Betonica officinalis L. in the Czech Republic. II. Seed production and quality and variability of total polyphenols content

KAREL DUŠEK*, ELENA DUŠKOVÁ, KATEŘINA SMÉKALOVÁ

Crop Research Institute
Department of Vegetables and Special Crops Olomouc
Šlechtitelů 11
783 71 Olomouc-Holice, Czech Republic

*corresponding author: phone: +420 585209963, e-mail: Dusek@genobanka.cz

Summary

The vriability of seed quality (1000-seed weight, germination capacity) and content of polyphenols were studied *in situ* and *ex situ* in Wood Betony (*Betonica officinalis*) from 10 natural sites of Czech Republic. The statistically significant differences in almost all studied characters were found between the plants from these localities *in situ* as well as *ex situ* in field cultivation in Olomouc. The cultivation of this species is convenient and also production of seeds was satisfactory in the isolation cages with bumble-bees as pollinators. In average 60.0±47.4 g of seeds per 20 plants were reached with 1000-seed weight 1.3±0.1 g and germination capacity 35.6±27.4 %. This seed quality was found to be comparable with naturally produced one. The content of total polyphenols ranged from 16.7 to 33.3 $mg.g^{-1}$ in dry matter.

Key words: *Betonica officinalis, Lamiaceae, Wood Betony, Stachys betonica, variability, pollination, seed quality, polyphenols*

INTRODUCTION

Betonica officinalis L. (syn. *Stachys betonica* Bentham, *Stachys officinalis* (L.) Trevisan; Lamiaceae - Wood Betony, Bishopswort) is a perennial herb relatively common in larger part of Europe. It contains relatively high amounts of tannin, bitter substances, small amount of essential oil and other active substances [1] and at the same time it was commonly used as a medicinal plant 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 [2, 3]. In addition to tannins, Jahodář [4] has also found iridoids, diterpenic lactones and methylbetains. Wood Betony is much less used for healing properties nowadays, and even Czech official medicine does not use it – it is not included in both Czech and European pharmacopoeia probably due to decreasing number of places of its natural occurrence areas and connected narrow stock for collecting [5]. Nevertheless *B. officinalis* is still commonly used in Chinese medicine [6, 7] and also in Russia [8]. It also plays an important role in natural phytocenosis – except from its high ornamental value it is highly melliferous [1] and it is as a perspective genus recommended also for founding flowering meadows and produce soil-conserving mixtures [9].

This paper is focused on possibility of seed production, variability or germination capacity as well as total polyphenols content of the *B. officinalis* populations from several localities in the Czech Republic, as a model sample of the situation in natural places of occurrence. The aim of this work was also to verify genotypic variability of *B. officinalis* in the Czech Republic.

MATERIALS AND METHODS

Plant material

For *B. officinalis* populations study, 10 natural sites in 4 protected landscape areas (or in the neighbouring localities) in the Czech Republic were chosen – the plant material used was the same as for previous article "*Betonica officinalis* L. in the Czech Republic. I. Variability of morphological characteristics" [10].

Seed production

In order to test the possibilities of seed production 20 plants coming from some original localities were kept isolated during the flowering period in 2009, it means from mid-May till the end of flowering. Technical isolation was realised by mobile isolation cages of size 2.0 x 3.0 x 1.7 m (width x length x high) covered by silon net. The bumble bees in small bumble-bee colonies were used as pollinators. When the seeds became mature (the beginning of September), whole plants were harvested by hand and dried in field drier of controlled airflow. Then seeds were crumbled by hand, cleaned on sieves and then carefully cleaned by hands and the 1000-seed weight was measured. The number of seeds was calculated with use of seed counter.

Determination of germination capacity

The germination capacity of original seed samples collected at natural localities in 2004 and 2005, was evaluated in laboratory conditions after drying. The germination conditions (filter paper dried by distil water, 20°C, 12 h light/12 h dark) based on method for *Salvia officinalis* [11]. No disinfection of seeds was realised and due to limited amount of available seeds only 1x 50 seeds were tested for each sample. Laboratory germination tests were started on February 4th, 2005 and January 12th, 2006 and maintained for 25 (2005) and 56 (2006) days, respectively. During the germination experiments so-called "maximal germination" was determined – the maximal germination capacity reached by individual sample in every experimental year [12].

The same method was also used for testing of germination capacity of seeds produced in controlled conditions. This experiment was started on 2.11.2009 and lasted for 82 days.

Total content of polyphenols

The total content of polyphenols was analysed in mixed sample from each group of plants separately and all the plant samples from the field nursery were prepared the same way for the evaluation of active substances content: the full flowering stems were harvested by hand, dried in the temperature lower than 35°C (drying house with controlled air circulation) and grinded by laboratory homogenizer. Each sample representing the locality and the source stage (mother plants/seeds) was mixed with the all plants of the group. The content of total polyphenols was measured by spectroscopy – a colour precipitate reaction of polyphenols with Folin-Ciocalteau phenol reagent assay was used [13]. Two repetitions of measurements were performed in each sample.

Statistical evaluation

Statistical evaluation of obtained data was done by Anova, one-factor analysis of variance with level of importance $\alpha=0.05$ resp. 0.01.

RESULTS AND DISCUSSION

Seed quality

The quality of seeds collected at the natural localities was tested after drying and cleaning. The 1000-seed weight varied from 0.594 g (České Středohoří – Mentaurov in 2005) to 1.3142 g (České Středohoří – Malečov in 2004) (tab. 1). An average weight is 1.0510 g per 1000 seeds and no statistically significant

difference was determined between both evaluated years (level of significance $\alpha=0.05$; F critical value=4.60; F value=0.85) neither between studied populations ($\alpha=0.05$; F critical value=3.50; F value=3.17).

Determined germination capacity of the seeds varied from 2 to 88% (average 33%) and also in this characteristics statistically significant differences between evaluated localities ($\alpha=0.05$; F critical value=3.73; F value=1.12) were not found. On the other hand, the difference between results obtained in both evaluated seasons (2004 and 2005) was statistically proved ($\alpha=0.05$; F critical value=4.60; F value=6.32). Selected dates of collection seem to be not a point influencing our results according acquired data – low and high values of both characteristics are presented at the beginning of collecting period as well as at the end.

Table 1.

Quality of seeds collected from the natural localities

original locality		2004			2005		
		collection date	1000-seed weight (g)	germination capacity (%)	collection date	1000-seed weight (g)	germination capacity (%)
Bílé Karpaty	Suchov	not evaluated due to small amount of seeds					
České středohoří	Malečov	22.9.	1.3142	14	30.9.	1.1609	40
	Mentaurov	22.9.	0.6102	2	30.9.	0.594	10
Moravský Kras	Blansek	not evaluated – seeds were send by local employers (unknown seed management)					
	Hády	14.9.	0.8327	22	27.10.	1.0000	52
	Jedovnice	14.9.	1.1775	34	27.10.	1.1636	64
Šumava	Dobrá	17.9.	1.1673	28	27.9.	1.0322	40
	Nebe I.	17.9.	1.0508	44	27.9.	1.3112	88
	Nebe II.	17.9.	0.8820	6	27.9.	1.3107	62
	Vinice	17.9.	0.9460	12	27.9.	1.2622	16
average			0.9976	20.3		1.1044	46.5

Seed production

Vegetative spread, although restricted in extent, is more important in the development of *B. officinalis* populations than regeneration by seed. Seed matures late in the year and is released slowly. The experiment showed that germination was delayed until spring, and was not associated with the formation of a persistent seed bank. The seed appears to be poorly dispersed, and *B. officinalis* is virtually restricted to old or semi-natural vegetation [14]. Nevertheless seed spread is necessary for production of seed mixtures. Therefore, the knowledge on controlled seed processing principles is irreplaceable.

Technical isolation of seed producing overgrowth by isolation cages brings

expected credit. Unlike bees, which are mentioned as natural pollinators in the literature [15], bumble-bees were used as pollinators during the experiment. It was proved that bumble-bees were also able to pollinate open flowers sufficiently. The seed yield and 1000-seed weight were satisfactory (tab. 2). From 11 to 150 g of seeds were produced from 20 *B. officinalis* plants in each isolation cage and the average 1000-seed weight was 1.3 g. Compared to seed produced in nature, the seeds produced under controlled conditions reached similar 1000-seed weight as well as germination. Of course, there were some exceptions (germination in seeds from locality Šumava – Nebe I. and Vinice) (fig. 1, 2). No statistically significant differences of 1000-seed weight and/or germination were proved in both groups of seeds – differences between localities as well as evaluated years (2004, 2005 and 2009) were unconvincing. Obtained seed germination (nearly 36%) seems to be low but is common in wild growing species. It was frequently proved that wild forms of cultivated crops as well as wild species itself have a lower germination capacity as compared to crop plants due to genetically controlled dormancy of seeds [16, 17]. On the other hand the laboratory germination could be also influenced by many seed pretreatments as for example cooling or disinfection and/or germination conditions like light conditions. Detailed study of ideal germination conditions of *B. officinalis* seeds was however not the aim of this study.

Table 2.

Seed production in controlled conditions

Origin	yield (g)	1000-seed weight (g)	germination (%)
MK Blansek	50	1.2516	64
MK Hády	150	1.3893	62
MK Jedovnice	53	1.4084	46
ŠU Nebe I.	11	1.0081	6
ŠU Vinice	36	1.3735	0
average ± standard deviation	60.0±47.4	1.3±0.1	35.6±27.4

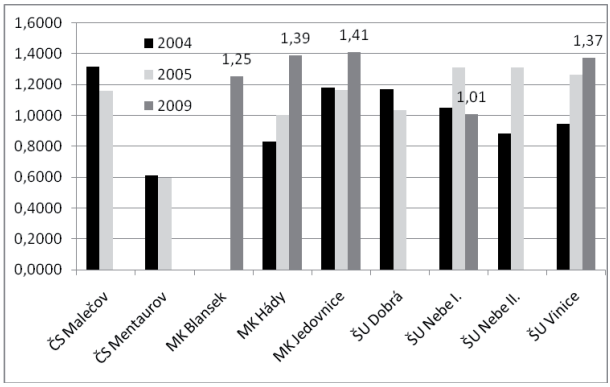

Figure 1. Comparison of 1000-seed weight of seeds from original localities and controlled conditions

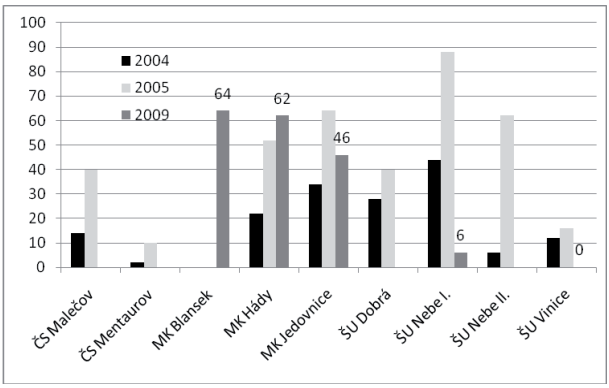

Figure 2. Comparison of germination of seeds from original localities and controlled conditions

Content of total polyphenols

According our experiment, *B. officinalis* in the Czech Republic reached on average 23.7 mg.g^{-1} of total polyphenols in dry matter (16.7–33.3 mg.g^{-1}) (tab. 3). Statistically high significant differences were found between samples from several natural localities (α=0.01; F critical value=3.51; F value=6.86) and statistically significant differences were found between plants from different protected landscape areas (α=0.05; F critical value=4.64; F value=3.14) as well. No statistically significant differences were found in results from evaluated years (α=0.05; F critical value=4.54; F value=3.52). The highest content of total polyphenols was found in plants coming from localities Šumava – Vinice and Bílé Karpaty – Suchov, in both evaluated years 2006 and 2007. In the same period the lowest value of this characteristic was found in plants from locality České Středohoří – Malečov.

Compared to this results 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 [18]. An average value of total polyphenols content was 17.5 mg.g^{-1} in this study and in this amount 2.0–3.9 mg.g^{-1} represents tannins. Matkowski and Piotrowska [19] present 15.6±0.9 mg tannins (expressed as gallic acid equivalents) per g of dry herb. These data are in accordance with our experimental results. Another literature source [1] reports a content of up to 19% of tannins in *Betonica* dry matter which means 190 mg.g^{-1}. However, this information could be possible in relation to its antiquity and in confrontation with our results and previous literature data it is considered controversial. The comparison of polyphenols content in *B. officinalis* and other species of Lamiaceae family implies the conclusion that *B. officinalis* does not content high amount of polyphenols (tab. 4).

Table 3.

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

protected landscape area/locality	2006 (mg.g^{-1})		2007 (mg.g^{-1})	
	average	st. deviation	average	st. deviation
BK Suchov	30.5	0.1	25.2	0.1
ČS Malečov	18.8	0.1	16.7	0.0
ČS Mentaurov	n	n	n	n
MK Blansek	24.4	0.2	20.8	0.3
MK Hády	24.3	0.1	n	n
MK Jedovnice	22.2	0.3	18.0	0.1
ŠU Dobrá	28.2	0.1	21.0	0.3
ŠU Nebe I.	21.2	0.2	21.7	0.3
ŠU Nebe II.	28.1	0.1	25.8	0.1
ŠU Vinice	33.3	0.3	25.7	0.2
average	25.6	4.4	21.9	3.3

n – not evaluated – plants in bad condition

Table 4.

Content of total polyphenols in some species from Lamiaceae family

source	plant species	type of sample	extraction	wavelength	content of total polyphenols (mg.g–$^{-1}$)
our experiment	*Betonica officinalis*	herb	ethanolic extracts (80%)	765 nm	16.07–33.3
[17]		herb	–	–	10.9–22.8
[18]		herb	methanolic extracts (–)	765 nm	15.6±0.9
[19]	*Salvia officinalis* ´Lincoln Grey´	non–flowering herb	ethanolic extracts (81%)	750 nm	18.4±0.8
[20]	*Thymus zygis ssp. gracilis*	herb	methanolic extracts (–)	765 nm	108.5–122.42
[18]	*Galeopsis speciosa*	herb	methanolic extracts (–)	765 nm	20.0±1.4
	Lamium album	flowers	methanolic extracts (–)	765 nm	23.0±1.7
	Lamium purpureum	flowers	methanolic extracts (–)	765 nm	25.7±1.0
	Leonurus cardiaca	herb	methanolic extracts (–)	765 nm	32.8±4.0
	Marrubium vulgare	herb	methanolic extracts (–)	765 nm	23.5±2.1
Parejo et al. (2002) in [21]	*Lavandula latifolia*	–	–	–	82.89±4.93
Proestos et al. (2005) in [21]	*Thymus vulgaris*	herb	–	–	19.2±0.3
[22]	*Nepeta cataria*	herb	methanolic extracts (62.5%)	–	19.2±0.3
[22]	*Origanum dictamnus*	leaves	methanolic extracts (62.5%)	–	13.6±0.4

– not known

CONCLUSIONS

1. The cultivation of this species is convenient and also production of seeds can be satisfactory in the isolation cages with use of bumble-bees as pollinators. On average, 60.0±47.4 g of seeds per 20 plants were reached with 1000-seed weight 1.3±0.1 g and germination capacity 35.6±27.4%. This seed quality was found comparable with natural-produced one.
2, The highest content of total polyphenols was found in plants coming from locality 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.
3. *B. officinalis* is not included in European pharmacopoeias but the reason is not evident. The most probable is decreasing of its natural occurrence areas and connected narrow stock for collecting. Also its therapeutic profile is not unique and we can find other medicinal plants with similar healing characters.

REFERENCES

1. Kresánek J, Krejča J. Atlas liéčivých rastlin a lesných plodov. Osveta 1977:126.
2. Phillips R, Foy N. Herbs. London, Basingstoke and Oxford 1992:162.
3. Bown D. New Encyclopaedia of Herbs and their Uses. London 2002:374.
4. Jahodář L. Farmakobotanika semenné rostliny. Praha 2006:148.
5. Opletal L. Problems of *Betonica officinalis* use in medicine. Verbal information, cited 15th June 2010.
6. Polienko EM. Chinese medicinal composition for stopping drug addiction and treating withdrawal symptoms. Patent of Russian federation RU 21 77799 C1 20020110. 2002.
7. Demande F. An oral Chinese medicinal composition containing plant as main ingredient for preventing and treating gynecologic diseases. Patent of France FR 2623397 A1 19890526. 1989.
8. Sukhanov A I. A herbal composition for the treatment of nephropathy and urine tract diseases. Patent of Russian federation RU 2137492 C1 19990920. 1999.
9. Šrámek P, Kohoutek A, Ševčíková M, Odstrčilová V, Jongepierová I. Zvyšování biodiversity travních porostů. Praha. Zemědělské informace 2001(21):21.
10. Dušek K, Dušková E, Smékalová K. *Betonica officinallis* L. in the Czech Republic. I. Variability of morphological characteristics. Herba Pol 2010; 56(2):7-20
11. ČSN 46 0610. Zkoušení osiva. Praha. Vydavatelství ÚNM, 1984.
12. Bláha L, Poková H. Test umožňující paralelní rychlé hodnocení klíčivosti druhů s odlišným stupněm dormance. In: Osivo a sadba. IX. odborný vědecký seminář; 2009 Feb 10; Prague, Czech Republic. 2009:153-7.
13. Pharmacopoeia bohemoslovenica. Praha, Avicenum 1987(1):412.
14. Grime J P, Hodgson J G, Hunt R. *Stachys officinalis* (L.) Trevisan. In: Comparative Plant Ecology. London. 1989:546-7.
15. Fern K. Plants For A Future: Edible & Useful Plants For A Healthier World. Clanfield 1997:320.
16. Hitchmough JD, Gough J. Corr B. Germination and dormancy in a wild collected genotype of *Trollius europaeus.* Seed Sci Technol 2000; 28(3):549-58.
17. Horovitz A, Bullowa S, Negbi M. Germination characters in wild and cultivated *Anemone coronaria* L. Euphytica 1975; 24:213-20.
18. Buchwald W, Dedio I. Ocena surowca z upraw próbnych *Betonica officinalis* L. Herba Pol 1998; 44(4):403-8.
19. Matkowski A, Piotrowska M. Antioxidant and free radial scavenging activities of some medicinal plants from the Lamiaceae. Fitoterapia 2006; 77:346-53.
20. Durling NE, Catchpole OJ, Grey JB, Webby RF, Mithcell KA, Foo LY, Perry NB. Extraction of phenolics

and essentials oil form dried sage (*Salvia officinalis*) using ethanol-water mixtures. Food Chem. 2007; 101:1417-1424.
21. Jordán MJ, Martínez RM, Martínez C, Moñino I, Sotomayor JA. Polyphenolic extract and essentials oil quality of *Thymus zygis* ssp. *gracilis* shrubs cultivated under different watering levels. Ind Crop Prod 2009; 29:145-53.
22. Proestos C, Boziaris I S, Nychas G-J E, Komaitis M. Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food Chem 2006; 95:664-71.

ACKNOWLEDGEMENT

Financial support of research goals MZE ČR No. 0002700602 and 0002700604 is gratefully acknowledged.

BETONICA OFFICINALIS L. W CZECHACH. II. PRODUKCJA MATERIAŁU SIEWNEGO ORAZ BADANIE JAKOŚCI I ZMIENNOŚCI ZAWARTOŚCI POLIFENOLI

KAREL DUŠEK*, ELENA DUŠKOVÁ, KATEŘINA SMÉKALOVÁ

Crop Reasearch Institute
Department of Vegetables and Special Crops Olomouc
Šlechtitelů 11
783 71 Olomouc-Holice, Czech Republic

*autor, do którego należy kierować korespondencję: tel: +420 585209963, e-mail: Dusek@genobanka.cz

S t r e s z c z e n i e

Badano zmienność jakości materiału siewnego (masa 1000 nasion, zdolność kiełkowania) oraz zmienność zawartości zawiązków polifenolowych czyśćca lekarskiego (*Betanica officinalis* L.) *in situ* i *ex situ* z 10 naturalnie występujących stanowisk w Czechach. W wyniku przeprowadzonych badań stwierdzono różnice pomiędzy materiałem roślinnym zebranym ze stanowisk naturalnych (*in situ*) oraz materiałem pochodzącym z uprawy polowej w Olomouc (*ex situ*). *Betonica officinalis* L. jest gatunkiem nie sprawiającym trudności w uprawie. Także wydajność produkcji nasion pod izolatorami z wykorzystaniem trzmieli jako zapylaczy jest zadowalająca. Z 20 roślin uzyskiwano średnio 60,0 ± 47,4g nasion; masa 1000 nasion wynosiła 1,3 ± 0,1g oraz zdolność kiełkowania wynosiła 35,6 ± 27,4%. Nasiona pochodzące z uprawy polowej posiadają porównywalną jakość z materiałem otrzymywanym z naturalnych stanowisk. Całkowita zawartość związków fenolowych wynosi od 16,7 do 33,3 mg.g^{-1}suchej masy.

Słowa kluczowe: *Betonica officinalis, czyściec lekarski, Stachys betonica, zmienność, zapylanie, materiał siewny, polifenole*