

Botany

Assessment of the Quality and Germination Capacity of Seed in the Plant Species of Conservation Concern Deposited in the National Seed Bank of Georgia

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The present paper deals with the results of assessment of seed collections of 47 species of Georgia's flora endemic to Georgia and Caucasus deposited at the National Seed Bank of Georgia (National Botanical Garden of Georgia). The studied species belong to the 14 botanical families and 24 genera. Restoring populations of endangered and especially rare endemic species in the wild has long been on the agenda of plant conservationists and for this aim it is very important to ensure safe storage of seeds obtained from plants growing in the natural environment. As a result of our experiments the germination capacity, seed quality and terms, necessary for the germination of seeds have been established for the particular plants species. Germination tests were carried out in the laboratory in conditions of controlled environment (temperature, illumination). Analysis of the experimental results revealed various factors that determine the duration of the dormancy period of seeds and their ability to germinate. The paper is richly illustrated with high-resolution photographs. © 2022 Bull. Georg. Natl. Acad. Sci.

Seed Bank, conservation, germination, eRH (equilibrium relative humidity)

At present more than 1750 species – 41.1%, of total 4275 [1]), or 43% of total 4130 [2], among them up to 500 Caucasus Endemics, from which more than 150 are endemics of Georgia, collected within the joint project under the aegis of Millennium Seed Bank Partnership (MSBP) of the Royal Botanic Gardens, Kew, are secured in the National Seed Bank of Georgia [3].

In the paper we summarize the results of assessment of seed collections of some endemic

plant species of conservation concern collected and deposited at the National Seed Bank under the joint grant project “Saving the Flora of Caucasus (2010-2020) implemented within the scope of the Millennium Seed Bank Partnership of the Royal Botanical Gardens, Kew [4]. The assessments were carried out under laboratory conditions following the methodology, which corresponds to the international standards. This work was implemented in the Department of Plant

Conservation of the National Botanical Garden of Georgia.

Materials and Methods

Upon arrival in the Seed Bank seed collections were evaluated visually using the Stereo Binocular Microscope Nikon SMZ 645. Each seed in the collection was checked individually in order to remove damaged and infested seeds. The 20 to 50 seeds, depending on size of seed collection, were cut and the seed quality was assessed (filled – perfect (good seed), half filled, empty or infested) and structure of the seed determined (embryological characteristics – presence or absence of endosperm, type of embryo, degree of embryo differentiation, its position). If there was doubt about the seed quality, it was placed in 1% TZ (tetrazolium chloride) solution with the aim to determine its viability using this biochemical method.

To establish the germination capacity seeds were sown in Petri dishes on 1% plain agar substrate. As the requirements for germination of wild seeds under natural conditions are unknown, depending on biological, ecological peculiarities and structure of the particular species the hardly germinating seeds were tested under conditions of variable temperature and illumination regime. Petri dishes were placed in the incubator mostly to the following regimes: temperature 24/14°C (day/night) and illumination 12/12 h (light/darkness) (I regime) or temperature 20/14°C (day/night) and illumination 8/16 h (light/darkness) (II regime), also on Petri dishes with filter paper in conditions of room temperature (20-23°C).

Some seeds required stratification. Seed sown on agar medium were placed to the fridge at 4°C to the certain period. Monitoring of the process of germination was carried out weekly.

Duration of experiments was from one to several weeks, sometimes it lasted even for months.

Evaluation and differentiation of seeds, which after the termination of the tests remained

ungerminated on the Petri dishes, were checked on viability by TZ staining. Seed was placed in TZ solution for 48 hr. If despite the created conditions seed did not germinate, though remained viable (alive) (this was determined by TZ at the end of experiment), after comparison with scientific literature sources we used to suppose about the presence of dormancy [5,6].

After termination of the experiment seed germination capacity and viability were determined using the corresponding formulas. Results of tests were recorded in special test monitoring sheets, where were entered also data on total number of seeds, the number of good quality seeds, duration of the tests, data on the state of seeds, remained on Petri dishes (moldy, empty, viable) as well as data of seed eRH before the cold storage in the Seed Bank, expressed in percent. The data were transferred to BRAHMS data base.

Seed collections initially were placed in silica gel or in the dry room in order to gradually reduce the moisture content. Equilibrium relative humidity was measured using the special device – hygrometer Rotronic. After reducing the moisture content to 15-20%, seed was sealed in aluminum foil bags and placed to the freezer at -20°C for the long-term storage. The duplicates of seed collections, processed and packed according to the standard seed bank procedures were sent to the Millennium Seed Bank of RBG, Kew.

Results and Discussion

A necessary precondition for the seed collection to be banked is high viability, as it should be stored for the long term. One of the main methods of determining viability of seed collections is testing on germination capacity. Seed germination under laboratory conditions implicates its ability to give normal seedling during the certain period. Thus determining the duration necessary for the seed to germinate is important also for the future to be able to turn the seed into plant. This is especially essential for the endemic species of conservation status.

Table. Results of testing germination capacity and viability of seeds of different plant species deposited in the National Seed Bank of Georgia

#	ID number of seed collection	Genus	Species	Germination %	Viability %	Duration of the experiment (weeks)	Note
1	2	3	4	5	6	7	8
Rosaceae							
1.	OCRSB:993	<i>Rosa</i>	<i>irysthonica</i>	0	-	17	I regime regime - Deep dormancy
						10	II regime - Deep dormancy
2.	OCRSB:1289	<i>Rosa</i>	<i>doluchanovii</i>	0	-	21	I regime - Deep dormancy
3.	OCRSB:996	<i>Rubus</i>	<i>adscharicus</i>	0	-	25	I regime - Dormancy
4.	OCRSB:999	<i>Rubus</i>	<i>cyri</i>	11.1	44.4	20	I regime Starts to germinate on 17 th week
5.	OCRSB:1002	<i>Rubus</i>	<i>miszczenkoi</i>	0	-	20	I regime prolonged seed dormancy
6.	OCRSB:1104	<i>Rubus</i>	<i>kacheticus</i>	0	-	16	I regime - Many fresh seeds after the experiment
7.	OCRSB:1106	<i>Rubus</i>	<i>moschus</i>	20.8	60	16	I regime - Starts to germinate on 8 th week
8.	OCRSB:1107	<i>Rubus</i>	<i>nakeralicus</i>	0	-	14	I regime - Embryo is alive, prolonged seed dormancy
9.	OCRSB:1110	<i>Rubus</i>	<i>platyphylloides</i>	5.8	64.7	17	I regime Starts to germinate on 13 th week
10.	OCRSB:1136	<i>Rubus</i>	<i>kudagorensis</i>	50	89	22	I regime - Starts to germinate on 8 th week, the last seedling appears on 18 th week
11.	OCRSB:1145	<i>Rubus</i>	<i>charadzeae</i>	70	90	21	I regime - Seed germination starts on 11 th week, the last seedling - on 17 th week
12.	OCRSB:1216	<i>Rubus</i>	<i>longipetiolatus</i>	68.1	100	31	I regime - Seed germination starts on 16 th week, the last seedling - on 29 th week
13.	OCRSB:1286	<i>Rubus</i>	<i>woronowii</i>	0	-	20	I regime prolonged seed dormancy - Many fresh seeds after the experiment
14.	OCRSB:1352	<i>Rubus</i>	<i>juzepczukii</i>	0	-	19	I regime prolonged seed dormancy - many fresh seeds after experiment
15.	OCRSB:1272	<i>Alchemilla</i>	<i>capillaceae</i>	30	100	18	I regime - Starts to germinate on 7 th week
16.	OCRSB:1335	<i>Alchemilla</i>	<i>impolita</i>	25	83	20	I regime - Starts to germinate on 10 th week
17.	OCRSB:1345	<i>Potentilla</i>	<i>suauetica</i>	38.4	100	22	I regime - Starts to germinate on 1 st week
18.	OCRSB:1551	<i>Potentilla</i>	<i>sosnowskyi</i>	47	76	13	I regime - Starts to germinate only on 1 st week
19.	OCRSB:1248	<i>Potentilla</i>	<i>kemulariae</i>	33.3	55.5	18	I regime - Starts to germinate on 10 th week
Leguminosae							
20.	OCRSB:1263	<i>Genista</i>	<i>suauetica</i>	30	90	16	I regime - Starts to germinate on 1 st week
21.	OCRSB:1395	<i>Astragalus</i>	<i>aspindzicus</i>	83.3	83.3	2	I regime - Germinated in two weeks
22.	OCRSB:1396	<i>Astragalus</i>	<i>meskheticus</i>	57	81	17	I regime - Starts to germinate on 1 st week
Rubiaceae							
23.	OCRSB:879	<i>Galium</i>	<i>praemontanum</i>	80	80	9 weeks	I regime Starts to germinate on 2 nd week

continued

1	2	3	4	5	6	7	8
24.	©CRSB:1274	<i>Galium</i>	<i>anfractum</i>	40	100	18 weeks	I regime Starts to germinate on 11 th week
Umbelliferae							
25.	OCRSB:930	<i>Astrantia</i>	<i>colchica</i>	7.6	53.8	26 weeks	I regime - Starts to germinate on 16 th week
26.	©CRSB:1549	<i>Peucedanum</i>	<i>adae</i>	90	90	4 weeks	I regime Starts to germinate on 1 st week
Compositae							
27.	©CRSB:1072	<i>Kemulariella</i>	<i>colchica</i>	25	25	17	I regime - Starts to germinate on 1 st week
28.	OCRSB:1137	<i>Tragopogon</i>	<i>meskheticus</i>	93.7	93.7	2	I regime - Fresh, newly harvested seeds tested before the cold storage
				76.9	76.9	2	I regime –Seeds tested after 4 years storage in the freezer
29.	OCRSB:1251	<i>Tragopogon</i>	<i>ketzkhovellii</i>	80	80	2	I regime - Starts to germinate on 1st week
30.	OCRSB:1285	<i>Tragopogon</i>	<i>makaschwilii</i>	100	100	1	I regime - All seeds sprouted within the 1 st week
31.	OCRSB:1355	<i>Tragopogon</i>	<i>colchicus</i>	81.8	81.8	2	I regime - Seeds sprouted in 2 weeks
32.	OCRSB:1139	<i>Podospermum</i>	<i>idae</i>	68	76	27	I regime - Starts to germinate on 1 st week
33.	OCRSB:1148	<i>Cirsium</i>	<i>oblongifolium</i>	40	100	15	I regime - Starts to germinate on 1 st week
34.	OCRSB:1155	<i>Cirsium</i>	<i>sosnowskyi</i>	50	100	10	I regime – Seeds sprouted in the first 2 weeks
35.	OCRSB:1312	<i>Hieracium</i>	<i>kharthlicum</i>	75	90	8	I regime - Starts to germinate in the 1 st week
36.	OCRSB:1275	<i>Hieracium</i>	<i>chromolepium</i>	100	100	2	I regime – Seeds sprouted in 2 weeks
37.	OCRSB:1484	<i>Thymus</i>	<i>sosnowskyi</i>	70	70	10	I regime Starts to germinate on 1 st week
Campanulaceae							
38.	OCRSB:805	<i>Campanula</i>	<i>kachetica</i>	95.7	95.7	3	I regime - Starts to germinate on 1 st week
39.	OCRSB:807	<i>Campanula</i>	<i>kemulariae</i>	82.5	87.5	12	I regime - Starts to germinate on 2 nd week
40.	OCRSB:936	<i>Campanula</i>	<i>fonderwisii</i>	40	90	12	I regime - Starts to germinate on 3 rd week
Euphorbiaceae							
41.	OCRSB:954	<i>Euphorbia</i>	<i>boissieriana</i>	0	-	20	I regime - fail
				60	75	9	II regime - Starts to germinate on the 2 nd week
Caryophyllaceae							
42.	OCRSB:1138	<i>Dianthus</i>	<i>charadzeae</i>	100	100	3	I regime - Starts to germinate on 1 st week
Helleboraceae							
43.	OCRSB:855	<i>Aquilegia</i>	<i>colchica</i>	94	99	3	I regime - Starts to germinate on 2 nd week
Polygalaceae							
44.	OCRSB:1260	<i>Polygala</i>	<i>albowii</i>	80	90	16	I regime - Starts to germinate on 2 nd week
Betulaceae							
45.	OCRSB:1261	<i>Betula</i>	<i>megrelica</i>	8	100	24	I regime Starts to germinate on 21 st week
Cistaceae							
46.	OCRSB:966	<i>Helianthemum</i>	<i>georgicum</i>	25	50	12	I regime Starts to germinate on 2 nd week
Scrophulariaceae							
47.	OCRSB:1061	<i>Euphrasia</i>	<i>kemulariae</i>	100	100	1	I regime Germinated in one week

I regime - 24/14°C (day/night) and illumination 12/12h (light/darkness).

II regime - 20/14°C (day/night) and illumination 8/16h (light/darkness).

The Table shows results of germination of seed of 47 endemic plant species of conservation concern belonging to different botanical families. Germination tests were carried out on freshly harvested seeds, during the first 1-2 weeks after the entry to the Seed Bank.

The results of tests show that several species of genera *Rosa* and *Rubus* of family Rosaceae show low germination percent or they fail to germinate during the prolonged time of the experiment. Seed coat in the mentioned species is thick and solid. In most of cases seed coat might be the reason for the physical dormancy, as the dense surface prevents the embryo from mechanical extension, hinders penetration of water or gas exchange, due to that the embryo is not supplied with necessary amount of oxygen. Testing of *Rosa irysthonica* and *Rosa doluchanovii* lasted for 17-21 weeks, though germination was not achieved. After the termination of tests seed viability was tested using the TZ test. The embryo was perfect and alive. Seed of the mentioned species might have been characterized by the durable period of dormancy. Dormancy period and interruption of germination in this genus is very often caused by the presence of inhibitors in the pericarp and physiological barriers in the embryo [7].

According to the literature application various methods, such as treatment with gibberellic acid, treatments with sulfuric acid, scarification or cold stratification for the germination of *Rosa multibracteata* does not yield positive results. Only storing in dry conditions for 68 weeks in combination with cold stratification for 16-24 weeks turned out to be the best method for the propagation of the mentioned species by seed (72-79%) [8].

Most of species of genus *Rubus* are characterized by thick layers of seed coat. Because of this we've done cutting of a small section of seed coat without damaging the embryo (its position within the seed was known for us from the preliminary checks) and continued the experiment in this way. In some cases this technique gave certain results. Germination percent of seed of 6 species

Rubus cyri, *Rubus moschus* (Fig. 1; 6), *Rubus platyphylloides*, *Rubus kudagorensis* (Fig. 1; 7), *Rubus charadzeae* (Fig. 1; 9), *Rubus longipetiolatus* (Fig. 1; 10), fluctuated from 11 to 70% (see Table 1). As to other 6 species: *Rubus adscharicus* (Fig. 1; 4), *Rubus miszczenkoi* (Fig. 1; 5), *Rubus kacheticus*, *Rubus nakeralicus*, *Rubus woronowii*, *Rubus juzepczuki* their seed fail to germinate within the given duration of tests, which in both cases was extended from 14 to 30 weeks, though TZ test has revealed a high percent of their viability from 80 to 100%.

On the basis of results of studies of seed structure the state of embryo, we can conclude that besides physical dormancy the mentioned species are characterized by physiological dormancy as well. According to different sources, scarification of 5 species belonging to the genus *Rubus* (*Rubus parvifolius*, *R. phoenicolasius*, *R. buergeri*, *R. takesimensis* and *R. corchorifolius*) with sulfuric acid followed by cold stratification at 4°C for 8 weeks turned out to be efficient only for 3 species *Rubus parvifolius*, *R. Phoenicolasius* and *R. takesimensis* but it did not have influence on others [9].

Alchemilla capillaceae and *Alchemilla impolita* seeds are characterized by the slow rate of germination, though their viability is high (83-100%). Under the same conditions seeds of two different species of genus *Potentilla* – *Potentilla suanetica* (NT) and *Potentilla sosnowskyi* start to germinate during the first week, though germination of seed of *Potentilla kemulariae* (Fig. 1; 11), occurs only from the 10th week. Seeds of all three examined species – *Genista suanica* (Fig. 1; 12, 14), *Astragalus aspindzicus* (Fig. 1; 13) and *Astragalus meskheticus* belonging to the family Leguminosae, start germination within the first week after sowing. Almost all seeds of *Astragalus aspindzicus* have germinated during two weeks, though in case of *Genista suanica* and *Astragalus meskheticus* the process of germination lasted for 16 and 17 weeks respectively. In the genus *Galium* *Galium praemontanum* seeds start to germinate from the 2nd week; seeds of *Galium anfractum* – from the 11th week (Table).



Fig. 1. Testing seed viability of different plant species deposited in the National Seed Bank of Georgia using different methods – cut test, Tz test, germination on agar and growing seedlings in pots: 1. *Rosa irysthonica*; 2. Cut test of *Rosa irysthonica* seeds; 3. *Rosa doluchanovii* (Tz test); 4. Cut test of *Rubus adscharicus* seed; 5. Cut test of *Rubus miszzenkoi* seed; 6. *Rubus moschus*; 7. *Rubus kudagorensis*; 8. Seeds of *Rubus kudagorensis*; 9. *Rubus charadzeae*; 10. *Rubus longipetiolatus*; 11. Sprout of *Potentilla kemulariae*; 12. Tz test *Genista suanica*; 13. Seeds of *Astragalus aspindzicus*; 14. *Genista suanica*; 15. *Tragopogon meskheticus* (after 4 years of cold storage); 16. Seeds of *Tragopogon meskheticus* -Tz test (before the cold storage); 17. Seedlings of *Tragopogon meskheticus* transplanted into pots; 18. *Tragopogon ketzkhoveli*; 19. *Tragopogon makaschwilii*; 20. Seeds of *Tragopogon makaschwilii* in Tz; 21. *Cirsium oblongifolium*; 22. *Cirsium sosnowskyi*; 23. Sprout of *Cirsium sosnowskyi*; 24. *Hieracium chromolepium*. Seed germination on agar medium and growing seedlings in pots: 25. *Hieracium kharthlicum*; 26. Seeds of *Hieracium kharthlicum* stained with Tz. 27. *Betula megrellica*; 28. Seeds of *Betula megrellica*; 29. Seeds of *Euphorbia boissieriana*; 30. *Cirsium sosnowskyi* and *Cirsium oblongifolium*.

The studied species of Compositae family are characterized by good germination capacity (Fig. 1; 15-24). High germination capacity of some representatives of Compositae family was also mentioned in our earlier work [10]. It is known that long term storage of seed causes decline in its

viability. We've checked germination capacity of several species, among them *Tragopogon meskheticus* (Fig. 1; 15, 16) after four years of storage in the seed bank. It was reduced from 93.7% to 76.9%, so this index remained within the norm (Fig. 1; 15).

For germination of seed of the species of Euphorbiaceae family – *Euphorbia boisseriana* decrease in duration of the light period from 12 to 8 hours, and the temperature from 24°C to 20°C turned out to be beneficial (Table).

Germination of seeds of *Betula megrelica* started after 21st week, though germination index was low (Fig. 1; 27-28). According to our earlier studies (microscopic and seed germination studies) in conditions, similar to the regime I (1% agar medium, temperature 24/14°C (day/night), illumination 12/12 hours (light/darkness) germination of *Betula medwediewii* started in 5 days and the germination percent was high – 75% [11, 12].

As seen from the results of experiment (Table), time, necessary for the germination and sprouting depends on the plant species and it is different even for the same species depending on conditions. Prolonged period of germination may be conditioned by a series of factors, among them solid seed coat, seed size, incompletely developed embryo (in freshly harvested seed), reduced level of metabolic processes, presence of growth inhibitors in the seed, absence of endosperm in the seed and others. Notion about the time, necessary for seed germination gives a general representation about the possibility of realization of its maximum

potential in the soil. The collections of the above discussed species were evaluated positively. Low germination percent does not mean that seeds are not suitable for the use for the plant regeneration. According to the general structure of seedlings, obtained under laboratory conditions – seedlings with well developed primary root, hypocotyl, epicotyl – we can judge whether it has a capacity for the further development after replanting to the soil. Most of normally developed seedling of Georgian endemic species are transplanted to the soil substrate in pots and they continue to develop successfully (Fig. 1; 30).

Thus while evaluation under laboratory conditions of the mentioned seed collections of high conservation concern the quality of the collection and its viability were assessed and the conditions and terms, necessary for the obtaining of normal seedlings established.

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ბოტანიკა

საქართველოს ეროვნულ თესლის ბანკში დაცული ზოგიერთი საკონსერვაციო სტატუსის მქონე სახეობის მცენარის თესლის ხარისხისა და აღმოცენების უნარის შეფასება

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წარმოდგენილ ნაშრომში მოცემულია საქართველოს ეროვნულ თესლის ბანკში (საქართველოს ეროვნული ბოტანიკური ბაღი) განთავსებული საქართველოსა და კავკასიის საკონსერვაციო სტატუსის მქონე 47 ენდემური სახეობის მცენარის თესლის კოლექციების შეფასების შედეგები. შესწავლილი სახეობები გაერთიანებულია 14 ბოტანიკურ ოჯახსა და 24 გვარში. ჩატარებული სამუშაოს შედეგად კონკრეტული სახეობებისთვის დადგენილია თესლის სიცოცხლისუნარიანობის ხარისხი, გაღივებისა და აღმოცენების უნარი და ვადები. იშვიათი და გადაშენების საფრთხის წინაშე მყოფი და, განსაკუთრებით, ენდემური სახეობების პოპულაციების აღდგენა ყოველთვის იყო მცენარეთა კონსერვაციის გადაუდებელი ამოცანა. ამ მიზნის მისაღწევად ძალზე მნიშვნელოვანია ველური ბუნებიდან მოპოვებული თესლის სათანადო შენახვის უზრუნველყოფა. ჩვენი ექსპერიმენტების შედეგად კონკრეტული სახეობებისათვის დადგინდა გაღივების უნარი, თესლის ხარისხი და გაღივებისათვის საჭირო დრო. ტესტირება ტარდებოდა ლაბორატორიაში კონტროლირებად პირობებში (ტემპერატურა, განათება). ექსპერიმენტების შედეგების ანალიზმა გამოავლინა თესლის სვენების პერიოდის განმსაზღვრელი ფაქტორები და მათი გაღივების უნარი. სამუშაო ილუსტრირებულია ექსპერიმენტის სხვადასხვა ეტაპის ამსახველი მაღალი ხარისხის ფოტოებით.

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