Scientific Papers. Series A. Agronomy, Vol. LVIII, 2015
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

SEED GERMINATION OF *Betonica bulgarica* Deg. et Neic UNDER THE INFLUENCE OF DIFFERENT TREATMENTS AND SEED QUALITY

Galia PANAYOTOVA, Neli GROZEVA, Mima TODOROVA, Marya GERDZHIKOVA

Trakia University, Faculty of Agriculture, Student campus, 6000 Stara Zagora, Bulgaria

Corresponding author e-mail: galia_panayotova@abv.bg

Abstract

Betonica bulgarica Degen & Nejceff is a Bulgarian endemic species protected under the Biological Diversity Act and included in the Red Data Book of the Republic of Bulgaria, vol.1. Plants and fungi in the category "endangered". The aim of this research was to study seeds germination of endemic species Betonica bulgarica Deg. et Neic, as well as 1000 seeds weight of four natural habitats from the Nature Park Sinite Kamani, Bulgaria. Harvested seeds from plants in the Sinite Kamani Nature Park were collected by means of insulators to assist the natural reproduction of the populations. The seed germination was studied in petri dishes after different temperature treatments - in a laboratory at 15°C, in a thermostat at 20°C and 25°C, at a temperature of 5°C, treated with water at 35°C, and direct sowing in the soil without any treatment. It was found that B. bulgarica is characterized by a prolonged period of germination and emergence. The best results were achieved after direct sowing in soil - 35.0%, followed by seeds placed for germination in laboratory conditions at 15°C - 25.0 %. Treatments of stratification and hot water at 35°C did not give good results for seed germination. The 1000-seed weight of four populations was an average of 0.971 g, from 0.840 to 1.055 g.

Key words: *Betonica bulgarica Deg. et Neic., seed germination, germination rate, weight of 1000 seeds.*

INTRODUCTION

The Nature Park Sinite Kamani is located in the Eastern Balkan Mountains on the southern slopes of the Sliven Balkan. The specific climate and lay conditions of the nature park at altitude between 290 and 1180 m determine the great diversity of flora. In the park, over an area of 11 308 hectares are established 1060 species of high plants from 430 genera and 96 families (Stoeva et al., 2002; Grozeva et al., 2004). 42 endemic species are protected by Biological Diversity Act of Bulgaria (2002) (Petrova et al., 2009, 2011; Tashev et al., 2010; Tashev, 2011).

The *Betonica bulgarica* Degen & Nejceff (Bulgarian Betony) from family *Lamiaceae* is a Bulgarian endemic species protected under the Biological Diversity Act (2002) and is included in the Red Book of Bulgaria, vol.1. Plants and fungi under the category „endangered" (Genova, 2011). It is known with localities in Stara planina (Middle and Eastern) and the Thracian plain (Koeva, 1970; Genova, 2011). According to Genova (2011) the species has good regeneration ability and area is 0.3-0.5 ha. It occurs on open grassy places within the forest and in the subalpine zone. No harvesting is allowed by its natural habitats. Populations involved in the composition of herbaceous communities with relatively small abundance.

For the first time *Betonica bulgarica* is reported for Eastern Stara planina by Grozeva et al. (2004) on the territory of Natural park Sinite kamani in Ablanovo area. According to data by the authors the population is small in number. In Bulgarian scientific herbaria (SOM, SOA, SO) there is one herbarium specimen of *Betonica bulgarica* from Eastern Stara planina, Natural park Sinite kamani - a meadow in the area of Karandila (SOM 167749, 19.07.2010, A. Petrova). The species was first described by the Hungarian botanist A. v. Degen and Ivan Neychev in 1906. Grozeva at al. (2004) has been reported for the first time the species for the Nature Park Sinite Kamani in Eastern Stara Planina. There are no clinical human trials supporting the use of Betony for any indication.

B. bulgarica is a perennial herbaceous plant to the family *Lamiaceae*. Stem is 30 to 60 cm, covered with bristles facing down. The leaves are ovate extended, heart-shaped at the base, along the edge acute serrated. The fruits are brown grass nuts, triangular, elongated, 4 mm long, 2 mm wide, the outside almost flat, the edges with narrow wings, which at the top edge

goes into irregularly toothed membranous appendage. Blooms in May-June, the seeds ripen in July-August. The species is reproduce by seeds and vegetatively (Velchev et al., 1992; Flora of the People's Republic of Bulgaria, 1989).

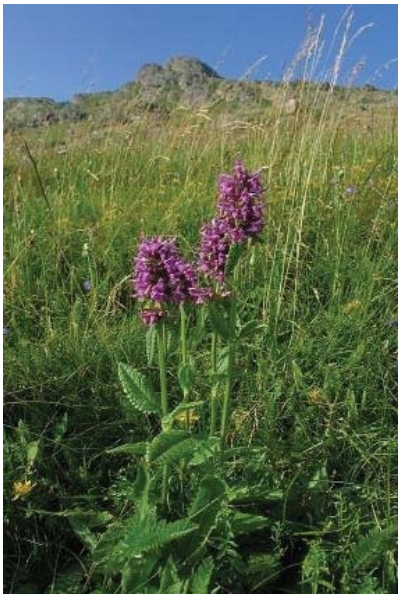

Figure 1. View of *B. Bulgarica*

Numerous procedures for data analysis of seed germination responses are scattered throughout the literature. The methods reviewed include the percent germination, germination index, coefficient of velocity, median response time, probit analysis, curve-fitting of cumulative germination, heat sums, survival analysis with life tables, logistic regression, proportional hazards regression and accelerated failure time analysis (Scott et al., 1984).

Detailed information on the different stages in the reproductive cycle of endemic, threatened and at the same time medicinal species may contribute to improved understanding of the phenomenon of endemism and at the same time assist conservation management decisions for the species under study (Navarro et al., 2003).

Each species has particular requirements for seed germination and germination requirements for native species are often unknown, particularly for rare or endemic species of which material is more difficult to obtain (Navarro et al., 2003; Cerabolini et al., 2004).

According to Escriba et al. (2004) the seed germination is a critical phase in the reproductive cycle, of great importance for species fitness and the variation in germination percentage and has been interpreted as an adaptation to ecological conditions. Temperature and light are the most important factors influencing the induction of seed germination (Baskin and Baskin, 1998). Senel et al. (2007) reported that the optimum temperature for seed germination of medical plants disturbed in Turkey - *S. dicroantha*, *V. bithynicum* and *V. wiedemannianum* was 20°C and darkness.

Emel et al. (2007) reported that the optimum temperature for seed germination of *S. dicroantha, V. bithynicum* and *V. wiedemannianum* was 20°C and darkness. The seeds of *V. bithynicum* and *V. wiedemannianum* incubated in darkness showed higher germination percentages than the seeds incubated with a 16:8 h photoperiod or continuous light, but the effect of application of darkness, photoperiod (16:8 h) or continuous light on the germination percentage of the seeds of *S. dicroantha* was not significant. In the case of *S. dicroantha,* seed weight significantly affected germination percentage, but not significant in the case of *V. bithynicum* and *V. wiedemannianum*. Exogenous GA_3 (20, 100, 200 mg L^{-1}) was comletely prevented germination the seeds of these species.

Yücel and Yılmaz (2009) reported that germination of *Salvia cyanescens* seeds was promoted by cold-wet process at -5ºC; low concentrations of NaCl and KNO_3 (0.5-1%) brought up high germination percentage, but higher concentrations inhibited the germination comparing with no treatment. Herranz et al. (1998) did not find a clear relationship between heat-shock germination response and post-fire regeneration strategy. This work supports that endemics species have germination more sensitive to fire than widely distributed ones.

According to Patanè et al. (2009) the increase in incubation temperature to 35°C determined a faster germination of Sorghum seed than at 25°C, despite the lower final germination percentage. With the lowering of temperature to 15°C germination percentage significantly declined. At 10°C seeds failed to germinate due to depressive effect of low temperature. Seed

priming enhanced germination and shortened the delay in germination time due to the increase in saline stress, at suboptimal temperatures only.

Dušek et al. (2010) reported that the 1000-seed weight of *Salvia officinalis* varied from 0.594 g (in 2005) to 1.3142 g (in 2004) and an average weight is 1.0510 g.

Thanos and Doussi (1995) recorded that 60 to 70% of the seeds of Sideritis syriaca ssp. syriaca germinated only in the dark at a warmer temperature range (20 to 25°C). At 30°C, only 40 % seeds germinated. There was no increase in the germination ratio of Sideritis seeds treated with hot water. No literature could be found concerning the hot water treatment.

Kozhuharova (2009) established that after treatment with gibberellic acid seeds of *Sideritis scardica* (Mountain tea) and *Sideritis recta* have good germination percentage and stratification does not manifest as an effective method.

B. bulgarica is close with *B. officinalis* L. (Stachys Betony), used as a medicine plant. According to Bown (2002) *B. officinalis* L. prefers a light moist neutral to acid soil in sun or light shade, rich heavy soils, hardy to at least -25°C. This herb is best sown at 41F/ 5°C to germinate in 30-90 days. Seed - sow spring in a cold frame and very easy, the plant can be successfully divided at almost any time of the year.

Betonica bulgarica Deg. et Neic of the territory of the Nature Park Sinite Kamani has not yet been the subject of special study. So far the species has not been studied in relation to seed germination and rate of germination for use ex situ conservation.

The aim of this research was to study seeds germination of endemic species *Betonica bulgarica* Deg. et Neic, as well as 1000 seeds weight of four natural habitats from the Nature Park Sinite Kamani, Bulgaria.

MATERIALS AND METHODS

To assist the natural reproduction of the populations of *Betonica bulgarica* using isolators are harvested seeds from ripe fruits of the plants. From preliminary expeditions in the Nature Park Sinite Kamani - Sliven were established populations of *B. bulgarica* in locality Ablanovo (N 42°42.628; E 26°17.251). Average altitude is 542 m above sea level.

Inflorescences capsules with placed isolators were collected in the month of September 2013 from natural populations, without the risk of reducing their reproduction. Collection was made after obtaining permission from the Ministry of Environment and Water of Bulgaia for the use of the exception from art. 40 of the Biodiversity Act, namely the collection of material of the protected plant species *Betonica bulgarica*. All the activities are in accordance with the Protected Areas Act (PAA), the Biological Diversity Act (BDA) and Ordinance № 8.

The study was conducted at the research laboratory of the Faculty of Agriculture at Trakia University - Stara. Zagora. The seeds were hulled, cleaned, inspected by microscopic technique for their physiological condition and stored in paper bags in the dark at room temperature. Sprouting seeds and damage by diseases and pests were not established.

For establishing seed germination and rate of germination were tested the following factors:

1. Germinate under laboratory conditions at 15°C;
2. Germinate in a thermostat at 20°C and humidity of 95% at 8 hours light and 16 hours dark;
3. Germinate in a thermostat at 25°C and humidity of 95% at 8 hours light and 16 hours dark;
4. Germinated after treatment with a temperature of 5°C for 7 days in a refrigerator, and then the seeds were placed at 18-20°C under room conditions;
5. Germinate after immersion of the seeds in water at 35°C;
6. Direct sowing in soil taken from natural habitats.

For the first five tested factors seeds were placed in Petri dishes between distilled water moistened filter paper. Twenty replicates of 25 seeds each were used. A periodic checking of germination was carried out. Radicule emergence was the criterion used for scoring a seed as germinated.

For establish seed weight four replication of 1000 seeds from four populations (Ablanovo, Slancheva poliana, Gorna Lift Stancia,

Microiazovir) were chosen at randomly and was weighed with a precision balance.
The data of each studied parameter was analyzed using Analyses of variance (ANOVA) and Principal component analyses (PCA).

RESULTS AND DISCUSSIONS

The results of our studies indicated that *B. bulgarica* was characterized with a prolonged period of seed germination (Table 1). The exceptionally low percentage of seed germination was established in all studies influences. The start of germination was observed 15 days after the placement of the seeds.
The highest percentage of germinated seeds was established in direct sowing in soil - 35.0%, followed by seeds placed for germination in laboratory conditions at 15°C - 25.0%. By increasing the temperature to 20°C seed germination was reduced to 19.2%, and at 25°C (a temperature which is favorable for a number of other cultures) after 37 days were not reported sprouted plants. Treatments by stratification and hot water at 35°C does not give good results on seed germination. When tested at 7-day low temperature 5°C germination was 1%, and under the effect of hot water at 35°C - 15.0%. Estrelles et al. (2004) also reported that at 35°C were not germinated seeds of Sideritis spinulosa, both at continuous and at varying temperatures 35/15°C, wherein germination was 35% at 25/15°C and 33% at 35/15°C under changing temperature conditions and at light. The results of our studies indicate that the germination is not increased by different types of treatment in comparison to direct sowing in soil taken from natural habitats.
The rate of germination of seeds varied and no permanent tendencies of increasing were observed. When placing on 27 January 2014 seeds for germination in a thermostat at 20°C and 95% humidity, the highest rate of germination was after 50-60 days (Table 2).
The mass of 1000 seeds is an indication of size of the seeds, for the opportunity all together to germinate and grow at accelerated rates. The seed weight is an important factor for successful germination (Kambizi et al., 2006; Malcolm et al., 2003; Perez-Garcia et al., 2006).

Table 1. Germination of seeds from *Betonica bulgarica* Deg. et Neic. under the influence of different temperatures

Treatment	Seeds for germination, total number	Sprouted seeds, number	Germination, %	Date of setting	Last date of sampling	Days of germination
1. 15°C	100	25	25	31.01	18.02	18
2. 20°C	100	19	19	27.01	11.04	74
3. 25°C	100	0	0	27.01	05.03	37
4. 5°C	100	1	1	18.02	09.04	50
5. 35°C	100	15	15	28.02	31.03	31
6. In soil	100	35	35	18.02	11.04	52
Total	600	95	15.8			43.7

Figure 2. Seeds in Petri dishes for germination

Figure 3. Germinated seeds of *Betonica bulgarica*

The 1000 seeds weight of *Betonica bulgarica* from harvest 2013 was on average 0.971 g, with variation in populations from 0.841 to 1.055 g (Table 3 and Figure 4). The plants by area Gorna Lift Stancia were characterized with the biggest seeds - 1.055 g, and with the lowest size were from the area Microiazovir. It was found that the variation within populations is much stronger (69.848%) than among the four populations (30.152%). The values are significantly different at $p < 0.05$ (Table 4). Figure 5 shows that with regard to the weight of 1000 seeds mostly (27%) were the cases within the range 0.9-1.0 g, followed by 23% in the range of 0.8-0.9 g. Single seeds have a lower weight of 0.7 g and greater than 1.2 g.

Principle Component analyses for establishing the power and contribution of the population of *Betonica bulgarica* regarding weight of 1000 seeds demonstrate that for principle components could be defined which have effect above 1. The seeds from population Slancheva poliana are the most important because this parameter is positive for first and second factors (Figure 6). Seeds from populations Gorna Lift Stancia and Microiazovir are the other parameters with the significant effect in PC 2. Fourth parameter (Ablanovo) have not substantial effect and contribution to the seed weight. Integrated PC analyses for the effect and contribution of the parameters to the seed weight demonstrated that the parameters could be separate in three groups as follows:

- first – seeds from Slancheva poliana - positive for F1 and F2;
- second – seeds from Gorna Lift Stancia and Microiazovir – negative for F1, positive for F2;
- third – seeds from Ablanovo - negative for F1 and F2.

Table 2. Rate of germination of seeds in a thermostat at 20°C

Date of sampling	Number of sprouted seeds	Date of sampling	Number of sprouted seeds
04.03	3	25.03	2
07.03	3	27.03	8
13.03	3	28.03	1
17.03	16	31.03	16
18.03	3	02.04	2
19.03	1	07.04	7
20.03	7	09.04	4
21.03	31	11.04	1
24.03	7		

Table 3. 1000 seeds weight of *Betonica bulgarica* from four populations

	1000 Seed mass, g
Ablanovo	1.001a
Slanch poliana	0.987a
Gorna Lift Stancia	1.055a
Microiazovir	0.841
All Grps	**0.971**
N	40
Std.Dev.	0.147
Variance	0.021
Std.Err.	0.023
Minimum	0.715
Maximum	1.430
Variation of 1000 seeds weight, g	
Between population (SSb)	30.152
Within population (SSv)	69.848

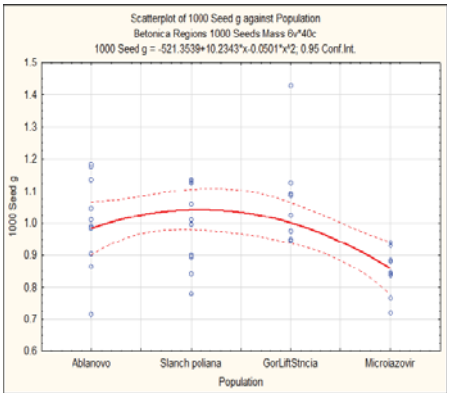

Figure 4. Scatterplot of 1000 seeds weight (g) for four populations of *Betonica bulgaric*

Table 4. Analysis of variance for 1000 seeds weight of four populations of *Betonica bulgarica*

	SS – Effect	df	MS	SS - Error	df – Error	MS - Error	F	P		
1000 Seed, g	37.346	3	12.449	2802.47	8482	0.330	37.678	0	SSb%	SSw%
Error	2802.47								1.32	98.68
Total	2839.82									

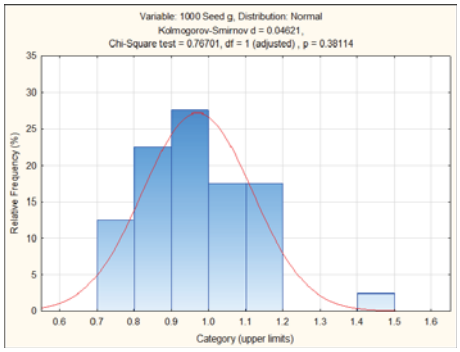

Figure 5. Frequency of weight of 1000 seeds, %

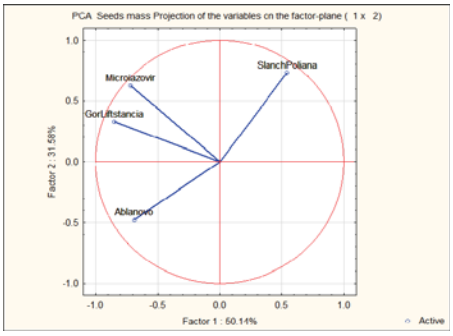

Figure 6. PCA for the distribution of population of *Betonica bulgarica* regarding weight of 1000 seeds

CONCLUSIONS

It was found that *B. bulgarica* is characterized by a prolonged period of germination and emergence. The best results were achieved after direct sowing in soil - 35.0%, followed by seeds placed for germination in laboratory conditions at 15°C - 25.0%. Treatments of stratification and hot water at 35°C did not give good results for seed germination. The 1000-seed weight of four populations were an average of 0.971 g, from 0.840 to 1.055 g.

ACKNOWLEDGEMENTS

This study is a part of project Restoration of habitats and preservation of biodiversity in Natire park Sinite kamani and was financed from Project 2P/14 "Complex population study of species of the genus Suaeda (Sueda) in Bulgaria".

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