Analysis of variation of phenotypical characters in *Hypericum maculatum* Crantz wild populations

E. Bagdonaitė,
J. Radušienė

Institute of Botany,
Žaliųjų ežerų 49,
LT-2021 Vilnius, Lithuania

The article presents an analysis of variation of phenotypical characters in wild populations of *Hypericum maculatum* Crantz., which showed that populations varied significantly in the majority of their morphological and productivity characters. The differences of characters among populations were specified by the Scheffe test. Within populations, a wide variability of inflorescence and sepal length and width was established.

The quantities of naphthodianthrones in the flowers of *H. maculatum* were determined by the high performance liquid chromatography method. The results of analysis showed a great variation of hypericin and pseudohypericin quantities among populations.

Key words: *Hypericum maculatum* phenotypical characters, hypericin, pseudohypericin, high performance liquid chromatography (HPLC)

INTRODUCTION

Hypericum maculatum Crantz is a perennial medicinal plant belonging to the family *Hypericaceae* L. The preparations of this plant are used as anti-inflammatory, antimicrobial, wound-healing and anesthetic means, though they have been known and used less than *H. perforatum*. Many active substance groups such as naphthodianthrones, flavonoids, tannins, xanthones, phenolic acids, etc. that can have a pharmacological effect were found in the raw material of *H. maculatum* [1].

The purpose of this work was to determine the variation amplitude of morphological characters, productivity, hypericin and pseudohypericin quantities in *H. maculatum* wild populations.

MATERIALS AND METHODS

The study material was gathered in July 2000 in Akmenė, Ignalina, Plungė, Šilalė and Švenčionys regions. The biometric analysis of 30 plants randomly selected for 15 characters was performed in 8 populations of *H. maculatum*. The populations are marked by a gathering number.

The variation of the characters was evaluated statistically [2]. The analysis of variance (ANOVA), the Scheffe test for comparing averages were performed, the variation coefficient (V%) was calculated. For determining the similarity of populations, a K–mean and hierarchical cluster analysis was used. The cluster analysis dendrogram was constructed on the grounds of agglomerative grouping by the method of single linkage distance between the groups, using squared Euclidean distances.

The quantitative analysis of naphthodianthrones was performed at the Research Institute of Medicinal Plants in Poznan (Poland). The content of hypericin and pseudohypericin was assessed in the methanolic flower extract by the high performance liquid chromatography (HPLC) method [3]. A Hawlett Packard chromatograph with a UV–VIS detector, LiChrospher RP–18 column (250 mm, 4 mm, 5 μm) was used. As eluent, the following solvents were chosen: methanol: ethyloctane: 0.1 M NaH_2PO_4 (pH 2.5). The column temperature 24 °C, eluent flow rate 1ml/min, injection volume 10 μl. The peak detection was performed in the wavelength of $\lambda = 590$ nm. The calibration schedules of quantitative determination were compiled according to standards.

RESULTS AND DISCUSSION

The analysis of variance revealed highly significant differences ($p < 0.001$) among *H. maculatum* populations regarding the majority of characters (Table). Peak values of the F statistic were observed for the height of plants, length and width of leaves, dry weight of flowers and leaves. Only the sepal width

Table. **Summary statistics of *H. maculatum* characters and the level of differentiation of populations by characters according to ANOVA**

Characters	M	SE	min	max	M min	M max	F	P
Height of plant, cm	53.16	0.77	33.00	76.00	41.15	61.55	24.35	0.001
Number of internodes	20.37	0.24	14.00	27.00	17.40	23.10	11.23	0.001
Length of 5th internode, mm	24.17	0.57	10.00	45.00	19.25	29.00	3.37	0.003
Length of inflorescence, cm	11.68	0.47	3.00	40.00	9.25	15.27	4.32	0.001
Width of inflorescence, cm	5.01	0.13	2.00	11.00	4.45	6.00	2.32	0.03
Length of petal, mm	9.24	0.09	5.00	12.00	8.40	9.90	4.02	0.001
Width of petal, mm	5.08	0.07	3.00	8.00	4.20	5.65	4.92	0.001
Length of sepal, mm	3.93	0.06	2.00	6.00	3.35	4.45	5.37	0.001
Width of sepal, mm	2.05	0.03	1.00	3.00	1.95	2.20	1.37	0.3
Length of leaf of 6th node, mm	20.48	0.36	11.00	35.00	17.05	25.70	16.16	0.001
Width of leaf of 6th node, mm	11.12	0.21	7.00	18.00	8.90	14.13	24.41	0.001
Dry weight of flowers, g	0.34	0.04	0.06	4.23	0.18	1.26	13.38	0001
Dry weight of leaves, g	0.62	0.06	0.12	4.58	0.34	2.17	24.23	0.001
Dry weight of shoots, g	1.35	0.12	0.29	12.31	0.59	3.96	12.61	0.001
Weight of raw material, g	1.33	0.13	0.35	12.05	0.85	4.61	17.13	0.001

M – mean; SE – standard error of mean; min – minimum; max – maximum; M min – minimum mean among populations; M max – maximum mean among populations; F – Fisher's statistics, P – significant level.
Note: degree of freedom among the groups equals 7, within the groups 147.

($p < 0.3$) averages did not differ significantly among the populations.

The character differences were specified by the Scheffe test. The height of *H. maculatum* plants varies from 33.0 to 76.0 cm. Among populations, the height averages alter less (41.1–61.5 cm) and form three statistically different homogeneous groups. Populations 00EB14, 00EB23, 00EB33 form a group of the smallest plants (41.1–48.3 cm) and populations 00EB17, 00EB28, 00EB30, 00EB32 form a group of the highest plants (56.3–61.5 cm). The variation of plant height within populations is small (V = 9–12%).

The main subcrown internode number averages make three statistical groups. The internode number average was least in population 00EB14 and largest in population 00EB32. Taking this character into account, population 00EB29 is most heterogeneous. The length of internodes varies from 10.0 to 45.0 mm (Table). The shortest internodes were found in population 00EB29, and the longest in population 00EB30.

The populations of *H. maculatum* analyzed according to the length and width of leaves fall into three homogeneous groups. The average length of leaves of most populations is 17.0–19.7 mm and the average width 8.9–11.1 mm. The leaves of average length and width are peculiar to population 00EB28 and long and wide leaves to population 00EB17.

The petal length and width averages form two homogeneous groups. The plants with long (9.2–9.9 mm) and wide (5.0–5.6 mm) petals prevail in most populations. In populations 00EB14 and 00EB32 petals are short and narrow. The amplitude of petal length (V = 7–17%) and width (V = 10–19%) variation within populations is narrow.

The sepal length average makes two homogeneous groups. The plants with longer sepals (3.7–4.4 mm) prevail. The sepal length (V = 17–23%) and width (V = 19–23%) variation is greatest in populations 00EB28, 00EB30 and 00EB33.

Within population, great variations in inflorescence length (V = 26–68%) and width (V = 25–38%) were determined. The boxplots (Fig. 1) show a very great inflorescence length variation (V = 49–68%) in populations 00EB23 and 00EB29. The long upper stroke in the diagram of population 00EB30 shows that there are exceptionally great meanings. This population differs from the others by a wide amplitude of inflorescence width variation (V = 34%).

The research results show that the dry flower, leaf, stem and raw material (the part of a plant 30 cm from the crown) weight of separate plants varies greatly. Evaluation of *H. maculatum* populations according to these quantitative characters was performed by the K–mean cluster analysis method (Fig. 2). All populations were joined into three clusters. The first cluster included populations 00EB14, 00EB17, 00EB23, 00EB28, in which the average dry weight of one plant flowers was 0.22–0.38 g, of leaves 0.45–0.57 g, stems 0.99–1.58 g, raw material 0.86–1.17 g. The clusterization of population 00EB30 was the

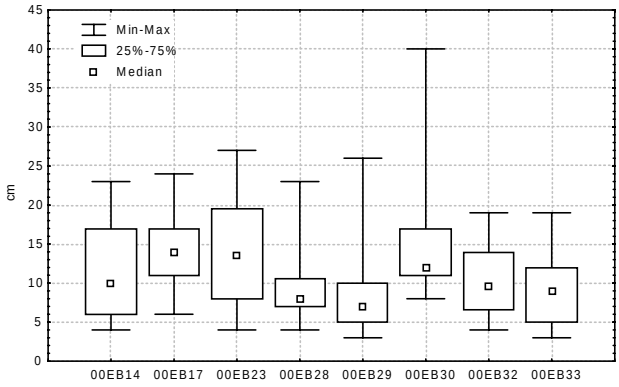

Fig. 1. Boxplots of inflorescence length in *H. maculatum* populations

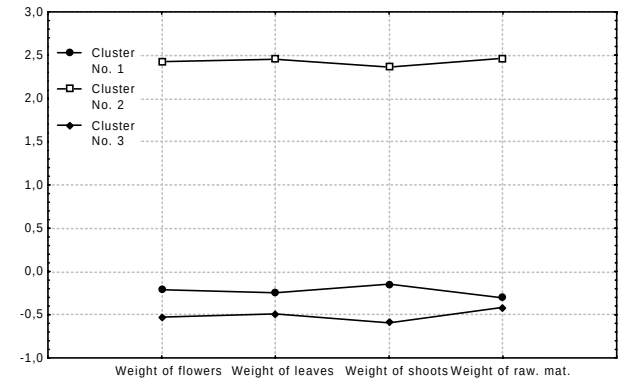

Fig. 2. Plot of means for each cluster of *H. maculatum* characters

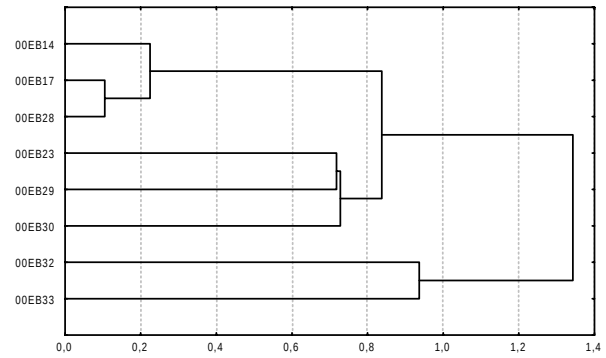

Fig. 3. The hierarchical cluster analysis dendrogram of *H. maculatum* populations according to the content of hypericin and pseudohypericin in flowers

most distant (the second cluster). The special case of this population could be explained by the largest weight of plant parts and the largest production of raw material. The characteristic feature of all populations (00EB29, 00EB32, 00EB33) of the third cluster was a low dry weight of plant parts and raw material.

The results of chemical evaluation show the variability of hypericin (0.266–0.495 mg/g) and pseudohypericin (0.609–2.098 mg/g) in raw material among populations. The contents of these compounds referred by other authors [4, 5] are higher (0.440–2.290 mg/g and 0.430–2.590 mg/g, respectively) than ours. The average quantity of hypericin in flowers is 0.382 ± 0.035 mg/g and pseudohypericin – 1.021 ± 0.183 mg/g. The hierarchical cluster analysis dendrogram (Fig. 3) revealed a connection between similar populations according to the quantity of the active substances analyzed. The populations in the dendrogram make three high level clusters. The first cluster includes populations 00EB14, 00EB17, 00EB28 containing minimum quantities of hypericin (0.266–0.275 mg/g) and pseudohypericin (0.609–0.780 mg/g). The second cluster consists of three populations (00EB23, 00EB29, 00EB30) containing a higher than the average quantity of hypericin (0.422–0.494 mg/g). The quantity of pseudohypericin below the average (0.738–0.930 mg/g). The third cluster, which includes 00EB32 and 00EB33 populations, is the most distant in the dendrogram. The distinction of these populations is predetermined by the largest quantity of pseudohypericin (1.535–2.098 mg/g). The content of hypericin does not differ significantly from its content in the second cluster.

The obtained data prove a great variation in the phenotypical characters of *H. maculatum.* The germplasm of the populations that differ from the others by a majority of useful characters (00EB30, 00EB32, 00EB33) will be kept under research and conservation in a field collection.

ACKNOWLEDGEMENTS

The authors are very grateful to Head of the Analytical Department of the Research Institute of Medicinal Plants in Poznan (Poland) Ass. Prof. Dr. E. Segiet-Kujawa, Dr. B. Zygmunt for the possibility to perform HPLC analysis.

References

1. Kitanov GM, Nikolov NTZ. Fitoterapia 1991; 62 (1): 92.
2. Зайцев ГН. Математика в экспериментальной ботанике. Москва, Наука, 1990.
3. Gaedcke F. Deutsche Apotheker Zeitung 1997; 137 (42): 3753–7.
4. Brantner A, Kartnig Th, Quehenberger F. Scientia Pharmaceutica 1994; 62: 261–76.
5. Kartnig Th, Gruber A, Sauer H. Planta Medica 1989; 55: 215.

E. Bagdonaitė, J. Radušienė

HYPERICUM MACULATUM CRANTZ. FENOTIPINIŲ POŽYMIŲ VARIJAVIMO ANALIZĖ NATŪRALIOSE CENOPOPULIACIJOSE

S a n t r a u k a

Hypericum maculatum Crantz fenotipinių požymių dispersinė analizė parodė, kad cenopopuliacijos skiriasi pagal augalų aukštį, lapų plotį ir jų ilgį, žiedų bei lapų sausą svorį, vaistinės žaliavos produkciją ($p < 0,001$). Požymių skirtumai tarp populiacijų konkretizuoti Scheffe's testu. Populiacijų viduje nustatyta plati žiedynų ilgio (V = 26–68%) ir jų pločio (V = 25–38%), taurėlapių ilgio (V = 17–23%) bei pločio (V = 19–23%) varijavimo amplitudė.

Hipericino ir pseudohipericino kiekiai *H. maculatum* žieduose buvo nustatyti didelio slėgio skysčių chromatografijos metodu. Pikų detekcija atlikta $\lambda = 590$ nm bangos ilgyje. Tyrimų rezultatai parodė didelę hipericino (0,266–0,495 mg/g) ir pseudohipericino (0,609–2,098 mg/g) kiekių įvairovę natūraliose cenopopuliacijose.