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Autecology and *ex situ* growth of *Onobrychis pindicola* Hausskn. subsp. *urumovii* Deg. & Dren. (Fabaceae) – endemic with medicinal potential

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***Abstract*:**

Kozuharova, E., Nash, R.: ***Autecology and ex situ growth of Onobrychis pindicola Hausskn. subsp. urumovii Deg. & Dren. (Fabaceae) – endemic with medicinal potential. Biologica Nyssana, 8 (1), September 2017: 83-92.***

Onobrychis pindicola subsp. *urumovii* Degen & Dren. is an endemic with very restricted distribution on just two mountains Pirin Mts. and Slavjanka Mts. SW Bulgaria. The taxon is evaluated as least concerned by the IUCN criteria but it is an element in several Natura 2000 habitats with conservation significance. The aim of this study is to investigate the microhabitat specifics of *O. pindicola* subsp. *urumovii*, namely slope, exposure, bed rock, soils, and vegetation as well as spatial distribution and phenology regarding the possible future cultivation. Basically *O. pindicola* subsp. *urumovii* demonstrates high tolerance to its environment although it is a calciphilous species. It grows successfully *ex situ* in experimental plots in the foothills of the mountains, which indicates prospects for successful cultivation. This will be important in case of future industrial necessity of the plant substance for medicinal purposes.

Key words: *ex situ,* microhabitat, *Onobrychis pindicola* subsp. *urumovii***,** phosphorus, soil pH

***Apstrakt*:**

Kozuharova, E., Nash, R.: ***Autekologija i ex situ razviće Onobrychis pindicola Hausskn. subsp. urumovii Deg. & Dren. (Fabaceae) – endemične vrste sa medicinskim potencijalom. Biologica Nyssana, 8 (1), Septembar 2017: 83-92.***

Onobrichis pindicola subsp. *urumovii* Degen & Dren. je endemit sa vrlo ograničenim rasprostranjenjem na samo dve planine, Pirin i Slavjanka, u jugozapadnoj Bugarskoj. Prema kriterijumima IUCN-a takson je procenjen kao takson niske verovatnoće ugroženosti, ali je graditelj nekoliko tipova staništa uključenih u Natura 2000 čije je očuvanje značajno. Cilj ove studije je istraživanje specifičnosti mikrohabitata *O. pindicola* subsp. *urumovii*, i to nagiba, ekspozicije, tipa staništa, zemljišta i vegetacije, kao i prostorne raspodele i fenologije u vezi sa mogućom budućom kultivacijom. U suštini *O. pindicola* subsp. *urumovii* pokazuje veliku toleranciju na svoje okruženje, iako je to kalcifilna vrsta. Uspešno raste *ex situ* na eksperimentalnim parcelama u podnožjima planina, što ukazuje na mogućnost uspešne kultivacije. Ovo će biti važno u slučaju industrijske potrebe za ovom biljnom sirovinom u medicinske svrhe.

Ključne reči: *ex situ,* mikrohabitati, *Onobrychis pindicola* subsp. *urumovii***,** fosfor, pH zemljišta

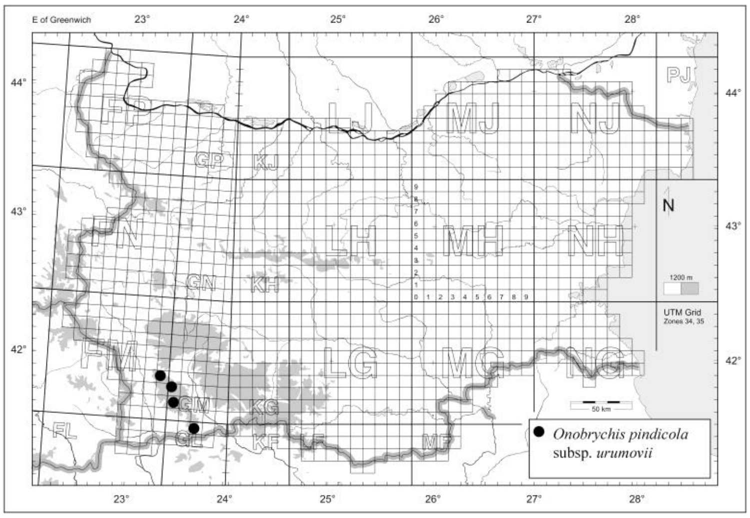

Fig. 1. Distribution of *Onobrychis pindicola* subsp. *urumovii* - UTM Grid map

Introduction

Onobrychis pindicola subsp. *urumovii* Degen & Dren. is an endemic with very restricted distribution (**Fig. 1**) on just two mountains Pirin Mts. and Slavjanka Mts. on the Balkan Peninsula, SW Bulgaria (V e l c h e v , 1992, Euro+Med PlantBase 2011). It is a perennial plant that forms dense tufts, has an almost vertical reddish-brown rhizome, and its stems are short or lacking. Its leaves are pinnately compound and normally bear four to seven pairs of lanceolate, hairy leaflets and a similar terminal leaflet, and its numerous purple flowers are borne on dense racemes. The legume is round and dentate (K o z u h a r o v , 1976).

Genus *Onobrychis* include species known basically as forage and honey plants. This is true particularly for *Onobrychis viciifolia* (K o z u h a r o v , 1976, T a s h e v & P a n c h e v a , 2009, Ł u c z a j , 2012). Ethnobotanical study reveals that *O. montana* (which is closely related to our research object *O. pindicola*) is highly valued as forage plant in the Alps (P i e r o n i & G i u s t i , 2009). Therefore we can consider *O. pindicola* important honey and forage plant for the mountain pastures on the Balkans.

Traditionally seed and aerial parts of *Onobrychis viciifolia* are mainly used as antidiarrhoeal (B o n e t at al., 1999, A g e l e t & V a l l e s , 2001). Leaves and flowers of *O. elymaitica* are used as anti-calculus against kidney problems (G h a s e m i at al., 2013). Antibacterial medicinal properties are reported for *O. cornuta* (J o u d i &

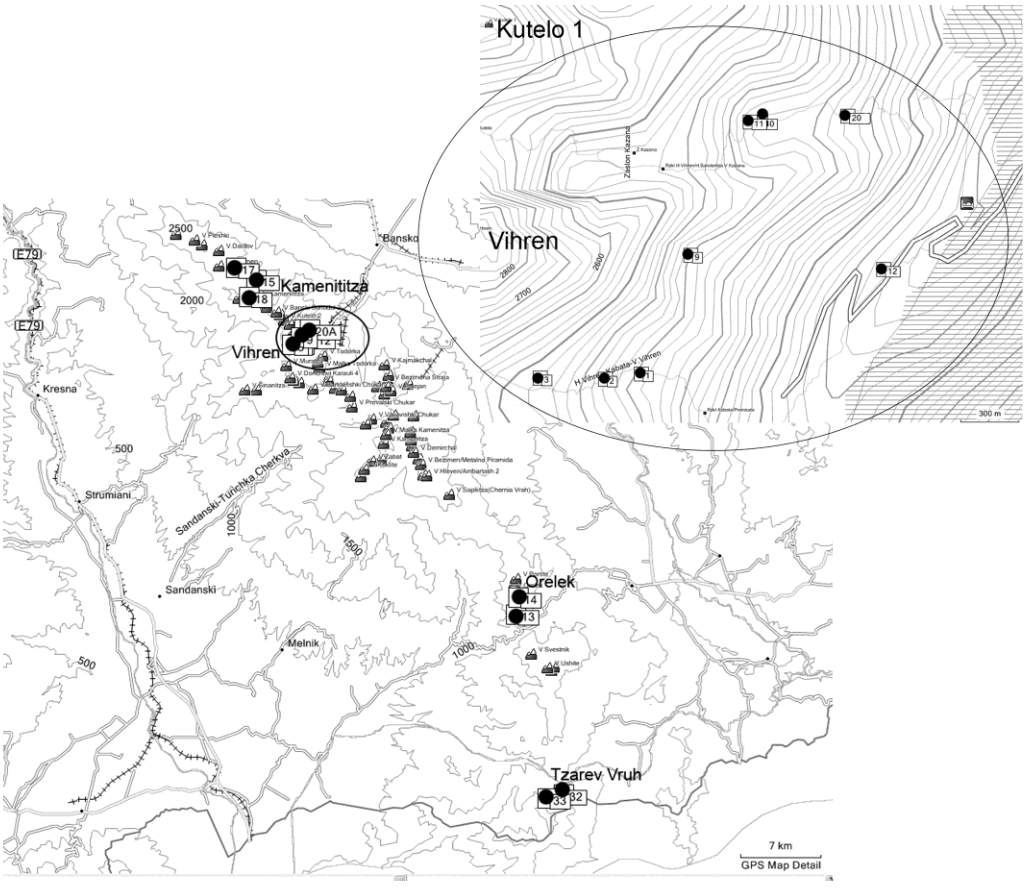

Fig. 2. Study sites in Pirin Mts and Slavjanka Mts.

Bibalani, 2010). Although Sanfions are known in Bulgaria as honey and forage plants (Kozuharov, 1976, Tashev & Pancheva, 2009) traditional application for medicinal purposes is not documented. Laboratory tests confirm that *Onobrychis viciifolia* possess anti-bacterial activity (Jones at al., 1999). In vitro tests show low toxicity of *O. viciifolia* (Ince & Filazi, 2009). *Onobrychis ebenoides* possesses strong antitumor activity as well as estrogenic effect – affinity to the estrogenic receptor (Halabalaki et al., 2000, 2006, 2008, Gutterson & Ralston, 2002, Katsanou et al., 2007, Papoutsi et al., 2007, Tchokouaha et al., 2010). Some Fabaceae species including *Astragalus* and *Oxytropis* can contain iminosugars. Iminosugars can be viewed as sugar mimics in which the ring oxygen atom of the parent sugar has been replaced by nitrogen. Such compounds have been the subject of extensive interest in the past three decades due to their therapeutic potential and may explain the benefits of some Fabaceae to health. The field of iminosugars continues to open up exciting new opportunities for therapeutic agent discovery and offers many new tools for precisely modifying carbohydrate structures

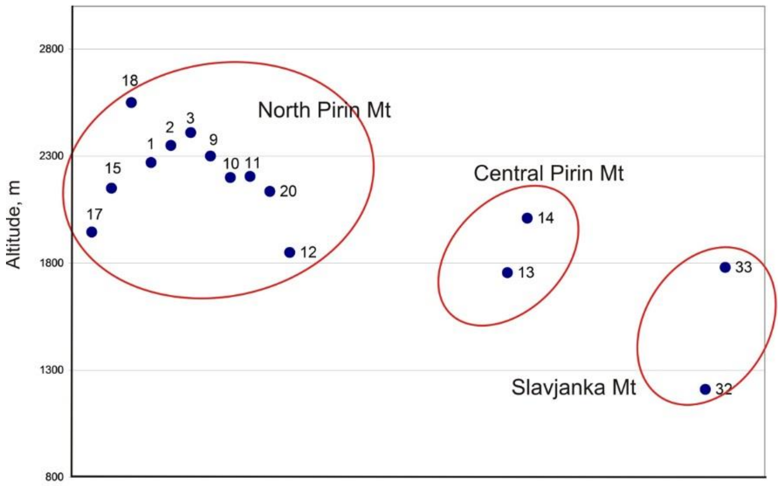

Fig. 3. List of study sites with information of their altitude

Table 1. Information about study sites: number of site, coordinates and characteristics

Number of site	altitude	Latitude	Longitude	Characteristics
1	2270	41°45'38.8	023°24'30.7	subalpine calcareous grasslands and habitat dominated by *Juniperus sibirica*
2	2350	41°45'37.9	023°24'23.4	subalpine calcareous grasslands
3	2410	41°45'38.1	023°24'10.3	subalpine calcareous grasslands
9	2300	41°45'58.4	023°24'40.6	subalpine calcareous grasslands and habitat dominated by *Pinus mugo*
10	2200	41°46'20.4	023°24'54.7	subalpine calcareous grasslands
11	2205	41°46'20.4	023°24'52.6	subalpine calcareous grasslands
12	1850	41°45'56.1	023°25'19.5	herbaceous communities within the coniferous forest belt, near to its upper limit (woods of *Pinus heldreichii*)
13	1755	41°33'02.7	023°36'54.0	subalpine calcareous grasslands and habitat dominated by *Juniperus sibirica*
14	2010	41°33'50.5	023°37'01.6	subalpine calcareous grasslands and habitat dominated by *Juniperus sibirica*
15	2150	41°48'38.8	023°22'07.3	subalpine calcareous grasslands
17	1945	41°49'13.1	023°21'00.2	herbaceous communities within the coniferous forest belt, near to its upper limit, woods *Pinus heldreichii*
18	2550	41°47'51.6	023°21'42.8	alpine calcareous grasslands and calcshist screes
20	2135	41°46'21.4	023°25'12.2	subalpine calcareous grasslands and habitat dominated by *Pinus mugo*
32	1210	41°24'41.1	023°39'35.3	herbaceous communities within the coniferous forest belt, near to its upper limit (woods *Pinus heldreichii*)
33	1780	41°24'28.8	023°38'41.3	herbaceous communities within the coniferous forest belt, near to its upper limit (woods *Pinus heldreichii*)

and modulating glycosidase activity *in vivo* (Blériot et al., 2005, Winchester, 2009, Mercer et al., 2009, Nash et al., 2011, Kato et al., 2015). Members of family Fabaceae family are particularly prospective from this point of view and analysis for iminosugars was included in this research because efforts are directed towards a greater range of structures and a wider range of biochemical targets. Although *Onobrychis pindicola* subsp. *urumovii* was assessed as least concern according to the IUCN criteria (Petrova & Vladimirov, 2008) knowledge about its conservation and cultivation should be accumulated simultaneously with the accumulation of knowledge about its possible medicinal application.

The aim of this study is to investigate the microhabitat specifics of *Onobrychis pindicola* subsp. *urumovii*, namely slope, exposure, bed rock, soils, and vegetation as well as spatial distribution and phenology regarding the possible future cultivation.

Material and methods

Study sites and habitat observations

The field observations were conducted in the marbleized karst regions of North Pirin Mts., namely the main watershed of North Pirin Mts. with its highest peaks, their slopes build of marble, the highest peak of Central Pirin Mts. and Slavjanka Mts. also build of marble. A big massive of silicate bed rocks separates the marble ridge of North Pirin Mts. and the marble Central Pirin Mts. (**Fig. 2**). The study sites are summarized in **Tab. 1**, **Fig. 2** and **Fig. 3**. They are all located in marble areas since *Onobrychis pindicola* subsp. *urumovii* is calciphilous. The period of investigations of wild populations was during the summers of 1995, 1996, 2001, 2002, 2005, 2014 and 2015. The *ex situ* experiments and observations were conducted during the period 2006-2010. The exact geographic location of all sites was determined using a global positioning receiver Garmin GPS 12, Datum WGS 1984, UTM projection (**Fig. 2** and **Fig. 3**, numbers marking the study sites correspond to the way points recorded with GPS receiver for this plant species in the field). Elevation was double checked with an altimeter. Slope and exposure were recorded and described both in the field and using the global positioning system (GPS) methods. Soil samples (two samples at each study site) were taken from the rooting zone of study plants. Each sample was taken from area of 20-30 cm^2 and 4 cm depth (**Tab. 2**). The soil characters were measured after a standard methodology at the Newcastle University in January 2002. The volume of 10 cm^3 of air dry soil (scoop filled and struck of level without tapping) was weighted on digital scales. The soil was ground to pass 2 mm mesh sieve. We transferred 5 cm^3 sieved soil into a bottle and added 100 cm^3 sodium bicarbonate reagent of pH 8.50 at 20 °C to extract the phosphorus. The concentration of the blue complex produced by the reduction, with ascorbic acid, of the phosphomolibdate formed when acid ammonium molybdate reacts with phosphate was measured spectrophotometrically at 880 nm. The number of μg of phosphorus equivalent to the absorbances of the sample and the blank determinations were calculated from the standard graph. The difference was multiplied by 100 to obtain the quantity of extractable phosphorus in the soil [mg P/kg soil]. Soil pH characters were measured using a pH electrode and meter at about 20 °C (between 20.2 °C and 20.8 °C for each sample). One-way ANOVA was applied for data analysis. Associated vegetation was recorded after Braun-Blanquet system. The plants were identified according to Jordanov (1963-2012).

Data analysis

The comparative analysis of the vegetation at the sites was done according to Jaccard (Muller-Domboa & Ellenberg, 1974). Jaccard coefficient measures the similarity of two sample sets. It uses the ratio of the intersecting set to the union set as the measure of similarity. Thus it equals to zero if there are no intersecting elements and equals to one if all elements intersect.

$$T = \frac{N_c}{N_a + N_b - N_c}$$

where: N_a - number of elements in set A, N_b - number of elements in set B, N_c - number of elements in intersecting set.

Ex situ experiments and observations

Seed was collected in September 2005 and kept cool (4 °C, in the fridge, but not frozen). Several sets of seeds were processed for germination at a natural light – dark photoperiod. Once the seeds had germinated, the seedlings were transferred individually to plastic pots filled with a mix of 30% rough marble sand, 30% sieved humus, 30% sieved good soil, 10% perlite or with a mix of 60% silty brown soil and 40% rough marble sand. When seedlings were at the 2-6 true leaf stage, they were transported to the experimental rock garden in the foot hill of Pirin Mts. near Dobrinishte village. They were planted singly into the flower beds with fine marble gravel top-dressing. The experimental rock garden was established in 2006 in hay meadow of approximately 500 m^2 near the river, situated at 865 m above sea level, at N 41°48’80,9” and E

23°33”67,4” (WGS84) on the steepest part of a hay meadow with an exposure to the north-east. The place was chosen with consideration for several factors: i) close enough to the river for watering; ii) away from potential floods; iii) moderately shaded; iv) the snow lies relatively long here, protecting the plants from the spring frosts, and providing a cool microclimate in summer.

Results and discussion

Habitat observations

Onobrychis pindicola subsp. *urumovii* grows in both herbaceous communities within the coniferous forest belt, near to its upper limit (study sites 12, 17, 32, 33), subalpine meadows (study sites 1, 2, 3, 9, 10, 11, 13, 14, 15, 20) and in the alpine belt (18). The altitude of the microhabitats ranges in wide diapason 1210-2550 m a.s.l. (**Fig. 3**). This habitat diversity is reflected in the habitus of the plants. The exposure of the microhabitats is diverse so the plant is tolerant to this factor.

Onobrychis pindicola subsp. *urumovii* grows on silty brown earth on the marble bed rock. It can be found on big flat terrains (study sites 10 and 11 big population with numerous individuals) or on very steep slopes (study site 18, small patches of few individuals) where it occupies the small flat surfaces resembling steps. The soil has often a skeleton structure. The granulometric composition of the soils, determined by sieving them (2 mm mesh sieve) revealed that the soils consisted of rough particles - sand and pebbles mixed with roots and dead vegetation in different ratios all listed in **Tab. 2**.

The analysis showed that the soil pH vary from neutral to slightly alkaline (**Tab. 2**). Soil pH is crucial both for the soil processes, soil productivity and vegetation diversity. Extractable phosphorus (P) in the soil where individuals of *O. pindicola* subsp. *urumovii* grow was at the average 23.08 mg per kg soil (min=28.26 max=20.98, **Tab. 2**). The average value of extractable phosphorus in the soil where individuals of *O. pindicola* subsp. *urumovii* grow was slightly higher that that of the soil in close vicinity. We measured extractable phosphorus of the soil where the individuals of *Oxytropis* species occur (Kozuharova et al., 2015). Both genera are members of Fabaceae family, and they grow together at study sites 3, 4 10, 11, and 18. We assumed that *O. pindicola* subsp. *urumovii* enrich the soil with extractable phosphorus. However there was not a statistically significant difference between groups as determined by one-way ANOVA ($F(1.28) = 1.758$, $p = 0.196$) and therefore such statement can not be supported at this stage of research.

Onobrychis pindicola subsp. *urumovii* is a member of plant communities where the following species participated with variable abundance: *Achillea ageratifolia*, *Acinos alpinus*, *Alyssum cuneifolium*, *Androsace villosa*, *Anthenaria dioica*, *Anthylis montana*, *Armeria alpina*, *Asperula longiflora*, *Aster alpinus*, *Aubrieta gracilis*, *Botrichium lunaria*, *Brassica jordanovii* (Nort Pirin Mts. only), *Calamagrostis arundinacea*, *Campanula alpina*, *Campanula cochlearifolia*, *Campanula*

Table 2. Features of the soil samples. Legend (-) absent in the sample, (+) sporadic or small, (++) plenty of material, (+++) dominates

Study site	Weight/volume [g/10 cm^3]	Roots and straw	Sand	Pebbles	Water absorption	pH	pH at temperature °C	mg P/kg soil
3	5.8	++	+	-	medium	6.83	20.8	22.15
3	5.2	++	+	-	medium	6.07	20.6	21.75
12	11.7	-	++	+	fast	7.81	20.6	21.29
12	11.3	-	++	++	fast	7.66	20.6	20.98
13	13	-	+++	++	fast	7.66	20.3	21.59
13	8.1	-	++	+	fast	7.62	20.5	21.39
14	7.6	++	+	++	medium	7.35	20.4	23.26
14	6.6	++	+	-	medium	6.95	20.3	23.05
15	3.5	++	+	-	slow	7.06	20.6	21.75
15	3.7	++	+	+	slow	6.63	20.5	25.63
18	9.9	-	+++	+	fast	6.97	20.6	28.26
18	10.6	-	+++	+	fast	7.19	20.4	26.93
20	7.4	++	++	+	slow	7.56	20.7	21.99
20	8.3	++	++	+	slow	7.43	20.6	23.05
average						**7.20**	average	**23.08**
stdev						0.48441	stdev	2.26

Table 3. Jaccard coefficient of similarity of the plant communities in which participates *Onobrychis pindicola* subsp. *urumovii* (N=10)

Study sites	12	20	9	10 &11	1	2 & 3	14	15	32
Number of plant species	26	15	35	17	26	16	19	14	8
12									
20	0.17								
9	0.07	0.16							
10 &11	0.23	0.28	0.24						
1	0.13	0.21	0.20	0.19					
2 & 3	0.11	0.11	0.13	0.22	0.14				
14	0.13	0.13	0.13	0.24	0.13	0.06			
15	0.18	0.16	0.07	0.15	0.08	0.07	0.10		
32	0.13	0.15	0.05	0.09	0.03	0.04	0.17	0.16	

velebitica, Carduus candicans, Carex sp. div., *Centaurea parilica* (Slavjanka Mts. only), *Centaurea rhenana, Cerastium decalvans, Chamaecytisus absynthioides, Clinopodium vulgare, Coeloglosum viride, Coronila varia, Daphne oleoides, D. Velenovskyi, Diannthus microlepis, D. cruentus, D. petraeus, Draba aizoides, Epilobium angustifolium, Epipactis helleborine, Euphorbia barrelierii, E. myrzenitis, E. salisburgensis, Festuca valida, Galium anizophylum, Galium molugo, Gearanium sylvaticum, G. macrorhysum, Genista depressa, Gentiana verna, Helianthemum nummularium, Hieracium villosum, Hypericum tetrapterum, Jasione bulgarica, Juniperus sibirica, Jurinea mollis, Knautia mydzorensis, Leontodon asper, Lerchenfeldia flexuosa, Linum capitatum, Luzula albida, Minuartia recurva, Nepeta nuda, Oxytropis campestris, Poa* sp., *Polygala major, Potentilla crancii, P. crysocraspeda, P.* gr. *recta, Rhinanthyus javorkae, Rhodax canum, Rumex alpinus, Saxifraga ferdinandi-coburgii, S. sempervivum, Scabiosa ochroleuca, Scutellaria alpina, Sedum hispanicum, S. ochroleucum, Senecio nemorensis, Sesleria comosa, Sideritis scardica, Silene ciliata, Stachis recta, Teucrium montanum, Thalictrum foetidum, Thymus* sp. div. Similarity of the plant communities in the study sites was not high (**Tab. 3**). The comparative analysis demonstrated that the plant communities in which participated *Onobrychis pindicola* subsp. *urumovii* have higher similarity when they were located nearby to each other at similar altitude (sites 12, 20, 10 and 11, **Tab. 3**). However comparatively high similarity was detected also between sites that were distantly situated (10, 11 and 14, **Tab. 3**). *Onobrychis pindicola* subsp. *urumovii* did not show adherence to particular complex of plant species. Requirement to particular plant communities was not observed. Interpretation of this fact is high tolerance of *Onobrychis pindicola* subsp. *urumovii* to its environment.

Fig. 4. *Ex situ* development of transplanted adult plant

Ex situ transplantation of adult plants, germination of seeds and ontogenesis

Transplanted adult plants pre-adapted successfully and developed well during the whole period of *ex situ* experiments – 5 years (**Fig. 4**). Legumes of *Onobrychis pindicola* subsp. *urumovii* do not dehisce to release the seed. Ours experiments revealed that scarification does not influence the effectiveness of germination. Germination was at the average 33% no matter scarified or not scarified the seed (N=30). Sowing on filter paper or in a soil resulted in similar

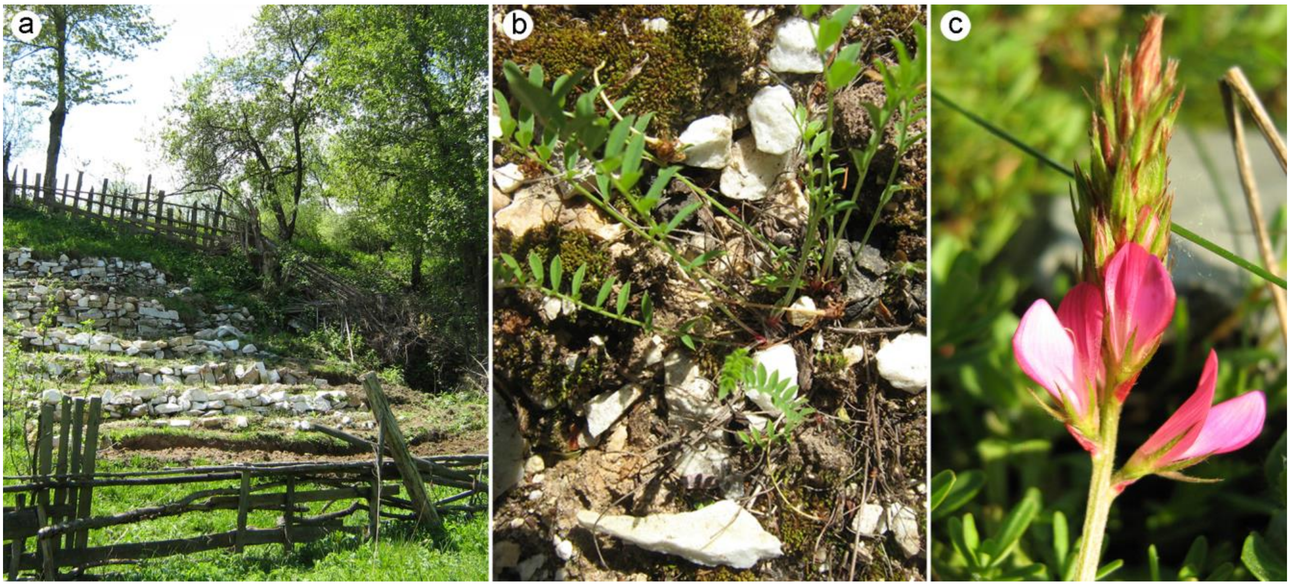

Fig. 5. a - Experimental flower beds for seedlings, b –virginal phase of a seedling., c – generative phase of a seedling

germination. Best survived the seedlings resulted from germination in the soil. Once the seedling survived to be planted in the experimental flower bed (**Fig. 5a**) in May 2006 they developed well the whole first season (**Fig. 5b**). The critical period was the winter and after the first winter survived less than 40% of the seedlings. They lived in vegetative phase for two seasons and reached generative phase during the third year (**Fig. 5c**). The seedlings remained small for all five seasons of the experiment – up to 5 sterile sprouts with 2-3 leaves each. This indicates that individuals with decade of sterile sprouts and with decades of leaves *in situ* were of significant age. Flowering phenology period *ex situ* took place about a month earlier compared to the wild populations (the experimental plot was located at 1000-1500 m lower altitude).

In summary the microhabitat specifics of *Onobrychis pindicola* subsp. *urumovii,* namely slope, exposure, bed rock, soils and plant communities in which it participates indicate that taxon is rather tolerant to its environment. The populations of *Onobrychis pindicola* subsp. *urumovii* in Pirin Mts. and Slavjanka Mts. have mosaic structure, they take vast areas on marble slopes and some patches consist of numerous individuals (e. g. study site 10 and 11). The populations of *O. pindicola* subsp. *urumovii* occupy habitats where the vegetation cover varies between 50% and 90%. *Onobrychis pindicola* subsp. *urumovii* participates in different herbaceous plant communities: 1) plant communities dominated by *Juniperus sibirica* or *Pinus mugo,* 2) plant communities of the ecotone zone of upper coniferous forest limit. This plant endemic to Pirin Mts. and Slavjanka Mts. seemed to be acting more like generalist. It is found in various herbaceous communities within the coniferous forest belt, near to its upper limit, subalpine meadows and in the alpine belt. The altitude of the microhabitats ranges in wide diapason 1210-2550 m a.s.l. Dullinger et al. (2000) state that plant community endemism of the North-eastern Limestone Alps, illustrates the fact that local and regional endemism may be due to different causal factors. Whatever determinants may be responsible for the occurrence of endemic taxa, the existence of an endemic flora does not provide a sufficient explanation for the existence of all endemic syntaxa present in the North-eastern Limestone Alps. The peculiar geomorphology of the region is responsible for the formation of at least a third of the endemic plant communities. Hence, we should be aware of the fact that regional endemism, though seeming homogeneous in a geographic perspective may be due to complex ecological as well as historical reasons. Regarding the distribution of endemic plants in the Northern Limestone Alps, no uniform trend could be detected. The endemic species pool contains plants restricted to azonal stands as well as typical species of climax grasslands and widespread generalists (Dullinger et al., 2000). *Onobrychis pindicola* subsp. *urumovii* falls in the second group. Being a generalist this endemic has the advantage to form big subpopulations with numerous individuals within its range. It forms large patches of numerous individuals/inflorescences which has a tremendously positive effect in the competition for pollinators. It is well known fact that bumblebees prefer cornucopian food plants (Heinrich, 1979, 1981, Hegland & Totland, 2012, Wester & Lunau, 2016). *Onobrychis* *pindicola* subsp. *urumovii* is actively visited by bumblebees (Kozuharova, 1999, Kozuharova & Raimondo, 2003).

Due to its generalist features *Onobrychis pindicola* subsp. *urumovii* falls in the category of abundant species even though restricted in distribution. Therefore it was assessed as least concern plant species according to the IUCN criteria (Petrova & Vladimirov, 2008). At the same time *O. pindicola* subsp. *urumovii* is an element in several habitats with conservation significance – Natura 2000 habitats: 6170 - alpine and subalpine calcareous grasslands, 8120 calcshist screes, 4060 dominated by *Juniperus sibirica*, 4070 dominated by *Pinus mugo*, 95A0 – woods of *Pinus peuce* and *Pinus heldreichii* (Roussakova, 2015).

Also in the past or in the present *Onobrychis pindicola* subsp. *urumovii* was not utilized except those parts of the populations which were in highland pastures on the territory of the Pirin National Park (Anonymous, 2004, 2009) and Slavjanka Mts.

Our preliminary results (Nash et al., in prep.) reveal that *Onobrychis pindicola* subsp. *urumovii* plant inhibits glucosidases and mannosidase, which is clear and almost certainly due to iminosugars because of how we prepared the samples using cation exchange resins. This is the first report of iminosugars in *Onobrychis*. All this shows that this plant has potential for medicinal use that is about to be discovered. Therefore future cultivation practices might be necessary. Our preliminary experiments in transplantation of adult individuals demonstrated good potential for adaptation. Seed germination was comparatively limited but scarification is not a factor for its optimization. Additional experiments are required in order to identify the best regimes for growing from seed.

Conclusion

Basically *Onobrychis pindicola* subsp. *urumovii* demonstrates high tolerance to its environment although it is a calciphilous species. It grows successfully ex situ in experimental plot in the foothills of the mountains, which indicates prospects for successful cultivation. This will be important in case of future industrial necessity of the plant substance for medicinal purposes. Implications for further research are investigation focused on *ex situ* experiments for optimization the regimes for germination and seedlings' growing.

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