საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 10, №1, 2016
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 10, no. 1, 2016

Botany

Seed Germination and Seedling Establishment in *Coluteocarpus vesicaria* (L.) Holmboe

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ABSTRACT. The objective of this study was to develop seed germination protocol and to investigate factors affecting seedling establishment for *Coluteocarpus vesicaria* (L.) Holmboe (Brassicaceae) - a rare perennial herb, which inhabits screes and stony sloops on 600-3200 m.a.s.l. The data obtained indicate that freshly harvested seeds are in the primary dormant condition. The effect of dry-warm after-ripening and cold stratification on seed germination was examined on seeds subjected 3 months of dry storage at 18° C and subsequent 1, 2 and 3 month of cold stratification resulted in 1.1%, 27.8% and 67.8% germination, respectively. Seedling survival after 3 years planting was 2% overall. Established seedlings form *cushion-like* clusters, are wintergreen and physocarpous in semiarid climate of Tbilisi.

Key words: *Coluteocarpus vesicaria* (L.) Holmboe**,** seed dormancy, germination protocol, seedling establishment.

Coluteocarpus vesicaria (L.) Holmboe 1907, Bergens Mus. Årbog (Årbok) 13: 6. – *Alyssum vesicaria* L. 1753, Sp. Pl.: 2: 651. – *Lagovskia physocarpa* v-Traut. 1858, *Bull. Cl. Phys.-Math. Acad. Imp. Sci. Saint-Pétersbourg 16: 321. - Noccaea vesicaria* (L.) Al-Shehbaz, comb. nov. 2014, Harv. Pap. Bot., 19, 1: 47.

Coluteocarpus vesicaria (L.) Holmboe (Brassicaceae) – is a perennial herb of oligotrophic Caucasian, Irano-Turanian genus *Coluteocarpus* Boiss. naturally distributed in Georgia, Azerbaijan, Armenia, Turkey and Iran. The species is characterized by the specific habitat requirements: it grows on screes, crevices of rocks and stony sloops. Noteworthy the broad altitudinal range of *C. vesicaria*, which extends from 600 to over 3200 m.a.s.l. In Georgia, populations of the species are quite rare [1]. Last botanical expeditions indicate that *C. vesicaria* become endangered. Restricted and specialized habitat of the species face multiple threats, most of which arise from human activity. According to current investigations [2-7] rare and less common species are the most vulnerable cluster exposed to anthropogenic pressure and effects of climate changes. The appropriate conservation measures should be taken to maintain and enhance existing populations of *C. vesicaria* in Georgia.

The most important prerequisites of the successful conservation comprise the detail knowledge about reproductive biology of the species, requirements for seed germination and corresponding data for potentially adaptive phenotypic traits of established seedlings. Little is known about seed germination of *C. vesicaria.* The objective of the present study was to fill this gap and develop seed germination protocol. The study is also the first attempt to investigate factors affecting seedling establishment of *C. vesicaria* in semiarid climate of Tbilisi.

Material and Methods

Mature fruits were collected in a 250 m^2 site at elevation of 1090 m. near village Tsaghveri (41° 48′ 02″ N, 43° 29′ 22″ E), on June 12, 2012. The following weather conditions prevailed at the time of seed dispersal: 20.3°C temperature mean, 69.5 mm. monthly precipitations. The seeds were separated from the pods at the laboratory and divided for the following treatments.

Experiment 1. To evaluate germinability of the freshly harvested seeds the seeds were sown on a filter paper moistened with distilled water in 9-cm glass Petri dishes. The alternating temperatures were chosen (17/10° C) with a 12-hour *photoperiod* in accordance with the prevailing natural conditions during the germination phenophase.

Experiment 2. To determine the effect of after-ripening on seed germination the seeds were dry-stored in paper bags and kept at room temperature (18 ± 1°C, 55-60% relative humidity) during 3 months.

Experiment 3. To determine the effect of duration of cold stratification on seed germination freshly harvested seeds were moist stratified (5°C, 1-3 months, dark) and then transferred to the conditions chosen for the experiment 1.

Experiment 4. To examine the role of combinational treatment on seed germination, seeds subjected 3 month of dry storage and subsequent 1, 2 and 3 month of cold stratification were sawn in conditions chosen for experiment 1. Three replications for a total of 90 seeds were assigned to each experiment. One-way ANOVA was used to detect the significant differences in germinated seeds. Means that showed significant differences were compared using Tukey's test at the 5 % level of significance. Seedling establishment was calculated as the proportion of established seedlings in relation to the number of sown germinable seeds. To provide the safe recruitment of limited number of survived seedling in semiarid climate of Tbilisi, seedlings were planted in two contrasting habitats: 1 – irrigated, with presence of existing vegetation, 2 - none irrigated, with scarce scree herbs.

Fig. 1. Imbibed seeds after coat removal

Fig. 2. Development of the root hairs after pre-chilling

Fig. 3. True leaf development in seedlings after combinational treatment

Results and Discussion

Cushion-like clusters of *C. vesicaria* were sporadically distributed in the study area, on dry scree slope inhabited with single specimens of xerophyllous vegetation: *Astracantha caucasica* (Pall.) Podlech, *Hyssopus officinalis* L., *Vincetoxicum funebre* Boiss. & Kotschy, *Euphorbia smirnovii* Geltman, *E. myrsinites* L., *Telephium imperati* subsp. *orientale* (Boiss.) Nyman.

Experiment 1. According to our results, the imbibition of freshly harvested seeds took place during the first 24 hours, however further development did not occur. Thus, the seeds of *C. vesicaria* were in primary dormant condition - the state induced in many plant species during seed development [8].

Experiment 2. The dormancy was not released by the seed after-ripening: after 3 months period of warm dry storage followed by wetting, the seeds were imbibed, but no radical protrusion was observed in subsequent 30 days period – the time span determined for germination of non-dormant seeds.

Experiment 3. Pre-chilling was ineffective in promoting germination. Thus, without preliminary dry storage radical protrusion was arrested. So the result was similar to data from experiment1. These data make it possible to categorize seed dormancy of *C. vesicaria* as primary endogenous morphophysiological dormancy [9,10]

To test the level of physiological component, the seed coat was removed after imbibition (Fig.1). The seeds remained intact indicating the occurrence of deep physiological dormancy [10]. Interestingly, cold-stratified seeds (experiment 3) respond to the coat removal a little differently: the radical growth was observed, thin root hairs developed (Fig. 2) and cotyledon unfolding had achieved. Hence, the wet pre-chilling as the only factor was more effective in the seed dormancy-breaking, than the dry-warm storage, however, complete germination did not occur and seedling development was arrested at true leaf development stage.

Experiment 4. *The data obtained indicate* that a combination of dry-warm after-ripening and cold stratification had substantial dormancy breaking effect. It should be noted, that the promotion of seed germination was significantly depended on duration of cold stratification. Thus, the highest percentage of the germination was achieved for seeds subjected to the longest period of cold stratification (Fig 3, 4). The differences in germination between duration of cold stratification were significant (Tab.1).

Seed dormancy is an inability of intact viable seed to complete germination under favorable conditions.

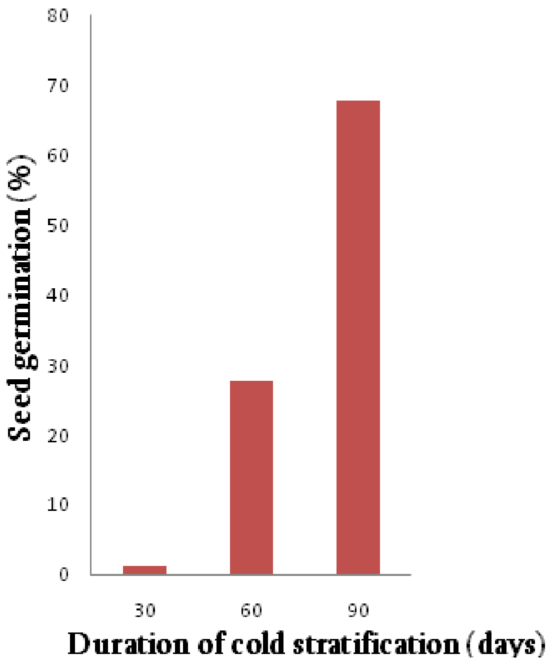

Fig. 4. The effect of combinational (dry -warm+ wet-cold) treatment on seed germination of *C. vesicaria*

Table 1. One-way ANOVA results for seed germination and survival of *C. vesicaria* seeds after combined treatment. Data were analyzed at 0.05 significance level

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
germinated seeds	Between Groups	608.000	2	304.000	53.647	.000
	Within Groups	34.000	6	5.667		
	Total	642.000	8			
survived seedlings	Between Groups	37.556	2	18.778	4.971	.053
	Within Groups	22.667	6	3.778		
	Total	60.222	8			

Mature water-permeable seeds are said to have primary dormancy that has been induced with the involvement abscisic acid during seed maturation on the mother plant [11]. Pre-chilling is believed to activate the gibberellin (GA)-synthesizing mechanism [12] and changes the inhibitor and promoter balance in seeds [13]. Physiological dormancy can be overcome by cold or warm stratification, soaking or after-ripening, depending on the species and how deep the dormancy is. Apparently, the release of dormancy by applying the combinational treatment is caused by the changes in metabolic and physiological activities in seeds, including bias of the ABA/GA balance towards the increasing of sensitivity and synthesis of gibberellins and other endogenous promoters. From our results, it is evident that dry storage strengthens the ability to respond to changes in the cold wet environment. The results of this study are also in accordance with the results of Kirmizi et al. [14], Conner [15] and Kucera et al. [16] reported that cold pre-chilling increases the level of seed sensitivity to gibberellins and thus function as a germination-promoters.

In addition, we assessed seedling establishment in the experimental plots. Overall survival is low, with c. 2% after 3 growing seasons. No significant correlation was revealed between the seedling survival and duration of cold pretreatment. Starting from the second year plant develops single reproductive shoot and begin to bloom forming clustered heads of pinkish white flowers (Fig. 5). The flowering period is quite short: it ends in 2 weeks.

C. vesicaria belongs to physocarpous pool of Brassicaceae, developing swollen fruits at matura-

Fig. 5. Buds before flowering.

Fig. 6. Mature bladdering fruits of *C. vesicaria* at harvest.

Fig. 7. Cushion-like habit of *C. vesicaria*.

tion (Fig. 6). The survived plants gradually form cushion-like clusters (Fig. 7). Thus, the plants being grown in semi-arid climate of Tbilisi are habitually similar to that of Karsani population and at the upper edges of altitudinal range. These data indicate general strategy of *C. vesicaria* for effective thermoregulation: cushion-like habit helps to resist the unfavorable environment both in semiarid climate of Tbilisi and in a harsh weather conditions. It is suggested that wide range is an adaptation of mountain species to long-term climatic fluctuations. In this connection, it is noteworthy, that the established plants of *C. vesicaria* remain wintergreen, that may be attributed to ecological adaptation to their new habitat.

Based on the experimental results the germination protocol for the seeds of *C. vesicaria* is proposed. The 3 months of dry-warm after-ripening followed by 3 months of wet-cold stratification had profound interacting effects on dormancy breaking and seed germination ability of *C. vesicaria*.

ბოტანიკა

ბუშტარას - Coluteocarpus vesicaria (L.) Holmboe თესლის გაღივება და აღმონაცენის დამკვიდრება

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კვლევის მიზანს წარმოადგენდა ოლიგოტიპური გვარ Coluteocarpus Boiss-ის იშვიათი პეტროფიტის, ვერტიკალური გავრცელების დიდი ამპლიტუდის (600-3200 მ.) მქონე სახეობის, ბუშტარას - Coluteocarpus vesicaria-ს თესლის გაღივების მეთოდიკის დამუშავება და აღმონაცენის დამკვიდრების შესწავლა თბილისის კლიმატურ პირობებში. თესლის აღების შემდგომი მომწიფება დაბალი ტენიანობის და მაღალი ტემპერატურის პირობებში ცივი სტრატიფიკაციის რეჟიმთან იყო კომბინირებული. მიღებული მონაცემების თანახმად, მწიფე თესლს ახასიათებს პირველადი მოსვენების მდგომარეობა და ის მოკლებულია გაღივების უნარს ნაყოფის მომწიფებისას. მშრალი შენახვისა (18°C, 3 თვე) და შემდგომი ცივი, ტენიანი სტრატიფიცირების პირობებში 5°C-ზე (1, 2 და 3 თვის განმავლობაში) თესლის გაღივების უნარი აღწევს, შესაბამისად, 1.1%, 27.8% და 67.8%-ს. მცენარე ყვავილობას იწყებს მეორე წლიდან, თბილისის სემიარიდული კლიმატის პირობებში ქმნის ბალიშა ფორმებს, ზამთარმწვანეა და ჯვარედინი დამტვერვისას ივითარებს ბუშტივით გაბერილ ჭოტაკებს.

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Received December, 2015