

1 **PERILLA SPP.: PHENOTYPICAL CHARACTERIZATION AND**
2 **BIOCHEMICAL COMPOSITION OF 3 STUDIED GENOTYPES**

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17 RESEARCH ARTICLE
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Abstract

Perilla is cultivated as one commercial oilseed crop. Now it has also been introduced to Europe, Russia and USA as an oilseed crop (R. K. Bachheti et.co, 2014). *Perilla* plant contains a number of important phytochemicals such as Rosmarinic acid, Luteolin, Chrysocoriol, Quercitin (Akriti Dhyani et co., 2019). Breeding and spreading for field production and also phenotypic and biochemical identification. 3 varieties of *Perilla spp.* were studied and bred by repeated individual selection. The 3 varieties have distinct phenotypic characteristics: V1 with green leaves, V2 with bicolour leaves and V3 with red leaves. The chemical composition demonstrates the presence of useful compounds for human nutrition such as antioxidants, polyphenols, minerals, essential oils, B-carotene, amino acids with impressive antioxidant activity. The varieties are also melliferous. The 3 varieties have strong characteristics of distinctiveness and a rich chemical composition

Keywords: antioxidants, oilseed, melliferous

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22 INTRODUCTION

23 During flowering, the frequency of pollinators foraging on *Perilla* was high. Honey
 24 bees comprised 93% of all insect visitors to this crop, with an average number of
 25 14 individuals per sq. m. (...) the utility of this plant to honey bees is in the late
 26 period of flowering given the scarcity of other blossoming plants (Cinzia Barbieri
 27 et.co., 2011). *Perilla* is cultivated as one commercial oilseed crop in other
 28 countries like Japan, Korea and northern India. Now it has also been introduced to
 29 Europe, Russia and USA as an oilseed crop (R. K. Bachheti et.co, 2014). *Perilla*
 30 plant contains a number of important phytochemicals such as Rosmarinic acid,
 31 Luteolin, Chrysocoriol, Quercitin, Chatechin, Caffeic acid and Ferulic acid. The
 32 presence of phytosterols, tocopherols, squalene and Polyunsaturated fatty acid

33 has also been reported from *Perilla* seed (Akriti Dhyani et co., 2019) Breeding and spreading for field production
 34 and also phenotypic and biochemical identification were our aims on this research.

35 MATERIALS AND METHODS

36 In the present research were studied 3 varieties of *Perilla spp.*, bred by repeated individual selection. The working
 37 methods consisted in phenotypic and biometric observations. The 3 genotypes have distinct genotypic
 38 characteristics; one is *P. frutescens var. crispa f. purpurea* with reddish purple leaves, *P. frutescens var. frutescens*
 39 with green leaves and *P. frutescens var. crispa f. viridis* with green and reddish side leaves.

40 The crop technology used was sowing in the first decade of March, in alveolar pallets with 70 alveoli. The planting
 41 was made in May, and the planting scheme used was 70 cm between the rows and 35 cm between plants. The
 42 planting distances were made considering the isolation distances between the 3 varieties because this is an
 43 allogamous plant, being part of the *Lamiaceae* family.

44 Another main objective of this study was to perform laboratory analyses in order to establish the chemical
 45 composition of the studied varieties. The volatile compound was done using the gas chromatography test on fresh
 46 leaves and shoots.

48 RESULTS AND DISCUSSIONS

50 *Perilla spp.* phenotypical and biometrical characteristics

52 *Perilla frutescens var. crispa f. purpurea* has a strong, fibrous-branching root that exploits a large volume of soil.
 53 The plant height can reach, on average, up to 2 meters, with a diameter at the base of the stalk measuring, on
 54 average, 2.2 cm. The stalk is flexible in vegetative stages, but it lignifies during the senescence period. The number
 55 of main stems is about 16 (table 1).

57 **Table 1.** Main characteristics of *Perilla spp.* plants of the 3 varieties

Characteristics	Red Perilla <i>Perilla frutescens var. crispa f. purpurea</i>	Intermediate Perilla <i>Perilla frutescens var. viridis</i>	Green Perilla <i>Perilla frutescens var. frutescens</i>
Plant height (cm)	195	258	216
Bush diameter (cm)	149	187	163
Stem length (first internode), cm	6	11	8
Tap root diameter (cm)	2.2	2.9	0.27
Vegetative weight (g)	2572	2798	2679

59 The plant diameter is about 149 cm. The leaves are simple, opposite, with broad oval shape pointy ends, serrated
 60 margins and a long petiole, or even absent on the leaves on the top (Figure 1). The leafstalks varies between 6.7-
 61 2.82 cm. The length of leaf in average, measured 13.2 cm, and the width was of 9.25 cm. The leaves have a strong
 62 anthocyanin colouring. The plant is self-pollinating, but is preferred by insects, especially bees, due to the pleasant
 63 smell. The seeds have a grey colour. The main characteristics variability shows that the highest standard deviation
 64 value of 7,35 was registered by leaf weight, result that proves a wide range of leaves dimensions (table 2).

66 **Table 2.** Main characteristics variability of Red Perilla-*Perilla frutescens var. crispa f. purpurea*

Characteristics	\bar{x}	S	C.V.%	$\bar{x}\pm S$
Leaf weight (g) per 10 leaves	10.55	7.35	69.7	17.9-3.2
Leaflet length (cm)	13.2	1.9	14.4	15.1-11.3
Leaflet width (cm)	9.25	1.75	18.9	11-7.5
Leaf petiole length (cm)	4.76	1.94	40.8	6.7-2.82
Leaf petiole diameter	0.195	0.055	28.2	0.25-0.14
Internode length (cm)	9.315	1.455	15.6	10.77-7.86



Figure 1. Leaves details

Note: \bar{x} - mean, SD-standard deviation, CV%- coefficient of variation

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Perilla frutescens var. *viridis* has a strong root, which exploits a large volume of soil. The stalk is green coloured, with a height of up to 2 meters and a plant diameter, on average, of 258 cm. The leaves are green, purple coloured on the side. The average size of the leaflet has a length of 16.5 cm and the width has a value of 11.6 (table 3) (Figure 2).

Table 3. Leaves characteristics for the 3 *Perilla* spp. varieties

Characteristics	Red Perilla <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>purpurea</i>	Intermediate Perilla <i>Perilla frutescens</i> var. <i>viridis</i>	Green Perilla <i>Perilla frutescens</i> var. <i>frutescens</i>
Main shoots no.	16	19	18
Main shoots length (cm)	96	127	113
Nodes diameter (cm)	0.8	0.14	0.12
Leaves no./shoot	157	179	162
Leaves no./plant	2512	3401	2916
Leaf marginal incision depth (cm)	0.45	0.29	0.27

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The flowers appear in the middle of August, grouped in racemes, and have a white colour.

Following the results, we recommend to grow *Perilla* species in the field in a well drain soil; it does not require high soil fertility. In what concerns main characteristics variability, the highest value of standard deviation was also registered by leaf weight characteristic (table 4).

Table 4. Main characteristics variability for Intermediate *Perilla*-*Perilla frutescens* var. *viridis*

Characteristics	\bar{x}	SD	C.V.%	$\bar{x}\pm S$
Leaf weight (g) per 10 leaves	23.8	13.9	58.4	37.7-9.9
Leaflet length (cm)	16.15	4.75	29.4	20.9-11.4
Leaflet width (cm)	11.6	2.8	24.1	14.4-8.8
Leaf petiole length (cm)	7.52	1.35	18.0	8.87-6.17
Leaf petiole diameter	0.315	0.105	33.3	0.42-0.21
Internode length (cm)	12.53	2.74	21.9	15.27-9.79



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Note: \bar{x} - mean, SD-standard deviation, CV%- coefficient of variation

Figure 2. Leaves details

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Perilla frutescens var. *frutescens* has a strong root, which exploits a large volume of soil. The stalk is green coloured, with a height of up to 2 meters, and a plant diameter, on average, of 163 cm. The leaves are green, coloured on both sides. The average size of the leaflet has a length of 15.5 cm and the width has a value of 10.2 cm (table 5).

Being a new acclimatized plant, during the vegetation period, no diseases and pests were registered.

Table 5. Flowers and seeds characteristics for the three *Perilla* spp. varieties

Characteristics	Red Perilla <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>purpurea</i>	Intermediate Perilla <i>Perilla frutescens</i> var. <i>viridis</i>	Green Perilla <i>Perilla frutescens</i> var. <i>frutescens</i>
Inflorescence length (cm)	10	13	12
Interflorete length (cm)	0.5	0.7	0.6
Seed diameter (cm)	0.15	0.18	0.22
Seed no/gram	661	307	253
Thousand grain weight (TGW) (g)	1.51	3.25	3.95
Seed no./schizocarp	4	4	4

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97 Leaves and shoots of *Perilla frutescens* var. *frutescens* and *crispa* were harvested during the vegetation period and
98 were subjected to chemical analyses. The analyses were performed on fresh material and were determined by gas
99 chromatography-mass spectrometry test.

100 The flowering began after the 1st of September and fruit setting started after 10th of October. The seed
101 reached maturity after 1st of November. In what concerns main characteristics variability, the highest value of
102 standard deviation was registered by leaflet length of 11,7 cm (table 6) (Figure 3).

103 **Table 6.** Characteristics variability for Green *Perilla-Perilla frutescens* var. *frutescens*

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Characteristics	\bar{x}	SD	C.V.%	$\bar{x}\pm S$
Leaf weight (g) per 10 leaves	16.75	6.3	37.6	27.2-6.3
Leaflet length (cm)	15.5	11.7	75.5	19.3-11.7
Leaflet width (cm)	10.2	5.8	56.9	14.6-5.8
Leaf petiole length (cm)	5.6	3.2	57.1	8-3.2
Leaf petiole diameter	0.215	0.13	60.5	0.3-0.13
Internode length (cm)	9.15	5.3	57.9	13-5.3



110 **Figure 3.** Leaves details

111 Note: \bar{x} - mean, SD-standard deviation, CV%- coefficient of variation

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114 *Perilla* spp. biochemical characterization

115 The result of the present study suggested that there were differences in chemical composition among the 3
116 varieties of *Perilla* spp. Compozitia chimica variata si nutrientii pe care ii contin genotipurile de *Perilla* se
117 datoreaza polenizarii eficiente si optime that occurred in the experimental field, due to pollinators such as bees and
118 other insects (table 7).

119 The β -caroten content is highly registered at red Perilla plant with a value of 25,1 mg/100g and the lowest value
120 of 10.8 mg/100g was registered by Green Perilla due to the fact that Red Perilla amount of nutrients show
121 nutritional importance.

122 Phenolic compounds are frequently occurring in *Perilla* plant expressed in gallic acid with the highest value of 7,1
123 registered by Intermediate Perilla, which shows the dietary and nutrition importance for the human health.

124 The content in total polyphenols varied from 7,1%, in *P. frutescens* var. *viridis* to 0,9% in *P. frutescens* var.
125 *frutescens*.

126 Antioxidant content recorded the highest value on *P. frutescens* var. *crispa*, with a content of 17.1%, and *P.*
127 *frutescens* var. *frutescens* had a value of 7.8%.

128 The chemical composition is also based on antioxidant activity which is highly important for human health
129 because of the positive effect over free radicals. The highest content of antioxidants are registered by red perilla
130 due to it's splendid colourfull foliage gaved by flavonoids and tannins content.

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Table 7. Chemical composition of the three *Perilla* spp. varieties (leaves)

Compound	Red Perilla <i>Perilla frutescens</i> var. <i>crispa</i> f. <i>purpurea</i>	Intermediate Perilla <i>Perilla frutescens</i> var. <i>viridis</i>	Green Perilla <i>Perilla frutescens</i> var. <i>frutescens</i>
Zn	1.5	2.1	2.2
Cu	20	12	2.5
Mn	42	13	6
Fe	16	14	42
Mg	255	330	180
Na	75	100	85
K	2600	4000	1900
Total ash	11.5	9.4	8.9
β -caroten content (mg/100g)	25.1	12.8	10.8
Tannins	0.33	0.34	0.41
Polyphenols expressed in gallic acid (%)	3.6	7.1	0.9
Antioxidant activity expressed in TROLOX equivalents	17.1	9.3	7.8
Amino acid content expressed in glutamic acid	0.3	0.5	0.2
Content in fatty substances (total lipids)%	3.2	3.5	4.2

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CONCLUSIONS

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Author Contributions: C.V. collected *Perilla* varieties, acclimatized them and coordinated the entire research theme, B.M. made the phenotypic and biometric measurements, centralized data, wrote the paper, C.B. Contributed data and analysis tools, G.N. Collected the data, M.P. conceived and designed the analysis, A.P. performed the analysis.

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Conflicts of Interest

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The authors declare no conflict of interest.

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