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Seed Germination, Growth and Morphological Parameters of *Betonica bulgarica* Deg. et Neic. Cultivated under Different Conditions

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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. Harvested seeds of the plants were collected by using insulators to assist the natural reproduction of the populations. The seeds germination and growth of *B. bulgarica* were studied in three soils: soil from natural habitat; in California red worms Lombricompost (CRWL) and in soil mixture of 2/3 soil + 1/3 CRWL in laboratory conditions. Leaf characteristics - length, width, petiole length, number of leaves and leaf area were studied. It was found that *B. bulgarica* was characterized by a prolonged period of germination and low rate of germination - 35.0 %. Best plant growth was seen in a soil mixture of 2/3 soil + 1/3 CRWL. The soil strongly influenced leaf length, leaf width and leaf area - 46.1 to 58.6 % of the total variation.

Keywords: *Betonica bulgarica* Deg. et Neic, germination, leaf length, leaf width, leaf area.

Introduction

The Nature Park Sinite Kamani is located in the Eastern Balkan (Stara planina) 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 the 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 its area is 0.3-0.5 ha. It is found 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) reported for the first time the species in the Nature Park Sinite Kamani in Eastern Stara Planina. In the Bulgarian scientific herbaria (SOM, SOA, SO) there is deposit with herbarium material of *Betonica bulgarica* from the Eastern Balkans (SOM 167749, Petrova). Locality data were not published. There were no clinical human trials supporting the use of Betony for any indication.

B. bulgarica is a perennial herbaceous plant from 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, on both sides pubescent, 6-14 cm long, 3-6 cm wide. At the base the leaves are with petioles longer than lamina, the lower 2-3 pairs of stem leaves have a short stalk and the supper leaves are sessile The inflorescence has concise and densely fibrous Sepal teeth of equal length of the tube. The pink petal has a narrow tube 9-10 mm long, slightly longer than the sepal. The staminal handles are fibrous, anthers - yellow. 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 go into irregularly toothed membranous appendage. It blooms in May-June, the seeds ripen in July-August. The species reproduces by seeds and vegetatively (Velchev et al., 1992; Flora of the People's Republic of Bulgaria, 1989).

Each species has particular requirements for seed germination and the germination requirements for local species are often unknown, particularly for rare or endemic species from which it is more difficult to obtain material (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 the 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 seed germination (Baskin and Baskin, 1998). Senel et al. (2007) reported that the optimum temperature for seed germination of medical plants distributed in Turkey - *S. dicroantha*, *V. bithynicum* and *V. wiedemannianum* was 20 °C and darkness. Yücel and Yılmaz (2009) reported that germination of *Salvia cyanescens* seeds was promoted by cold-wet process at -5 ºC; low concentrations of NaCl and KNO_3 (0.5-1 %) brought up high germination percentage, but higher concentrations inhibited the germination compared to no treatment. Herranz et al. (1998) did not find a clear relationship between heat-shock germination response and post-fire regeneration strategy. This work supported that endemics species have germination more sensitive to fire than the widely distributed ones.

B. bulgarica is close with *B. officinalis* L. (Stachys Betony), used as a medicine plant. According to Bown (2002) *B. officinalis* L. prefers 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/ 5ºC to germinate in 30-90 days. Seeds were sown in spring in a cold frame and very easily the plant can be successfully divided at almost any time of the year.

Betonica bulgarica Deg. et Neic on 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 in ex situ conservation. Essential elements of technology of cultivation have not been studied.

The aim of this research was to study seeds germination of endemic species *Betonica bulgarica* Deg. et Neic, as well as the growth and development of plants in three soils, relating to the conservation of the species in Bulgaria.

Materials and Methods

To assist the natural reproduction of the populations of *Betonica bulgarica* were used insulators 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* at the Ablanovo locality (N 42º42.628; E 26º17.251). The average altitude is 542 m above sea level.

Inflorescences with placed insulators were collected in September 2013 from natural populations, without risk of reducing their reproduction. The study was conducted at the research laboratory of the Faculty of Agriculture at Trakia University in Stara Zagora. The seeds were hulled, cleaned and stored in paper bags in dark place at room temperature. After a three-month rest, the stored seeds were placed in Petri dishes between distilled water-moistened filter paper at room temperature (18-25°C). Twenty replicates were used with 25 seeds each. A periodic checking of germination was carried out. Radicule emergence was the criterion for scoring a seed as germinated.

To establish seed weight four replications of 100 seeds were chosen at random and were weighed with a precision balance.

Seeds of *B. bulgarica* were sown on 25April 2014 in laboratory conditions in plastic containers in three types of soil: 1.) soil from the site of their natural habitat; 2.) California red worms Lombricompost (CRWL); 3.) soil mixture of 2/3 soil + 1/3 CRWL. The containers with sown seeds were periodically sprinkled, replanted and

weeded in order to create optimal conditions for plant germination, growth and development. Pests or diseases control were not carried out. Biometric measurements of the plants were carried out in triplicate with a 20 days period between the phases (27 June, 17 July and 6 August), respectively 61, 83 and 103 days after the sowing date.

The initial growth of the *B. bulgarica* was studied. Leaf characteristics - leaf length and width, petiole length, number of leaves and leaf area were examined. Ten plants were used for each soil at the three true leaf stages. Leaf area was determined by the weighing method.

All the activities were carried out in accordance with the Protected Areas Act (PAA), the Biological Diversity Act (BDA) and Ordinance No 8.

Soil samples from the 0-20 cm layer were taken from the studied area and an agrochemical analysis was performed. The samples were air-dried, plant residues and stones were removed, after which the samples were crushed and sieved for particles sized less than 2 mm.

Soil samples were analyzed for: pH using air-dry samples with 1:2,5 soil:water ratio; mineral nitrogen (NH_4^+-N + NO_3^--N) content using spectrophotometer JENWAY 6705 UV/VIS; available phosphorus and available potassium content by the Egner-Riem method. The concentration of available potassium was determined by AAS using AAnalyst 800 Atomic Absorption Spectrometer, Perkin Elmer.

The data for each studied parameter was analyzed using SPSS 10.0 for Windows. Analyses of variance (ANOVA) and Principal component analyses (PCA) were used to analyse the relationship between soil and growth stages. Correlations were examined using simple linear regression.

Results

The results of our studies indicated that *B. bulgarica* was characterized with a prolonged period of seed germination (**Table 1**). The start of germination was observed 15 days after the placement of the seeds. The percentage of germinated seeds at room temperature was extremely low. When set for germination of 500 seeds on 27 February 2014, the highest rate of germination was after 50-60 days. The rate of germination of seeds varied and no permanent tendencies of increasing were observed.

The 1000 seeds weight of Betonica bulgarica from harvest 2013 was on average 5,59 g, with variation in individual replications from 5.02 to 5.80 g.

Table 1. Germination of seeds from *Betonica bulgarica* Deg. et Neic. at room conditions

Seeds for germination, number	Date of setting	Date of sampling	Days of germination	Sprouted seeds, number	Germination, %
500	18.02.2014	27.02.2014	10	-	-
		04.03	15	1	0,2
		10.03	21	6	1,2
		17.03	27	18	3,6
		20.03	30	39	7,8
		24.03	41	62	12,4
		28.03	45	93	18,6
		02.04	50	131	26,2
		07.04	55	156	31,2
500		11.04.2014	**59**	**175**	**35,0**

The soil type of Ablanovo natural habitat was Chromic Luvisols (WRBSR, 2006) and was characterized with moderate soil structure, with crumb structure in the upper surface horizon, with sandy clay loam soil texture, which determines a favorable air regime and good water-permeability. Carbonates were not detected. The reaction of the soil was slightly acidic to neutral, with pH values between 6,6 and 6,7. The soil was characterized with high humus content.

The mineral nitrogen (ammonium and nitrate) values ranged from 8.2 to 17.9 $mg.kg^{-1}$. The amount were significantly below 40 mg.kg-1 which characterized the soil as poorly supplied. The results from the chemical analysis showed that the soil was poorly to moderately supplied with available phosphorus - from 6.97 to 15.63 mg P_2O_5.100 g-1 soil and with good

level of exchangeable potassium. The data on chemical composition and reaction of soil from natural habitat determined that *Betonica bulgarica* was not demanding about the soil reaction and the supply of nutrients. Favorable environment for its development were soils with light mechanical composition and good permeability (**Table 2**).

The agrochemical analysis of Lombricompost (CRWL) showed extremely high values of the main macronutrients. The content of mineral nitrogen was 82.82 mg.kg-1, and predominantly nitrate nitrogen. The average amount of available phosphorus and available potassium was very high. Biosoil was characterized with alkaline reaction and with a very high content of total humus - 10.28 %.

Table 2. Agrochemical characteristics of the soil types

Soil type	pH (H2O)	N-NH_4 mg.кg^{-1}	N-NO_3 mg.кg^{-1}	N_{min} mg.кg^{-1}	P_2O_5 mg.100 g^{-1}	K_2O mg.100 g^{-1}	Humus, %
Soil	6,6	6,50	9,78	16,28	13,55	20,28	3.74
CRWL	8,16	8,65	74,17	82,82	64,56	85,16	10,28

Average for the study the formed leaves on one *B. bulgarica* plant were 7,3 - from 4 to 12 in number (Table 3). Plants grown in soil + CRWL had 11,2 % greater number of leaves compared to those grown only in soil, and 49,1 % more than those cultivated in CRWL.

Leaf length in the three soils significantly increased with the progress in plant age to reach 12,34; 14.81 and 16.12 mm in the first, second and third stage of development, respectively. The leaf length of plants grown in a soil mixture of 2/3 soil + 1/3 CRWL was 18,58 mm on average, 14,34 and 119,62 % over the leaf length for sandy clay loamy soil and CRWL, respectively.

The differences in leaf width under the influence of plant stage and soil type were significant (Table 3 and 4). The average width of the leaves was 13,3 mm and varied from 4,60 to 26,83 mm. In soil mixture of 2/3 soil and 1/3 CRWL, the leaf width was 105.60 % higher than the plants grown in CRWL. The mean values of leaf width after 103 days recorded its maximum and was 14,86 mm.

The average leaf area in this study was 18,6 cm^2, ranging from 1,35 to 35 49,15 cm^2 (Table 3). Proven impact on leaf area was observed from soil - 60.32 % of the total impact of factors (Table 4). In the second stage the plants reached an average of 20.93 cm^2, and in the third stage - 21,27 cm^2, i.e. the daily rate of growth of vegetative mass compared to the second one was 0,017 cm^2.

The mean values of leaf petiole length of *B.bulgarica* grown in sandy clay loamy soil was 17,16 mm. These plants had longer petiole than those grown in CRWL with 8.06 mm in the 1st and 2nd season. The leaf petiole length significantly increased with the progress of plant age up to 103 days for the three soil types. In all plant stages the differences between the three soils in leaf petiole length were true.

The data illustrated in Table 3 showed that the vegetative growth of *B. bulgarica* plants in sandy clay loamy soil was higher than those grown in CRWL. The best plant growth was seen in a soil mixture of 2/3 soil 1/3 CRWL and it was true in the three plant stages. As for the number of leaves on one plant over the period, the impact of age of plants was not proven.

The highest coefficient of variation was found for leaf area - 176,15 %, medium - for leaf petiole length, leaf length and leaf width, and the lowest -for leaves number / plant - 3,0 %.

With respect to all examined leaf characteristics - leaf length and width, petiole length, number of leaves and leaf area, it was found that the soil environment had 37.31 to 60.32 % effect out of the total impact of factors (Table 4). The age of the plants had a much lower effect - from 7.02 to 8.74 %, except for the number of leaves.

Propagated plants reached 5-6 leaf stage, were planted in their natural habitats in the Nature Park Sinite Kamani - Sliven. The return of the plants for grown in their natural habitats was accompanied by systematic monitoring to track the process of adaptation and development of imported plants in the population, so that in case of difficulties to be promptly take action to assist this process.

The results of correlation analysis showed a statistically significant relationships between leaf area, leaf length, leaf width and petiole length and the relationship between leaf length and leaf width (R = 0,937) was the most strongly. Moderate correlation was observed between

leaf area, leaves number, leaf length and leaf width. The leaves number showed no influence of petiole length. The established correlations are contributing to a better understanding of the relationships between morphological characteristics and influence of these parameters on the overall physiological status of *B. bulgarica* (Table 5).

Table 3. Influence of plant stage and soil on the *B. bulgarica* leaf characteristics, n = 90

Stage	Soil	Leaves number/ plant	Leaf length, mm	Leaf width, mm	Leaf area, cm^2	Leaf petiole length, mm
1	Soil	7.5a	13,0	12,7	14,7	12,7
1	Soil+ CRWL	7.8a	15,7	14,5	22,9	14,9
1	CRWL	5.5b	8.32a	7.51a	3.07a	7.83a
2.	Soil	7.1a	17,5	15,5	28.02b	19.14b
2	Soil+ CRWL	9,0	18.45b	16,6	29.26b	18.22b
2	CRWL	5.8b	8.52a	8.12a	5.50a	7.40a
3.	Soil	8.4c	18.24b	17.69c	26,2	19.64b
3	Soil+ CRWL	8.8c	21,6	18.43c	32,9	23,7
3	CRWL	5.8b	8.53a	8.46a	4.70a	8.96a
All Groups		**7,3**	**14,4**	**13,3**	**18,6**	**14,7**
SD		*1,7*	*5,65*	*5,07*	*13,27*	*7,83*
VC, %		*3,0*	*31,95*	*25,68*	*176,15*	*61,25*
SE		*0,2*	*0,60*	*0,53*	*1,40*	*0,82*
Min		*4,0*	*5,00*	*4,60*	*1,35*	*4,00*
Max		*12,0*	*29,29*	*26,83*	*49,15*	*41,00*

*Differences are statistically significant at P<0.05

Correlation analysis was used not only for the analysis of soil-plant relationships, but also for the study of changes in interrelationships with connected land use change (**Fig. 1, 2, 3**). The comparative research into natural and cultivated ecosystem components - soil and plant was conducted within the limits of adequate three soil groups.. The average values of the studied morphological parameters of *B. bulgarica* grown in CRWL strongly differentiated from those in the other two soil types. In the, space of the main components, plants in a CRWL formed separate group remote from the other two groups of plants grown in sandy clay loam soil and in soil+CRWL. Analysis confirmed that with the highest correlation coefficient (R = 0.9913) was the relationships between the leaves number and leaf area.

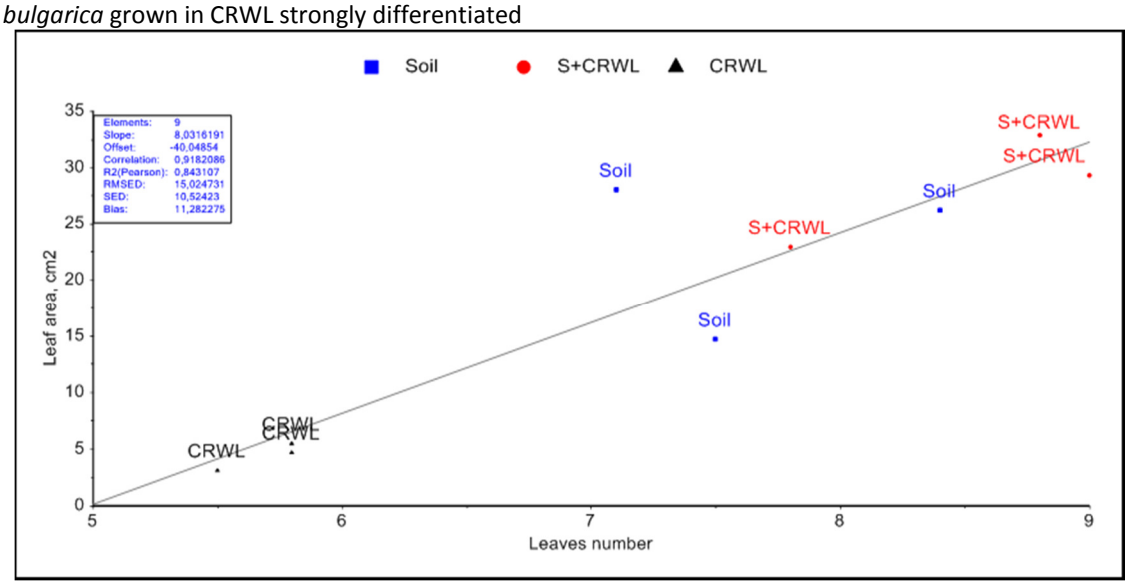

Figure 1. Relationship between leaves number and leaf area in the plants of *B. bulgarica*

Table 4. Influence of factors (stage and soil) on the *B. bulgarica* parameters, n = 90

Factors	SS	DF	MS	F	P	Partial eta squared	Non-certanly	Observed power (0.05)	%
Number of leaves									
Stage	8,07	2	4,03	2,58	0,08	0,06	5,16	0,50	2,98
Soil	126,47	2	63,23	40,42	0,0	0,49	80,85	1,00	46,68
Stage*Soil	9,67	4	2,42	1,54	0,2	0,07	6,18	0,46	3,57
Error	126,70	81	1,56						46,77
LAI, cm^2									
Stage	1131,26	2	565,63	9,86	0,001	0,20	19,73	0,98	7,22
Soil	9455,9	2	4727,95	82,46	0,00	0,67	164,91	1,00	60,32
Stage*Soil	445,40	4	111,35	1,94	0,11	0,09	7,77	0,56	2,84
Error	4644,43	81	57,34						29,63
Leaves length, mm									
Stage	224,00	2	112,00	11,09	0,00	0,22	22,18	0,99	7,88
Soil	1686,70	2	843,35	83,50	0,00	0,67	166,99	1,00	59,31
Stage*Soil	114,84	4	28,71	2,84	0,03	0,12	11,37	0,75	4,04
Error	818,12	81	10,10						28,77
Leaves width, mm									
Stage	160,40	2	80,20	7,93	0,00	0,16	15,86	0,95	7,02
Soil	1262,89	2	631,44	62,43	0,00	0,61	124,86	1,00	55,25
Stage*Soil	43,12	4	10,78	1,07	0,38	0,05	4,26	0,32	1,89
Error	819,30	81	10,11						35,84
Leaves Petiole length, mm									
Stage	476,22	2	238,11	7,11	0,001	0,15	14,23	0,92	8,74
Soil	2033,96	2	1016,98	30,39	0,00	0,43	60,77	1,00	37,31
Stage*Soil	230,34	4	57,58	1,72	0,15	0,08	6,88	0,50	4,23
Error	2710,96	81	33,47						49,73

Table 5. Correlations among studied parameters of the *B. bulgarica* leaves, n = 90

Parameters	Means	Std. Dev.	Leaves Number	Leaf area, cm^2/cm^2	Leaf length, mm	Leaf width, mm	Petiole length, mm
Leaves Number	7,30	1,745	1,0	0,617	0,512	0,443	0,370
Leaf area, cm^2	18,58	13,27	-	1,0	0,912	0,886	0,839
Leaf length, mm	14,43	5,652	-	-	1,0	0,937	0,858
Leaf width, mm	13,28	5,068	-	-	-	1,0	0,904
Petiole length,mm	14,71	7,826	-	-	-	-	1,0

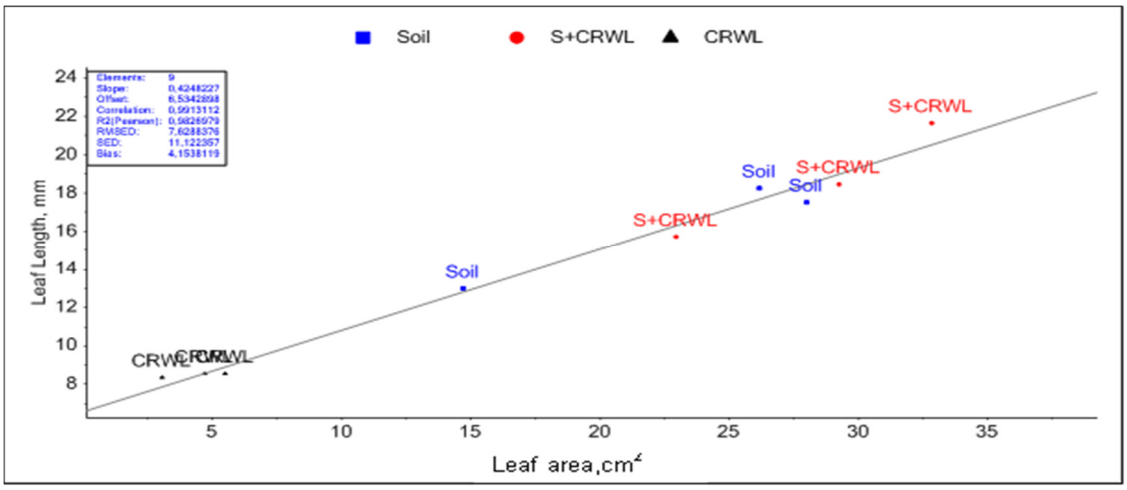

Figure 2. Relationship between leaf area and leaf length in the plants of *B. bulgarica*

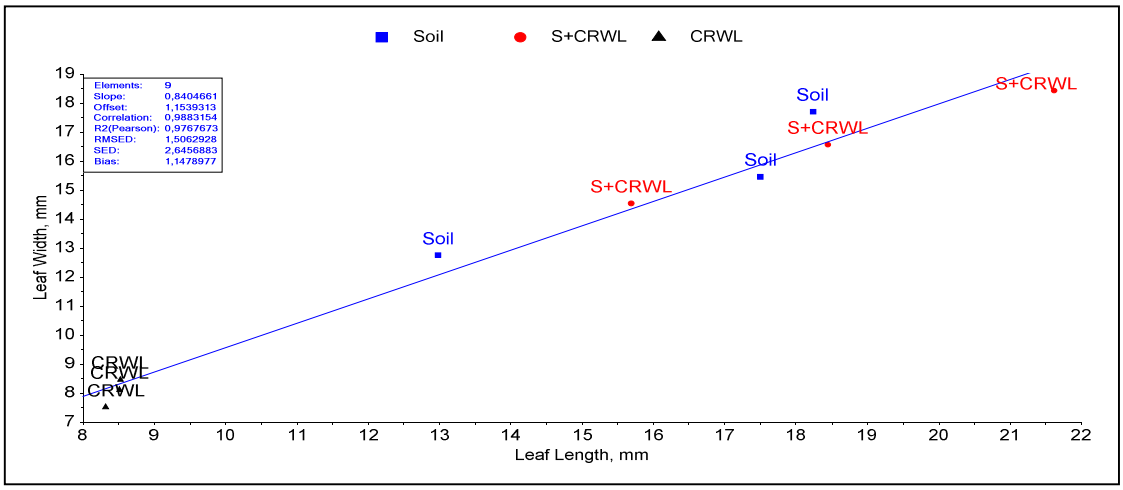

Figure 3. Relationship between leaf length and leaf width in the plants of *B. bulgarica*

Conclusion

It was found that *B. bulgarica* was characterized by a prolonged period of germination and low rate of germination - 35.0 %. Best plant growth was seen in a soil mixture of 2/3 sandy clay loam soil + 1/3 Lombricompost. The soil strongly influenced leaf length, leaf width and leaf area - 46.1 to 58.6 % of the total variation.

By implementing the above *in*-situ and *ex*-situ measures, stabilization of the populations of the Bulgaria endemic *Betonica bulgarica* on the territory of Natural park Sinite kamani will be achieved.

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References

Baskin, C. and J. Baskin, 1998. Seeds Ecology, Biogeography and Evolution of Dormancy and Germination. Academic Press, San Diego, pp. 666.

Bown, D., 2002. New Encyclopedia of Herbs & Their Uses, The Herb Society of America, Dorling Kindersley Publishers Ltd, pp. 424.

Biological Diversity Act, Republic of Bulgaria, 2002. *State Gazette* No. 77/9.08.2002 (Bg).

Cerabolini, B., R. Andreis, R. Ceriani, S. Pierce and B. Raimondi, 2004. Seed germination and conservation of endangered species from the Italian Alps: *Phsoplexis comosa* and *Primula glascescens.* Biol. Conserv., 117: 351-356.

Escriba MC, E Laguna, M Guara, 2004. Seed germination trends of endemic vascular plants in the Valencian community (Spain). 4th European Conference on the conservation of wild plants, Planta Europa IV Proceedings, 17-20th September 2004, Valencia (Spain).

Estrelles E, F. Albert, A. Navarro, J. Prieto, A.M. Ibars, 2004. Germination behaviour of Labiatae SW distributed in the Iberian Peninsula. 4th European Conference on the conservation of wild plants, Planta Europa IV Proceedings, 17-20th September 2004, Valencia (Spain).

Flora of the People's Republic of Bulgaria, vol IX, 1989. Published by BAS. Editor C. Velchev (Bg).

Genova E. 2011. Betonica bulgarica Deg. et Neic. – In: Peev D (ed.), Red Data Book of the Republic of Bulgaria, vol. 1. Plants & Fungi, http://e-ecodb.bas.bg/rdb/en/vol1/Betbulga.html (12.01.2014).

Grozeva, N., M. Georgiewa, M. Vulkova, 2004. Flowering plants and ferns. In: Stoeva, M. (ed.), Biological diversity of Sinite Kamani Nature Park. Kontrast – Bogomilovo, Stara Zagora, pp 9-112 (Bg).

Koeva Y. 1970. Genus Betonica –In: Jordanov D (ed.), Flora Reipublicae Bulgaricae, vol 9, Editio Acad. „Prof. Marin Drinov", Serdicae: 412-416 (Bg).

Navarro, L. and J. Guition, 2003. Seed germination and seedling surviv of two threatened endemic species of the northwest Iberian Peninsula. *Biol. Conserv.,* 109: 313-320.

Herranz, J.M., P. Ferrandis, J. Martinez-S´anchez, 1998. Influence of heat on seed germination of seven Mediterranean Leguminosae species. Plant Ecol.136, 95–103.

Petrova A, Trifonov G, Venkova D, Ivanova M. 2009. Records 51-74. – In: Vladimirov V & al. (comps). New floristic records in the Balkans: 10. *Phytol.Balcan.,* 15(1): 128-132.

Petrova AS, Getova N, Grozeva N, Venkova D. 2011. Reports 73–93. – In: Vladimirov, V. & al. (comps). New floristic records in the Balkans: 17. *Phytol. Balcan.,* 17(3): 361–384.

Senel E., Y. Ozdener and D. Incedere, 2007. Effect of Temperature, Light, Seed Weight and GA3 on the Germination of Verbascum bithynicum, Verbascum wiedemannianum and Salvia dicroantha. *Pakistan Journal of Biological Sciences*, 10 (7): 1118-1121.

Stoeva, M. et al. 2002. Management Plan of Nature Park Sinite Kanani, Sliven. 124

Thanos CA, MA Doussi, 1995. Ecophysiology of seed germination in endemic Labiates of Crete. *Israel J. Plant Sci.,* 43: 227-237.

Tashev A, Aleksandrova A, Dochshev D. 2010. New record of Quercus coccifera L. in Bulgaria*. Gora,* 8: 16-18 (Bg).

Tashev A. 2011. Reports 108–113. – In: Vladimirov V & al. (comps). New floristic records in the Balkans: 17. *Phytol. Balcan.,* 17(3): 361–384.

Uçar E, K. Turgut, 2009. In Vitro Propagation of Some Mountain Tea (*Sideritis*) Species. Akdeniz Unversity, *J. Agric. Facul.* 22(1): 51-57

Velchev, V., S. Kozuharov, and M. Ancev, eds., 1992. Atlas of the Endemic Plants in Bulgaria, Sofia. Publishing House of the Bulgarian Academy of Science, pp. 204.

World referense base for soil resources. 2006. A framework for international classification, correlation and communication. Food and agriculture organization of the United Nations – Rome, Italy.

Yücel E, G. Yılmaz, 2009. Effects of different alkaline metal salts (NaCL, KNO3), acid concentrations (H2SO4) and growth regulator (GA3) on the germination of *Salvia cyanescens* Boiss. & Bal. Seeds. *G.U. J. Sci.* 22(3): 123-127.