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VARIABILITY OF MORPHOLOGICAL CHARACTERISTIC AND CONTENT OF ACTIVE SUBSTANCES IN *BETONICA OFFICINALIS* L. IN THE CZECH REPUBLIC

VARIABILITA MORFOLOGICKÝCH ZNAKŮ A OBSAHU AKTIVNÍCH LÁTEK U *BETONICA OFFICINALIS* L. V ČESKÉ REPUBLICE

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The aim of work was a verification of genotypic variability of *Betonica officinalis* L. in the Czech Republic and plants from 10 natural sites in 4 protected landscape areas (PLA) in the Czech Republic were used for experiments.

Height and width of plants, length and width of leaf, length of inflorescences and content of total polyphenols were evaluated in plants from natural localities and *ex situ* in field nursery in Olomouc. All obtained data was statistically evaluated and statistically significant differences were found between *Betonica* plants from different PLA and different localities inside of one PLA but also in between plants from the same population.

Key words: Wood Betony, *Betonica*, morphological characters, polyphenols

Species-rich grasslands are important and irreplaceable parts of our cultural landscape, but have declined substantially as a result of modern farming methods and/or industrial activities (Hopkins and Clements 2008). Maintaining, restoring or re-establishing flower-rich grassland areas is a very popular topic in the Czech Republic at this time because the agriculture utilisation was broken in many areas and in the country there are also many other places (slag hoppers, waste sites etc.) which need to be recovered. Non managed lands immediately come in on the different stage of air-raid woody species invasion (Kvítek et al. 1997). Unfortunately, it is not usually possible to recreate species-rich grassland by simply changing management and hoping that the area will revert to a flower-rich meadow. It is therefore necessary to introduce seeds or plants if a species-rich sward is to be created in a reasonable time (Hopkins and Clements 2008).

According to Šrámek et al. (2001) it is generally not desirable to distribute the uniform seeds or seed mixtures to a whole country, because the uniform seeds should break a phytogeographic and regional character of overgrowth. Therefore the use of autochtonous seeds is recommended, because the creation of the analogue of original overgrowth is a main goal of this tendency (Kvítek et al. 1997).

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As one of the suitable species for the sowing of species-rich grasslands on dry acid sites and sandy soils was presented Wood Betony (*Betonica officinalis* L.) (Hopkins and Clements 2008). It is a very desirable component of wild flower grasslands because of its nutrition value which improves the quality of grazing lands and hay, its antierosive effects as well as its huge aesthetic value. As a medicinal plant it was commonly used in the treatment of a wide range of disorders (especially as a nervine and tonic for treating maladies of the head and as an external application to wounds) in the past (Bown 1995). It is a perennial plant relatively common in the larger part of Europe (Ball 1972) and some natural populations in the Czech Republic were studied in our research goal.

The aim of this paper was a study of variability of morphological characteristic and content of total polyphenols of *Betonica officinalis* L. in the Czech Republic and the valuation of individual genotypes for purposes of species-rich grasslands restoring.

T a b l e 1

Origin of evaluated plants

Protected landscape area	Locality	North	East	Altitude [m]
A České Středohoří (ČS)	1 – Hradiště-Mentaurov	50°33´51.8´´	14°07´23.3´´	380
	2 – Babinské louky	50°35´54.6´´	14°07´38.6´´	545
B Bílé Karpaty (BK)	3 – Suchovské mlýny	48°52´98.6´´	17°34´26.1´´	373
C Moravský Kras (MK)	4 – Jedovnice	49°19´95.4´´	16°45´96.1´´	471
	5 – Hádecká planina	49°13´10.5´´	16°40´31.7´´	407
	6 – Blansek	49°17´02.6´´	16°44´50.9´´	464
D Šumava (ŠU)	7 – Vinice-Kašperské Hory	49°08´23.8´´	13°32´13.2´´	669
	8 – Nebe I.-Kašperské Hory	49°08´23.0´´	13°34´27.1´´	757
	9 – Nebe II.-Kašperské Hory	49°08´28.2´´	13°34´45.8´´	807
	10 – Dobrá-Stožec	48°53´52.7´´	13°50´00.5´´	732

T a b l e 2

The evaluation of morphological characteristics of plants at the natural localities

Protected landscape area/ Locality	No. of evaluated plants (2004/2005)	High of plants* [cm]		Width of plants* [cm]	
		2004	2005	2004	2005
ČS/Hradiště-Mentaurov	6/10	70.2±10.0	61.1±11.1	42.2±8.3	29.3±4.7
ČS/Babinské louky	10/10	86.8±12.9	86.2±11.2	27.7±4.9	30.0±5.3
BK/Suchovské mlýny	12/13	64.7±8.2	42.6±16.1	30.5±5.9	19.4±6.3
MK/Jedovnice	3/10	56.1±14.7	71.0±10.2	23.3±2.5	32.0±6.1
MK/Hádecká planina	6/10	45.4±6.7	78.6±10.2	26.3±6.5	41.0±7.2
MK/Blansek	8/0	63.7±12.0	n	25.9±4.5	n
ŠU/Vinice-Kašperské Hory	10/10	96.8±15.0	56.3±10.1	42.6±14.3	21.0±4.0
ŠU/Nebe I.-Kašperské Hory	6/10	92.4±4.8	87.8±9.3	37.2±15.2	40.4±3.4
ŠU/Nebe II.-Kašperské Hory	10/10	86.5±5.2	90.9±8.7	35.9±6.0	43.9±9.5
ŠU/Dobrá-Stožec	8/10	74.3±5.7	75.1±11.0	45.7±9.2	36.4±4.0
$\overline{x}$	–	75.9±19.0	71.2±19.6	34.2±11.3	32.2±10.2

*average and standard deviation

n – Blansek locality was not studied in 2005

MATERIAL AND METHODS

Plants from 10 natural sites in 4 protected landscape areas (PLA) in the Czech Republic (Table 1, Fig. 1) were used for experiments and all characteristics were evaluated separately on plants from original localities and plants which were as the mother plants transferred into the *ex situ* field nursery in Olomouc (N 49°34´25.6´´; E 17°16´52.4´´; 250 m above see). The height and width of plants, length and width of leaf and length of inflorescences were evaluated in particular plants, content of total polyphenols was analysed in mixed samples from each group of plants separately.

The evaluation of the morphological characteristics of plants at the natural localities was done in 2004 and 2005. The collection missions of the plant material were realised in 2004 and mother plants (at least 10 plants per locality) were used for the founding of the field nursery in Olomouc. All plants were grown under the same field and climatic conditions in the field nursery and nursed (watering, weeding, harvesting etc.) in the same way. The evaluation of morphological characters in the field nursery was made in 2006 and 2007.

Fig. 1. Origin of evaluated plants

For the evaluation of active substances content all the plant samples from the field nursery were prepared in the same way: the stems in full flowering stage were harvested by hand, dried in a temperature lower than 35°C (drying house with controlled air circulation) and grinded by a laboratory homogenizer. Each sample representing the locality was mixed from the all plants from the locality. The content of total polyphenols was measured by a spectroscopy – a colour precipitate reaction of polyphenols with Folin-Ciocalteau phenol reagent assay was used (Kolektiv autorů 1987). The two repetitions of measurement were used in each sample.

Statistical evaluations of obtained data was carried out by Anova, one-factor analysis of variance with significance level $\alpha = 0.05$ resp. 0.01.

RESULTS

The evaluation of the morphological characteristics of plants at the natural localities (Table

T a b l e 3

Statistical differences in heigth of plants in 2004 between the plants at the natural localities

Protected landscape area/Locality		2	3	4	5	6	7	8	9	10 ŠU Dobrá-Stožec
ČS/ Hradiště-Mentaurov	(1)	+	–	–	++	–	++	++	++	–
ČS/Babinské louky	(2)		+	++	++	++	–	–	–	+
BK/Suchovské mlýny	(3)			–	++	–	++	++	++	–
MK/Jedovnice	(4)				–	–	++	++	++	++
MK/Hádecká planina	(5)					++	++	++	++	++
MK/Blansek	(6)						++	++	++	+
ŠU/Vinice-Kašperské Hory	(7)							–	–	++
ŠU/Nebe I.-Kašperské Hory	(8)								+	++
ŠU/Nebe II.-Kašperské Hory	(9)									++

– no statistical differences; + statistically significant differences ($\alpha = 0.05$); ++ statistically high significant differences ($\alpha = 0.01$)

2) showed statistically high significant differences (a = 0.01) between plants from different localities within one PLA as well as between plants from different PLAs in both evaluated characteristics – height and width of plants. An example of this evaluation is presented in Table 3. On the other hand, statistically significant differences were not found between results from evaluated years.

The evaluation of the morphological characteristics of plants in the field nursery brought different results. In 2006 the height of plants was 40–90 cm and statistical differences were found only between a few localities (Table 4). No statistical differences were found between plants from different PLAs. In 2007 the height of plants was 43–84 cm and no statistical differences were found between localities and even between different PLAs (Table 5). The same results were reached in the evaluation of plant width – it was 27–101 cm and statistical differences were found only between a few localities in 2006 (Table 4). In 2007 the width of plants was 20–88 cm and no statistical differences were found between localities and even between different PLAs (Table 5). These results proved that the plants with different origins can acquire identical parameters in the same soil and cli-

T a b l e 4

Statistical differences between the plants in field nursery in height and width of plants in 2006

Protected landscape area/ Locality		2	3	4	5	6	7	8	9	10 ŠU Dobrá-Stožec
		(height/width of plants)								
ČS/ Hradiště-Mentaurov	(1)	n	n	n	n	n	n	n	n	n
ČS/Babinské louky	(2)		–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
BK/Suchovské mlýny	(3)			–/–	–/–	–/–	–/–	–/–	–/–	+/–
MK/Jedovnice	(4)				++/+	+/+	+/–	+/–	+/–	++/–
MK/Hádecká planina	(5)					+/–	–/–	++/+	–/+	–/+
MK/Blansek	(6)						+/–	–/+	–/+	+/+
ŠU/Vinice-Kašperské Hory	(7)							+/–	–/–	–/–
ŠU/Nebe I.-Kašperské Hory	(8)								–/–	++/–
ŠU/Nebe II.-Kašperské Hory	(9)									–/–

– no statistical differences; + statistically significant differences (a = 0.05); ++ statistically high significant differences (a = 0.01)
n – not evaluated – plants were in bad condition

T a b l e 5

Statistical differences between the plants in field nursery in height and width of plants in 2007

Protected landscape area/ Locality		2	3	4	5	6	7	8	9	10 ŠU Dobrá-Stožec
		(height/width of plants)								
ČS/ Hradiště–Mentaurov	(1)	n	n	n	n	n	n	n	n	n
ČS/Babinské louky	(2)		–/–	–/–	n	–/–	–/–	–/–	–/–	–/–
BK/Suchovské mlýny	(3)			–/–	n	–/–	–/–	–/–	–/–	–/–
MK/Jedovnice	(4)					–/–	–/–	–/–	–/–	–/–
MK/Hádecká planina	(5)						n	n	n	n
MK/Blansek	(6)						–/–	–/–	–/–	–/–
ŠU/Vinice-Kašperské Hory	(7)							–/–	–/–	–/–
ŠU/Nebe I.-Kašperské Hory	(8)								–/–	–/–
ŠU/Nebe II.-Kašperské Hory	(9)									–/–

– no statistical differences; + statistically significant differences (a = 0.05); ++ statistically high significant differences (a = 0.01)
n – not evaluated – plants were in bad condition

matic conditions and therefore the height and width of plants are not characters suitable enough for the determination of biodiversity of plant population.

Also the length and width of leaf and length of inflorescences were evaluated in particular plants in the field nursery (Table 6). Statistically high significant differences between localities within one PLA as well as between plants from different PLAs were found in all three evaluated characteristics. In spite of these results we cannot confirm the heterogeneity of studied populations because the statistically significant and high significant differences were found also between the particular plants from the same original locality which means that biodiversity

T a b l e 6

The length and width of leaf and length of inflorescences evaluated in particular plants in field nursery

Protected landscape area/Locality	No. of evaluated plants 2006/2007	Length of leaf [cm]		Width of leaf [cm]		Length of inflorescences [cm]	
		2006	2007	2006	2007	2006	2007
ČS/Hradiště - Mentaurov	0/0	n	n	n	n	n	n
ČS/Babinské louky	5/5	20.7 ± 5.4	16.1 ± 2.6	4.6 ± 1.2	4.2 ± 0.8	17.7 ± 7.2	24.4 ± 8.9
BK/Suchovské mlýny	6/7	25.3 ± 6.5	16.5 ± 3.3	5.8 ± 1.4	4.2 ± 1.0	11.8 ± 5.8	36.0 ± 11.5
MK/Jedovnice	3/4	20.7 ± 3.1	18.8 ± 4.4	4.5 ± 0.8	4.7 ± 1.5	19.3 ± 6.4	27.1 ± 11.3
MK/Hádecká planina	3/0	22.2 ± 3.5	n	4.9 ± 0.6	n	17.6 ± 5.5	n
MK/Blansek	4/4	20.6 ± 3.5	14.7 ± 2.9	4.6 ± 0.6	4.8 ± 1.1	15.2 ± 4.4	23.0 ± 11.9
ŠU/Vinice-Kašperské Hory	4/4	23.8 ± 5.1	16.1 ± 3.0	5.5 ± 1.2	4.3 ± 0.8	13.2 ± 4.6	18.0 ± 5.6
ŠU/Nebe I.-Kašperské Hory	5/5	24.5 ± 4.9	17.9 ± 3.3	4.6 ± 0.6	4.3 ± 0.7	11.9 ± 3.9	26.8 ± 7.8
ŠU/Nebe II.-Kašperské Hory	4/5	29.8 ± 4.8	18.8 ± 3.7	7.0 ± 1.8	5.0 ± 1.0	10.2 ± 4.0	15.8 ± 9.7
ŠU/Dobrá-Stožec	5/5	22.6 ± 4.8	16.2 ± 4.1	4.8 ± 1.0	3.6 ± 0.8	10.1 ± 4.3	14.8 ± 5.8
S; $\bar{x}$ ± st. deviation	39/39	22.7 ± 5.3	16.9 ± 3.6	5.2 ± 1.3	4.4 ± 1.0	13.4 ± 5.6	23.6 ± 11.8

n – not evaluated – plants were in bad condition

T a b l e 7

Content of total polyphenols in *Betonica officinalis* L. plants in field nursery

Protected landscape area/ Locality	2006 [mg.g^{-1}]		2007 [mg.g^{-1}]	
	$\bar{x}$	s	$\bar{x}$	s
ČS/Hradiště-Mentaurov	n	n	n	n
ČS/Babinské louky	18.8	0.1	16.7	0.0
BK/Suchovské mlýny	30.5	0.1	25.2	0.1
MK/Jedovnice	22.2	0.3	18.0	0.1
MK/Hádecká planina	24.3	0.1	n	n
MK/Blansek	24.4	0.2	20.8	0.3
ŠU/Vinice-Kašperské Hory	33.3	0.3	25.7	0.2
ŠU/Nebe I.-Kašperské Hory	21.2	0.2	21.7	0.3
ŠU/Nebe II.-Kašperské Hory	28.1	0.1	25.8	0.1
ŠU/Dobrá-Stožec	28.2	0.1	21.0	0.3
$\bar{x}$	25.6	4.4	21.9	3.3

n – not evaluated – plants were in bad condition

of plants within the one population can have the same rate as biodiversity of plant populations.

The evaluation of the total polyphenols content showed that *Betonica officinalis* L. plants contain 16.7–33.3 mg.g^{-1} of dry material in

T a b l e 8

Statistical differences between the content of total polyphenols

Protected landscape area		2	3	4	5	6	7	8	9	10 ŠU Dobrá-Stožec
ČS/ Hradiště-Mentaurov	(1)	n	n	n	n	n	n	n	n	n
ČS/Babinské louky	(2)		+	-	++	-	++	+	-	-
BK/Suchovské mlýny	(3)			++	++	++	-	-	-	-
MK/Jedovnice	(4)				++	-	++	+	-	++
MK/Hádecká planina	(5)					++	++	-	-	++
MK/Blansek	(6)						++	-	-	-
ŠU/Vinice-Kašperské Hory	(7)							-	-	++
ŠU/Nebe I.-Kašperské Hory	(8)								-	-
ŠU/Nebe II.-Kašperské Hory	(9)									-

n – not evaluated – plants were in bad condition
–, +, ++ – symbols are identical with table 5

T a b l e 9

Content of total polyphenols in some species from *Lamiaceae* family

Source	Plant species	Type of sample	Extraction	Wavelength [nm]	Content of total polyphenols [mg.g^{-1}]
our experiment	*Betonica officinalis* L.	herb	ethanolic extracts (80%)	765	16.07–33.3
Buchwald and Dedio (1998)	*Betonica officinalis* L.	herb	–	–	10.9–22.8
Matkowski and Piotrowska (2006)	*Betonica officinalis* L.	herb	methanolic extracts (-)	765	25.7±1.0
Durling et al. (2007)	*Salvia officinalis* L. ´Lincoln Grey´	non-flowering herb	ethanolic extracts (81%)	750	18.4±0.8
Jordán et al. (2009)	*Thymus zygis* Loef. ex. L. subsp. *gracilis* (Boiss.) R. Morales	herb	methanolic extracts (-)	765	108.5–122.42
Matkowski and Piotrowska (2006)	*Galeopsis speciosa* Mill.	herb	methanolic extracts (-)	765	15.6±0.9
	Lamium album L.	flowers	methanolic extracts (-)	765	23.0±1.7
	Lamium purpureum L.	flowers	methanolic extracts (-)	765	23.5±2.1
	Leonurus cardiaca L.	herb	methanolic extracts (-)	765	32.8±4.0
	Marrubium vulgare L.	herb	methanolic extracts (-)	765	20.0±1.4
Parejo et al. (2002) in Jordán et al. (2009)	*Lavandula latifolia* Medik.	–	–	–	82.89±4.93
Proestos et al. (2005) in Jordán et al. (2009)	*Thymus vulgaris* L.	herb	–	–	19.2±0.3
Proestos et al. (2006)	*Nepeta cataria* L.	herb	methanolic extracts (62.5%)	–	19.2±0.3
Proestos et al. (2006)	*Origanum dictamnus* L.	leaves	methanolic extracts (62.5%)	–	13.6±0.4

– not known

average (Table 7). Statistically high significant differences between localities within one PLA (Table 8) as well as between plants from different PLAs were found in this characteristic but not any statistically significant differences were found between results from evaluated years. The highest content of the total polyphenols was found in plants from localities Šumava/Vinice – Kašperské Hory and Bílé Karpaty/Suchovské mlýny in both evaluated years 2006 and 2007. The lowest value of this characteristic was in the same period found in plants from the locality České Středohoří/Babinské louky.

DISCUSSION

The results obtained in our experiments were confronted with literature data. Unfortunately *Betonica officinalis* L. is not a species often studied in scientific papers or there are presented only other types of results (for example essential oil analysis) and therefore the comparison with latter data from scientific journals was difficult.

Height of plants in literature is presented as 20–100 cm (Dostál 1989) but during our measurements in natural localities the plants between 21 and 135 cm were found. In the field nursery the cultivated plants had 40–92 cm which means that the cultivation in identical field and climatic conditions causes the decreasing of morphological variability between plants in this character. Other results were obtained in the evaluation of the width of plants. In natural localities the plants 12–77 cm wide were found whereas in cultivation they reached 20–101 cm. These differences could be explained by the absence of competition with other plants in field condition – the *Betonica* plants could used a larger area for its growing on the field. Any information about width of plants and as well as about length of inflorescences is missing in literature so the comparison was not possible in these characteristics. In natural localities the inflorescences were long between 2 and 41 cm whereas in the field nursery they reached 3–63 cm which can be also explained by better growing conditions.

The length of *Betonica* leaves is presented as 3–12 cm (Dostál 1989) but in natural localities the plants with leaves 8–56 cm long were found. The plants with longer leaves were found mainly in damper places. In the cultivated plants the length of leaves was set at 8–40 cm. The similar results were got in width of leaves measurement. Whereas literature (Dostál 1989) presents 0.5–5.0 cm our plants had 2.4–14.0 cm wide leaves in natural localities and 2.0–14.0 cm in field nursery. This comparison showed that the width of leaves is a character not influenced by cultivation. A big difference between our results and data cited in literature can be explained only in one way: literature data is related only to the leaves on the stalk whereas our results coming from measurement of basal leaves which are much larger and where a very long leaf petiole is often presented.

The content of total polyphenols was determined as 10.9–22.8 $mg.g^{-1}$ in dry matter of *Betonica* plants coming from several European botanical gardens (Buchwald and Dedio 1998). An average value of the total polyphenols content was 17.5 $mg.g^{-1}$ in this experiment and from this amount 2.0–3.9 $mg.g^{-1}$ represent tannins. Matkowski and Piotrowska (2006) on the other hand found in *Betonica officinalis* L. 25.7 ± 1.0 $mg.g^{-1}$ of total polyphenols expressed as gallic acid equivalent. Compare to these results our plants reached on average 23.7 $mg.g^{-1}$ of the total polyphenols in dry matter (16.7–33.3 $mg.g^{-1}$) which agree with the literature data. Another literature source (Kresánek and Krejča 1977) on the other hand reports a content of up to 19% of tannins in *Betonica* dry matter which means 190 $mg.g^{-1}$. This information is however possible in relation to its old-age and in confrontation with our results and previous literature data consider as controversial. Beside other species of *Lamiaceae* family *Betonica officinalis* L. has average or below the average content of total phenols (Table 9) but these results are unfortunately not directly comparable due to different types of plant material and methods used for those studies.

As a recommendation for future study we can summarise that also other secondary metabolites (for example essential oil content and quality) should be studied to confirm genetically based differences between plants from different localities in the Czech Republic.

CONCLUSIONS

The evaluation of variability of morphological characters and the content of total polyphenols in *Betonica officinalis* L. proved statistical significant and highly significant differences between populations from chosen natural localities and protected landscape areas.

Unfortunately the statistically significant and highly significant differences were in some morphological characteristics proved also between the individual plants from one locality so the question of biodiversity between the populations stays unsolved. In spite of results we cannot confirm the heterogeneity of studied populations because the biodiversity of plants within the one population can have the same rate as biodiversity of plant populations.

The highest content of total polyphenols was found in plants coming from the locality of Kašperské hory – Vinice (protected landscape area Šumava) and Suchovské mlýny (Bílé Karpaty). The content of total polyphenols was set up to 33.3 $mg.g^{-1}$ of dry matter.

On the basis of current information we cannot declare that populations of *Betonica officinalis* L. in the Czech Republic are genetically different from each other. The final evaluation of genotypic variability of *Betonica officinalis* L. in the Czech Republic can be probably solved only by methods of molecular genetics.

On the other hand, on the basis of current information we also cannot recommend using uniform *Betonica* seeds for purposes of species-rich grasslands restoring.

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SOURHN

Ačkoliv bukvice (*Betonica officinalis* L.), léčivá rost-

lina používaná na celou řadu chorob (zvláště jako nervinum a tonikum k léčení nemocí hlavy a pro vnější ošetření ran), byla poměrně běžná v celé Evropě, počet a velikost jejích populací se v současné době snižuje. Tento trend se projevuje i v České republice a proto byly studovány jeho příčiny, stejně jako možnost reintrodukce původních genotypů na dané lokality, variabilita morfologických znaků a obsah aktivních látek u vybraných rostlin. Cílem práce bylo také ověření genotypové variability bukvice v České republice, a proto byly studovány rostliny z 10 lokalit ve čtyřech CHKO České republiky.

Na původních lokalitách a *ex situ* v polní školce v Olomouci byla hodnocena výška a šířka rostlin, délka a šířka listů, délka květenství a obsah celkových polyfenolů. Všechna získaná data byla statisticky vyhodnocena a byly zjištěny statisticky průkazné rozdíly mezi rostlinami z různých CHKO, rostlinami z různých lokalit v rámci jedné CHKO i mezi rostlinami v rámci jedné populace.

Klíčová slova: bukvice, *Betonica*, morfologické znaky, polyfenoly