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RESEARCH ON PHENOTYPIC AND BIOCHEMICAL VARIABILITY IN NEW GENOTYPES OF *PERILLA FRUTESCENS*

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Abstract

Perilla frutescens originates in Asia and is widely grown in China, Japan, South Korea, Vietnam and India. China is considered to be the center of origin; therefore, in some areas of Asia, Perilla Nanking or Sisho is considered a sacred plant. In Europe, Perilla sp. has entered rather late, appreciated for its economic properties, and used for its oils or as an ornamental plant, although, it occupies quite small areas.In Romania, the plant was studied after 2010 by the Breeding and Biodiversity Laboratory Buzau, obtaining three genetically stable genotypes from which two genotypes are the subject of this work. The genotypes taken in the study were phenotypically analysed, while biochemical laboratory analyses were also performed. Regarding the content of volatile oil, there were no differences between genotypes (0.4 mL/kg), while the content in total polyphenols has variations from 3.94 to 6.26%; the contents in flavones ranged from 1.19% to 0.49, and the content in pectin also varied from 7.68 to 11.29%.

Key words*: breeding, biodiversity, volatile oil, polyphenols, flavones.*

INTRODUCTION

The genus Perilla is part of the Lamiaceae family, also called Labiatae, or the mint family, with 236 genera and more than 7000 species, is the largest family of the Lamiales order. *Perilla frutescens* (L.) Britt. originates from Asia being widely grown in China, Japan, South Korea, Vietnam and India. China is considered to be the primary gene center, in some areas of Asia, Perrilla Nanking or Sisho is regarded as sacred plant. The largest cultivated area is found in Korea. In Europe, Perilla sp. has entered rather late, appreciated for its economic properties, and used for its oils or as an ornamental plant (Nitta et al., 2003), although, it occupies quite small areas. Perilla sp. is divided according to the morphology of the plant and its use in two varieties: *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa. P. frutescens* var. *frutescens* is used to obtain oils and is known in the countries of East Asia as Ren in China, Dllggae in Korea and Egoma in Japan. Only in Korea the leaves are consumed. Perilla seeds are used like sesame seeds from ancient times in China, Korea and Japan (Lee and Ohnishi 2001, 2003; Nitta et al. 2003). *P. frutescens* var. *crispa* is part of Chinese herbs, and is called Cha-jo-ki in Korea, Shiso in Japan and Zisu in China (Lee and Ohnishi 2001, 2003). It is also used as a condiment for pickles in Japan. In conclusion, these two species have been an important crop in East Africa since ancient times. (Lee and Ohnishi 2003; Nitta et al. 2003). In southern China, the leaves of crispa variety are used mainly because of its medicinal properties (Tan et al. 2012; Wang and Guo 2012; Wei et al. 2015). For instance, the leaves have detoxifying properties and have been used for cooking crab and fish for over 2000 years (Yu șicolab. 2016). In addition, seeds and leaves of crispa varieties have been considered effective in the treatment of cough, common cold, asthma and digestive problems (Yu et al. 2016). Another study (Asif M., 2011, Lands William E. M., 2005) suggested that Perilla oil is known to have one of the highest concentrations of omega-3 fatty acids and it is beneficial for human health and for preventing various diseases, such as cardiovascular disease, cancer, inflammatory and rheumatoid arthritis.

Taxonomic studies have been the foundation of genetic resources management in many aspects. Research on the plant taxonomy is based on comparing the agro-morphological characteristics. Thus, until now, characteristics such as leaf size, seed size, plant height, number of branches, colour of flowers and leaves, degree of pubescence and aroma of the plant are used to differentiate *frutescens* variety from *crispa* variety. Of these, the seed size is considered the most reliable characteristic that distinguishes *P. frutescens* var. *frutescens* from *P. frutescens* var. *crispa* and *P. frutescens* var. *frutescens* the wild variety. Rao and Hodgkin (2002) suggested thatmorphological analyses are dependent on environmental factors.

Perilla frutescens in pedoclimatic conditions of Romania is an annual plant. *Perilla frutescens* is a plant with multiple uses, appreciated for its therapeutic and food uses, but is also valued for its ornamental and aromatic purpose.

In Romania, the plant was taken into study after 2010 at the Breeding and Biodiversity Laboratory, Buzau, obtaining until now a number of three genetically stable genotypes and two of them made the subject of this work. The research was extended in collaboration with University of Agronomic Science and Veterinary Medicine of Bucharest.

The studied genotypes were analysed from a phenotypic point of view, at the same time biochemical laboratory analyses were performed for two varieties, crispa variety and frutescens variety.

MATERIALS AND METHODS

In the study were used two genotypes that have distinct genotypic characteristics, one is *P. frutescens* var. *crispa*, with reddish purple leaves and *P. frutescens* var. *frutescens* with green and reddish side leaves.

The crop technology used: was sowing in the first decade of March, in plastics pots with 70 cubes with a volume of 50 mL/cube in a mixture of peat and sand. The planting was made at the beginning of May, and the planting scheme used was 70 cm between the rows and 35 cm between plants. A special care was made using one mechanical hoeing and two manual hoeing for loosen soil and weed. Throughout the plant season, no chemical treatments were applied for management of diseases and pest. So far, no pathogens have been identified to endanger the crop.

One of the main objectives of the study was to observe the adaptability process in pedoclimatic conditions of Romania. It is known that only varieties that acclimatize can survive to produce progeny from which a new population may become established.

During the vegetation period, biometric and phenological observations were made. Another main objective of this study was to performed laboratory analyses in order to establish the chemical composition of the studied varieties. The volatile compound was done using gas chromatography on fresh leaves and shoots on 23rd of September.

The content of flowers and seeds in volatile oils, polyphenols, flavones, antioxidants, pectin, amino acids, total lipids and calcination residue was made on 4th October.

RESULTS AND DISCUSSIONS

Perilla frutescens var. *crispa* (Figure 1) has a strong, fibrous-branching root that exploits a large volume of soil. The plant height can reach, on average, up to 2meters, with a diameter at the base of the stalk measuring, on average, 2.8cm. The stalk is flexible in the vegetative stages, but it lignifiesduring the senescence period. The number of main stems varies between 16 and 18.

Figure 1. *Perilla frutescens* var. *crispa* crop detail

The plant diameter has varied from 1.10-1.40meters. The leaves are simple, opposite, with broad oval shape pointy ends, serrated margins and a long petiole, or even absent on the leaves on the top (Figure 2). The leafstalks

measure, on average a length of 9.12 cm. The length of leaf in averaged, measured 16.12 cm, and the width was 10.24 cm. The leaves have a strong anthocyanin colouring. It has been shown that the red overtones are mainly due to the presence of malonilshisonin (Meng L. et al., 2006).The average number of leaves per main stem was 155 leaves.

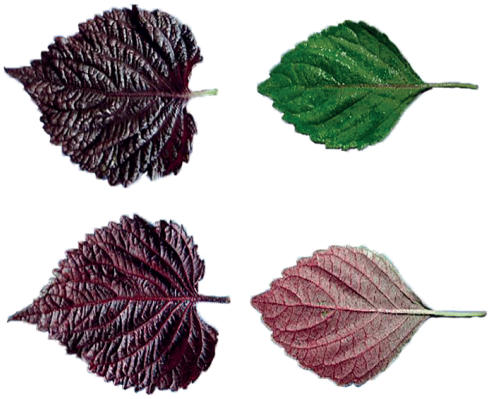

Figure 2. Different types of leaves belonging to *P. frutescens* var. *crispa* (left) and *P. frutescens* var. *frutescens* (right)

The beginning of flowering began on 17th of August. The plant is self-pollinating, but is preferred by insects, especially bees, due to the pleasant smell (Figure 3). The seeds have a grey colour and in one gram can be found 213 seeds.

Figure 3. Flowers of *P. frutescens* var. *crispa*

Perilla frutescens var. *frutescens* (Figure 4) has a strong root, which exploits a large volume of soil. The stalk is coloured green, with a height of up to 2 meters am with a plant diameter, on average, of 1.33 m. The leaves are green, coloured purple on the side. The average size of the leaflethas a length of 15.93 cm and the width has a value of 9.87. The average number of leaves on the main shoot was 149 leaves.

Figure 4. *Perilla frutescens* var. *frutescens* crop detail

The flowers appear in the middle of August, grouped in racemes, and have a white colour. The seeds have a grey colour and the mass of one thousand seeds is 4.26 g (Figure 5).

Figure 5. Seeds of *Perilla frutescens* var. *frutescens*

Following the results, it is recommended to grow the Perilla species in the field in a well drain soil; it does not require high soil fertility.
Being a new acclimatized plant,during the vegetation period, no diseases and pests were registered.
Leaves and shoots of *Perilla frutescens* var. *frutescens* and *crispa* were harvested during the vegetation period and were subjected to chemical analyses. The analyses were performed on the fresh material and were determined by gas chromatography-mass spectrometry analysis. In Tables 1 and 2 are shown the volatile compounds of both Perilla species.

Table 1. Volatile compound of *P. frutescens* var. *frutescens*

Compound	RT	Area %	Peak Area	Peak Height	S/N
3 Octenol	23.64	0.30	10557125	3057045	4108.03
Linalool	26.21	0.76	26410282	7791242	10469.82
Cariophylene β	27.27	4.10	142334173	39113802	52560.85
Cariophylene α	29.03	0.51	17604740	4906300	6593.05
Germacrene	29.97	0.13	4382556	1167577	11568.98
Hexanoyl furan	30.47	2.16	74893076	20620856	27710.16
Cas 16076-65-9	30.85	0.35	12206287	3469820	4662.72
Hexanoyl furan	32.10	88.58	30764663044	73178087	983361.87
Farnesoljhvc	34.11	0.10	3551027	911590	1224.99
Trimethylpropylsilane	35.17	0.40	13720037	3900422	5241.36
Cariophyleneoxid	35.70	0.29	9904768	2721065	3656.55
Cariophyleneoxid	35.88	1.31	45586931	12253122	16465.66
Nerolidol	37.08	0.32	11152956	2033704	2732.88
Spathulenol	38.65	0.40	13735148	363560	4885.17
Cadinol α	46.94	0.10	3558601	519375	697.93
Phytol	47.18	0.20	6870902	1471880	1977.90

The result of the study suggested that there were differences in volatile compounds among the *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* grown in Buzau site. Chemical polymorphism indicated that genetic and environmental factors should be considered to ensure consistent quality. Xie et al. (2012) also states that the yield, chemical compositions and bioactivities may be influences by genetic and environmental factors. The quality of the volatile compounds is directly influenced by the relationships between the variation in chemical composition and the bioactivity.

Table 2. Volatile compound of *P. frutescens* var. *crispa*

Compound	RT	Area%	Peak Area	Peak Height	S/N
Perillene	22.74	0.95	36088223	10347198	15206.46
3 Octenol	23.62	0.31	11616928	3380426	4967.68
Linalool	26.20	1.31	49516537	14551536	21385.25
Cariophylene β	27.26	2.96	112324437	30744782	45227.27
Cariophylene α	29.02	0.20	7617124	2087524	3067.87
Germacrene	30.46	0.98	37031232	10277782	15104.45
Hexanoyl furan	30.85	0.29	10854233	3093804	4546.72
Isoledene	31.09	0.10	3754014	954715	1403.07
Hexanoyl furan	32.09	90.87	3444728173	783466079	1151398.34
Trimethylpropylsilane	35.16	0.30	11333517	3176587	4668.38
Cariophyleneoxid	35.69	0.20	7714819	2105306	3094.00
Cariophyleneoxid	35.88	0.97	36632271	9869422	14504.31
Nerolidol	37.07	0.16	5923843	1077019	1582.81
Spathulenol	38.64	0.10	3968968	980909	1441.56
Cadinol α	40.64	0.18	6673555	1797402	2641.50
Phytol	47.18	0.13	4913071	903840	1328.30

The flowers and seeds of *P. frutescens* var. *frutescens* and *P. frutescens* var. *crispa* were studied for the chemical content in: volatile oil, total polyphenols, flavones, antioxidants, pectin, amino acids, lipids and calcination residue.
Based on the chemical properties, these active compounds in Perilla could be classified either as hydrophilic (phenolic compounds, flavonoids, anthocyanin) or hydrophobic (lipophilic) ones (volatile compounds, triterpenes, phytosterols, fatty acids, tocopherols and policosanols).
Perilla is an important oleaginous plant; the seeds contain about 45% oil and most are loaded with unsaturated fatty acids. The laboratory analysis showed that the lipid content in seeds ranged from 25.36% to 33.79% (*P. frutescens* var. *frutescens*).

Phenolic compounds are frequently occurring in Perilla plant. They have a wide structural variability with a broad range of pharmacological activities.
Some of these products have been studied and proven to be an efficient source of phenolic antioxidants. A recent study (Gai F. et al., 2017) showed that a greater accumulation of phenolic component occurs at the complete flowering stage.In the present work, the content in total polyphenols varied quite from 3.94%, in *P. frutescens* var. *frutescens* to 6.26% in *P. frutescens* var. *crispa.*
The total flavone content also registered quite large differences between the studied genotypes, thus, *P. frutescens* var. *frutescens* had a value of 0.49%, and in *P. frutescens* var. *crispa* content in flavone was 1.19%. Recent studies show that flavonoids from *P. frutescens* have a strong antioxidant activity and have pharmacological properties (Peticilă et al., 2019)
In Table 3 are presented the results of chemical analyses of flowers and seeds of studied Perilla varieties.

Table 3. Chemical compositions of flowers and seeds of Perilla sp.

Compound	***Perilla frutescens* var. *crispa***	***Perilla frutescens* var. *frutescens***
Volatil oil (mL/kg)	0.4	0.4
Total polyphenols (%)	6.26	3.94
Total flavones (%)	1.19	0.49
Antioxidants (%)	12.01	8.76
Pectin (%)	7.68	11.29
Amino acids (%)	0.51	0.41
Total lipids in seeds (%)	25.36	33.79
Residue calcination	7.0	7.5

Antioxidant content recorded the highest value on *P. frutescens* var. *crispa*, with a content of 12.01%, and *P. frutescens* var. *frutescens* had a value of 8.76%.
In terms of pectin content, the highest value was recorded by *P. frutescens* var. *frutescens* and had a value of 11.29%, and to *P. frutescens* var. *crispa* was 7.68%.
The content in volatile oils had the same value of 0.4% for both varieties studied.

CONCLUSIONS

The seeds and seedlings offered promotionally at Vegetable Research Development Station Buzau, Romania attest that the Perilla sp. can be grown throughout the territory of our country both in greenhouse conditions and also in the field. This species may be soon become a niche crop, expanding throughout the country.
The acclimatized and genetically stabilized genotypes will be forward to ISTIS Bucharest for approval and patenting.

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