, all cultivated Romanian varieties has the origin from wild flora (Ancu et al., 2017).

The plant has a great environmental plasticity and is extremely resistant to adverse ambient conditions, thus, it can be used for afforestation and wasteland management, which has led to its large-scale planting. It is resistant to urban conditions. Furthermore, as a xerophyte species, it tolerates drought, cold (up to −40°C), heat (up to 40°C), soil salinity, and air pollution. Sea buckthorn is a fruit species with a high nitrogen fixation capacity, thus improving soil quality; moreover, because of its extensive root system, it exhibits soil-binding properties (Ilhan G et al., 2021).

This study aims to identify new biotips for horticulture studies, in breeding programs, as well to ensure the preservation of germoplasm resources of interest to spontaneous species of Hippophae rhamnoides out of the natural enviroment from the center of Romania. Therefore, in this study, we attempted to determine the basic agro-morphological and biochemical traits of 4 seed-propagated sea buckthorn genotypes that grow naturally in the flora of Leordeni area from Argeş County, located in the center of Romania and the results were compared with 'Piteşti 1'cultivar.

MATERIALS AND METHODS

Research Institute for Fruit Growing Pitesti-Maracineni conducted numerous studies of exploring the wild flora in different areas of the country to identify new biotips for horticulture

studies, to be used in breeding programs, as well to ensure the preservation of germoplasm resources of interest to spontaneous species out of the natural enviroment from Romania.

For this purpose, four biotypes named ’Leordeni 3’, ’Leordeni 4’, ’Leordeni 5’ and ’Leordeni 6’, were selected during 2020-2021 in Leordeni area from Argeş County. All these native biotypes were subjected to study in order to evaluate the fruits quality traits and the results were compared with ’Piteşti 1’ cultivar.

The samples were harvested at the optimal stage of maturity, between the second and the last decade of September.

The average weight of a fruit was determined by the weighing of a sample of 20 fruits with an electronic balance with an accuracy of 0.01 g, for each genotype and expressed in g/fruit.

The fruit length and diameters were measured using a calliper of a sample of 20 fruits for each genotype and expressed in mm.

The fruit size index was determined by measuring the length, large and small diameter (the longitudinal and polar diameter) for all 20 fruits of a sample for each genotype. The index size was calculated by formula: (height + large diameter + small diameter)/3 (Botu, 1997).

The fruit firmness was determined with a non-destructible penetrometer on a number of 20 fruits per genotype and expressed in units HPE.

Chemical analyzes and laboratory determinations were performed in three repetitions, this consisted in determining the soluble dry matter, organic acids, total sugar, vitamin C and total polyphenols.

Soluble solids content was determined in fruits juice by using a digital refractometer (Kern) and the results were reported as °Bx at 20°C (AOAC, 1999).

Total dry matter content was determined after extraction of water content for 8 hours at 105°C (AOAC, 1999) and expressed as g/100 g DW.

The organic acids (%) were measured by titration of the sampled fruits juice with a solution of 0.1 N NaOH, the results were expressed in g malic acid/100 g FW; ascorbic acid by the iodometric method and expressed in mg/100 g FW and total sugars (%) by the Fehling-Soxhlet method, 1965 (AOAC, 1968).

The acidity was determined by titration using of the sampled fruits juice (Ermakov et al., 1987); the results were expressed in g malic acid/100 g FW.

The pH of fruit juice was measured by squeezing the sea buckthorn fruits, using a multimeter C- 561, calibrated with 7 and respectively 4 pH solutions (AOAC, 1999).

The determination of total polyphenols was performed spectrophotocolorimetrically, by the Folin-Ciocâlteu method (Singleton et al, 1999) and was expressed as mg GAE/g fresh fruit.

The results were statistically calculated by Duncan´s Test at a significance level = 0.05.

RESULTS AND DISCUSSIONS

PHYSICAL CHARACTERISTICS OF THE FRUIT

Average weight of the fruit (g)

Regarding the average weight / fruit, the average of the 2020-2021 study years showed a significant difference between the genotypes studied as follows: 'Pitești 1' (0.57 g/fruit), 'Leordeni 3' (0.56 g/fruit) and 'Leordeni 6' (0.59 g/fruit) were statistically different from the genotypes 'Leordeni 4' (0.43 g/fruit) and 'Leordeni 5' (0.44 g/fruit) (Table 1).

Table 1. The evolution of average fruit weight (g)

Genotypes	Weight (g)		
	2020	2021	2020-021
Pitesti 1	0.57±0.08^{a}	0.57±0.06^{a}	0.57±0.07^{a}
Leordeni 3	0.60±0.08^{a}	0.52±0.04^{b}	0.56±0.07^{a}
Leordeni 4	0.43±0.04^{b}	0.42±0.02^{d}	0.43±0.03^{b}
Leordeni 5	0.41±0.06^{b}	0.46±0.03^{c}	0.44±0.05^{b}
Leordeni 6	0.60±0.03^{a}	0.57±0.05^{a}	0.59±0.04^{a}
Coeficient pearson	p=0.000	p=0.000	p=0.002

For 2020 and for 2021 the genotypes 'Leordeni 6' (0.60 g/fruit)/(0.57 g/fruit) and 'Pitești 1' (0.57 g/fruit)/(0.57 g/fruit) recorded the highest values. The results are in accordance with data from the literature Topala et al. (2020) obtained an average value of a fruit of 0.53 ± 0.17 g.

Size index of the fruit

In the case of the size index, the 'Pitești 1' genotype (10.31) had the highest average compared to the other genotypes studied in the two years, the lowest average values being

registered by the 'Leordeni 4' selections (8.62), respectively 'Leordeni 5' (8.79) (Table 2).

Table 2. The evolution of size index

Genotypes	Size index		
	2020	2021	2020- 2021
Pitesti 1	10.59±0.45^{a}	10.02±0.47^{a}	10.31±0.54^{a}
Leordeni 3	10.12±0.44^{b}	9.09±0.51^{b}	9.60±0.70^{c}
Leordeni 4	9.01±0.34^{c}	8.24±0.17^{c}	8.62±0.47^{d}
Leordeni 5	8.82±0.50^{c}	8.77±0.30^{b}	8.79±0.40^{d}
Leordeni 6	10.14±0.31^{b}	9.88±0.27^{a}	10.01±0.31^{b}
Coeficient pearson	p=0.000	p=0.000	p=0.000

For the year 2020 the genotype 'Pitești 1' (10.59) was followed by 'Leordeni 6' (10.14) and 'Leordeni 3' (10.12); the genotypes 'Leordeni 4' (9.01) and 'Leordeni 5' (8.82) remain the smallest.

The year 2021 had the same leader: 'Pitești 1' (10.02) followed by 'Leordeni 6' (9.88); the genotypes 'Leordeni 3' (9.09) 'Leordeni 5' (8.77) and 'Leordeni 4' (8.24) had lower averages.(Ancu et al., 2017) had similar results with values ranging between 7.07-9.80.

Table 3. The evolution of firmness

Genotypes	Firmness		
	2020	2021	2020- 2021
Pitesti 1	39.73±4.03^{a}	36.93±6.63^{a}	38.33±5.53^{a}
Leordeni 3	27.49±7.60^{b}	31.05±6.52^{b}	29.27±7.13^{b}
Leordeni 4	34.41±7.22^{a}	29.55±3.29^{b}	31.98±6.00^{b}
Leordeni 5	27.84±8.90^{b}	37.61±8.70^{a}	32.73±9.92^{b}
Leordeni 6	37.70±6.81^{a}	29.43±5.10^{b}	33.57±7.23^{b}
Coeficient pearson	p=0.000	p=0.007	p=0.203

Firmness of the fruit (N)

Analyzing the average values of the two years of study in the case of Firmness we can see according to the data in Table 3 that the genotype 'Pitesti 1' (38.33 N) had the highest value compared to the other selections.

BIOCHEMICAL CHARACTERISTICS OF THE FRUIT

Soluble dry matter content (°Bx)

From the data recorded in Table 4 it is observed that the genotypes significantly influenced the soluble dry matter content in the two years of study obtaining five statistical classes with values between 11.10°Bx in 'Leordeni 5' and 12.86°Bx in 'Leordeni 3'.

In 2020 the genotypes 'Leordeni 3' (12.43) and 'Leordeni 6' (12.26) had the highest values and in 2021 they were exceeded: 'Leordeni 3' (13.28) and 'Leordeni 6' (12.98). Similar results were found at (Ancu et al., 2017) which has an average variation between 5.28 and 9.76.

Table 4. The evolution of soluble solid °Bx

Genotypes	Soluble solid °Bx		
	2020	2021	2020- 2021
Pitesti 1	11.46±0.97^{bc}	12.50±0.73^{b}	11.98±0.99^{b}
Leordeni 3	12.43±0.48^{a}	13.28±0.44^{a}	12.86±0.63^{a}
Leordeni 4	11.97±0.67^{ab}	11.20±0.42^{c}	11.59±0.67^{c}
Leordeni 5	10.88±0.59^{c}	11.32±0.39^{c}	11.10±0.54^{d}
Leordeni 6	12.26±0.55^{a}	12.98±0.27^{a}	12.62±0.56^{a}
Coeficient pearson	p=0.000	p=0.000	p=0.000

pH of the fruits

For pH, mean values varied between the two years of study; according to them, the highest value was registered by the genotype 'Leordeni 5' (2.95) and the lowest being at 'Pitești 1' (2.60) (Table 5). Similar results were found at (Ancu et al., 2017) which has an average variation between 2.33 and 2.99.

Table 5. The evolution of pH

Genotypes	pH		
	2020	2021	2020- 2021
Pitesti 1	2.68±0.07^{c}	2.52±0.11^{c}	2.60±0.12^{c}
Leordeni 3	2.86±0.11^{b}	2.79±0.09^{a}	2.83±0.10^{b}
Leordeni 4	2.89±0.12^{b}	2.70±0.04^{b}	2.80±0.13^{b}
Leordeni 5	3.11±0.12^{a}	2.78±0.04^{a}	2.95±0.19^{a}
Leordeni 6	2.93±0.09^{b}	2.73±0.09^{ab}	2.83±0.13^{b}
Coeficient pearson	p=0.000	p=0.000	p=0.000

Table 6 shows the evolution of the average values of the total organic acid content, Vitamin C content, total sugar content and total polyphenol content of the sea buckthorn fruits studied.

Total dry matter content (%)

Total dry matter content was significantly influenced by genotype. The mean values of the total dry matter content are grouped into four different series (Table 6). There is a significant decrease in the total dry matter content of the 'Leordeni 5' genotype (18.49%) compared to the other genotypes studied. The highest total dry matter content was recorded in

the 'Leordeni 6' genotype (21.61%). The results are in accordance with the data in the literature (Olteanu et al., 2007) showing a variation of the total dry matter content of sea buckthorn fruits from 15 g % to 19.43 g %.

Total titratable acidity expressed as malic acid (%)

It is found that the average total content of organic acids decreased significantly starting from the genotype 'Pitesti 1' (3.32%) followed by the genotype 'Leordeni 6' and 'Leordeni 5' (3.01% and 2.88%). The lowest average content of organic acids expressed as malic acid in fruit was obtained in the 'Leordeni 2' genotype (2.60%). Variations in the organic acid content of fruits have been reported in the literature from 0.26% to 2.89% (Kawecki et al., 2004). The improvement of fruit quality is determined by the content of sugar and organic acids (Raffo et al., 2004).

Total sugar content (%)

Total sugar content was significantly influenced by genotype. The average values of the total sugar content of sea buckthorn fruits are grouped into three different series. The genotype with the highest average total sugar content was 'Pitești 1' (2.37%) followed by 'Leordeni 3' and 'Leordeni 6' (2.33% and 2.26% respectively). The lowest total sugar content was obtained in the genotype 'Leordeni 4' (2.07%). Kawecki et al., 2004, obtained a total sugar content in sea buckthorn fruits between 1.99% and 2.78 %.

Table 6. The evolution of the average values of the biochemical values

Genotypes	Soluble solid content (%)	Titratable Acidity (%)	Total Sugar %	Sugar/Acidity ratio	Vitamina C (mg/100g FW)	Total Phenolic content (mg GAE/g FW)
Pitesti 1	20.02 ± 0.20^{c}	3.32 ± 0.09^{a}	2.37 ± 0.12^{a}	0.72 ± 0.05^{b}	91.53 ± 1.36^{c}	14.71 ± 0.14^{c}
Leordeni 3	20.57 ± 0.10^{b}	2.60 ± 0.09^{d}	2.33 ± 0.09^{a}	0.90 ± 0.01^{a}	93.28 ± 0.92^{cd}	16.233 ± 0.09^{b}
Leordeni 4	19.92 ± 0.06^{c}	2.81 ± 0.06^{c}	2.07 ± 0.06^{c}	0.73 ± 0.01^{b}	142.56 ± 0.45^{a}	18.973 ± 0.09^{a}
Leordeni 5	18.49 ± 0.14^{d}	2.88 ± 0.13^{bc}	2.14 ± 0.07^{bc}	0.74 ± 0.02^{b}	113.96 ± 5.67^{b}	10.897 ± 0.36^{d}
Leordeni 6	21.61 ± 0.08^{a}	3.01 ± 0.08^{b}	2.26 ± 0.09^{ab}	0.75 ± 0.01^{b}	96.80±1.01c	16.043 ± 0.09^{b}
Coeficient pearson	p=0.000	p=0.000	p=0.008	p=0.000	p=0.000	p=0.000

Sugar/organic acid ratio

According to (Tang et al., 2001) increasing the sugar/organic acid ratio is an important factor in improving the flavor of sea buckthorn fruits. Sea buckthorn fruits are known to have a low sugar content and a high acid (Rongsen, 2006). Ohkawa et al., 2009, obtained a ratio of sugar/acid content between 0.34 and 1.14. In the studied genotypes, the highest ratio of sugar/acid content was obtained for the 'Leordeni 3' genotype (0.90).

Vitamin C content (mg/100 g FW)

Vitamin C content was significantly influenced by genotype. There are four different homogeneous series of Vitamin C content in the sea buckthorn genotypes studied (Table 6). The genotype with the highest Vitamin C content was 'Leordeni 4' (142.56 mg/100 g FW) followed by 'Leordeni 5' (113.96 mg/100 g FW) and 'Leordeni 6' (96.80 mg/100 g FW). The 'Pitesti 1' genotype had the lowest Vitamin C content (91.53 mg/100 g FW).

Total polyphenol content (mg GAE/g FW)

In the sea buckthorn genotypes, the content of total polyphenols varied between 10.90 mg GAE/g FW (genotype 'Leordeni 5') and 18.97 mg GAE / g FW (genotype 'Leordeni 4'). Similar results were found at (Criste et al.,

2020) with average values ranging between 10.12 and 18.66 mg GAE/g FW.

The evaluation of Table 7, highlights the correlations obtained between some biometric indicators of sea buckthorn fruits. Thus we can observe that there is a positive correlation, distinctly significant between the average weight of the fruit and the size index (r = 0.758), between the average weight of the fruit and the soluble dry matter content (r = 0.444). Also, the average weight of the fruits correlates negatively significantly with the pH of the fruit juice (r = - 0.231) and positively significantly with the textural firmness (r = 0.245). It is observed that the size index correlates positively, distinctly significantly with the soluble dry matter content (r = 0.274) and with the textural firmness (r = 0.327). Also, the size index has a significant negative correlation with the pH of fruit juice. There is also a significant negative correlation between the soluble dry matter content and the pH of the fruit (r = 0.215).

Table 7. Correlations

		Average fruit weight (g)	Size index (mm)	Soluble solid °Bx	pH	Firmness (N)
Greutate medie fruct (g)	Pearson Correlation	1				
Indice de marime (mm)	Pearson Correlation	0.758(**)	1			
Substanță uscată solubilă % Brix	Pearson Correlation	0.444(**)	0.274(**)	1		
pH	Pearson Correlation	-0.231(*)	-0.237(*)	-0.215(*)	1	
Fermitate (N)	Pearson Correlation	0.245(*)	0.327(**)	-0.008	-0.176	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

CONCLUSIONS

This study encompass four sea buckthorn genotypes 'Leordeni 3', 'Leordeni 4', 'Leordeni 5', and 'Leordeni 6', that grow naturally in the flora of Leordeni area from Argeş County, located in the center of Romania, and 'Piteşti 1'cultivar from Research Institute for Fruit Growing Pitesti-Maracineni. The results displayed important nutritional and bioactive compounds in the sea buckthorn berries.

Over the studied period the 'Leordeni 4' genotype had recorded the highest values of Vitamin C and total phenolic content and 'Leordeni 6' had good results at fruit weigh, size index, soluble solid °Bx and firmness versus the 'Piteşti 1' cultivar and studied genotypes, for that we recommend this genotypes for spread in culture and for further breeding programs targeting different breeding purposes.

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