Self-renewal Capacity of Several Species of the Genus *Sternbergia* Possessing Medicinal Properties

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Abstract: The present work dealt with sexual reproduction capacity of three relic species of the genus *Sternbergia* (family Amaryllidaceae) distributed in the Caucasus, i.e., *Sternbergia lutea* (L.) Ker Gawl. ex Spreng., *Sternbergia fischeriana* (Herb.) Roem. and *Sternbergia colchiciflora* Waldst. & Kit.. Under this study, the natural populations of species, which possess valuable medicinal and ornamental properties, have been assessed by experts as vulnerable, and *S. colchiciflora* is included in the red list of endemic plants of the Caucasus, as having status of the critically endangered species. The situation is aggravated by the poor self-regeneration capacity of these species, which is one of the main factors responsible for the sustainability of the population of this or that species in the wild. Self-regeneration capacity for sexual reproduction was investigated in the listed species of the genus *Sternbergia* using common methods of embryology and reproduction biology. In conditions of the National Botanical Garden of Georgia (NBGG), the species *S. lutea* and *S. fischeriana* prove to be completely infertile and propagate vegetatively by bulblets, while *S. colchiciflora* revealed the ability for propagation by seed. Long-term conservation of seeds of the studied species *S. colchiciflora* in the Caucasus Regional Seed Bank (CRSB) and establishing of living collections of this species at the experimental plot were chosen as the method for safeguarding this critically endangered species.

Key words: *Sternbergia*, medicinal properties, vulnerable species, micro- and macro-sporogenesis, ovule, germination, plant reproduction, *ex-situ* conservation.

1. Introduction

Georgia has one of the highest concentration of plant species known from any temperate part of the world. The number of species in the Caucasus amounts to 6,350 and the number of endemic species to 1,500 [1, 2]. Georgia's flora comprises more than 4,100 species of the vascular plants. High endemism level of Georgia's flora reflects its richness; 21% of the local flora is endemic and consists of about 900 species. Among them, about 600 species are endemic to the Caucasus and about 300 are endemic to Georgia [3]. The Caucasus and Georgia, in particular, have been attributed to biodiversity "hotspots" [1, 2, 4].

The country holds a great number of species of medicinal value, with over 700 species utilized in Georgian traditional medicine and 200 taxa registered in the official pharmacopoeia [5].

The oldest recordings of Georgian medicinal plants come from Greek mythology. There is a mention of curing properties of "herbs" in such Georgian legends as Amiran (2nd millennium BC), the search for the goldenfleece and the magical gardens in Colchis [6]. It is known that royal gardens of medicinal plants were the predecessors of botanical gardens. In feudal Georgia, medicinal plants were used in hospitals that at the time existed in monasteries. Important medicinal books were created in Georgia in the Middle Ages, such as "medical book—Karabadini" by Zaza Panaskerteli-Tsistsishvili (15th century) and "Iadigar-Daudi" by David Bagrationi (16th century). These works contain information on the curative properties and methods of preparation for a number of wild plants and crops [5, 7-9].

In the National Botanical Garden of Georgia (NBGG), which history counts almost four centuries, establishing of collections of medicinal plants and

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their propagation has been carried out for years. At a certain stage of development, the historical garden was referred to as “physic garden”.

Study of biological peculiarities of medicinal plants, investigation of capacity for reproduction by seed, creation of living collections, propagation and *ex-situ* conservation are priorities of our work, as hundreds of plant species in Georgia are threatened with extinction or serious genetic erosion. The vast majority of medicinal plants in Georgia are collected from wild habitats, and in some cases, are heavily exploited, potentially threatening further existence of selected species. Increasing pressure on natural areas, due to increased deforestation, erosion, air pollution and dam-building, threatens the habitats of important medicinal plants.

The present work dealt with three relic species of the genus *Sternbergia* distributed in the Caucasus, i.e., *Sternbergia lutea* (L.) Ker Gawl. ex Spreng., *S. fischeriana* (Herb.) Roem. and *S. colchiciflora* Waldst. & Kit.. *S. lutea* and *S. fischeriana* are distributed in Azerbaijan (Apsheron peninsula) and *S. fischeriana* in

Georgia Shavi Mta and Makhatas Mta [10]. The latter is located in environs of Tbilisi, though during the fieldwork expeditions of recent years, the species was not found on the territory and indicated in the flora of Georgia. Distribution of the studied species in the Caucasus is shown on the map below (Fig. 1). *S. colchiciflora* is included in the red list of endemic plants of the Caucasus as having status of the critically endangered species [2].

Species belonging to the genus *Sternbergia* possess valuable curative properties due to the content of alkaloids and thus have various applications in medicine [11-13]. This puts additional stress on natural populations of these species.

Study of peculiarities of seed forming processes and establishment of reproduction strategy is of crucial importance for the survival of plants, reproduced mainly by seeds. This will serve as a basis for the development of particular, efficient protection measures. Questions of reproduction biology of species under study have not been researched so far. *Ex-situ* conservation activities for these species were

Fig. 1 Occurrence of species of genus *Sternbergia* distributed in the Caucasus.

carried out for the first time as well. Processes of seed formation of a species and growth of seedlings developed from seeds are known to be influenced by different factors, such as, climate, human impact, genotype factors and characteristic to particular species. This is a complex of factors, which affect the species directly or indirectly and are responsible for its success or failure in its distributional range.

This research was aimed at the assessment of self-renewal capacity of three species of the genus *Sternbergia*, the establishment of their seed bank and *ex-situ* living collections.

2. Materials and Methods

Flowering biology and seed forming capacity were studied on plant material of *S. lutea* (Figs. 2a-2f) and *S. fischeriana* (Fig. 3a), transplanted years ago from Azerbaijan (Talysh) to the collection plot of the

Department of Plant Conservation of NBGG. Studies of *S. colchiciflora* were carried out on plants both those grown on the collection plot of NBGG and obtained from the single wild population located at Shavi Mta (Fig. 1).

The condition of *ex-situ* site, collection plot of the Department of Plant of NBGG was as following: GPS coordinates at 41°41'150" N, 44°48'140" E, above sea level 504 m; mean annual temperature 12.6 °C, mean minimum temperature in January -0.5 °C and absolute minimum -18 °C; mean annual precipitation 518 mm; summer and winter moderately dry.

Investigations were carried out both in the field and under laboratory conditions using the approved novel methods of embryology and reproduction biology. Observations on seed germination and seedling biology were made on seedlings sown on Petri dishes and in pots outdoors. Material for embryological investigations

Fig. 2 Flowers and generative structures of *S. lutea*.

was processed and studied using common cyto-embryological methodology [14, 15]. Pollen germination on artificial medium was tested on 10%-15% saccharose solution at 20-25 °C, and the maximum exposition time was 24 h. Temporal fixation required for embryological studies was done from the beginning of flower shoot development to final flowering. The following fixing agents were used: formalin-acetic acid-alcohol (FAA), Carnoy's fluid and formalin-propionic acid-alcohol (FPA). Microscopic studies were carried out using CARL ZEISS (Germany) light microscope.

Seed productivity was determined according to the technique described by Rabotnov [16]. Fifty fruits with seeds already entered into the maturation phase were collected from each population, and the developed seeds as well as undeveloped ovules were counted. Observations on seed germination capacity were made at ambient temperature (18-20 °C). Germination capacity, seed collection and deposition in the Caucasus Regional Seed Bank (CRSB) were performed after the method in Ref. [17].

3. Results and Discussion

Capacity for sexual reproduction in any plant species is directly connected with the process of normal formation of female and male generative structures, as well as with normal course of pollination and fertilization. Processes of micro- and macro-sporogenesis, i.e., formation of male (microgametophyte or pollen grain) and female generative sphere (female gametophyte or embryo sac), proceed under the ground, before the flower emerges on the ground surface.

Meiosis in *S. lutea* and *S. fischeriana* proceeds with deviations, very often 4-5 univalents are formed. In anaphase, bridges are marked and chromosome retardation takes place. Formation of the male sphere under the ground proceeds until the mononuclear stage, where the common membrane is not disintegrated and the microspores are presented as

linearly arranged tetrads. Quite often, pentads are observed, too. In *S. lutea*, division of microspore nucleus takes place a week prior to the emergence of the bud above the ground (Figs. 2e and 2f), while in *S. fischeriana*, the same process takes place in October, two months prior to the beginning of anthesis.

The processes of micro- and macro-sporogenesis (formation of microspore and megaspore mother cell) proceed simultaneously in both species (in September). Meiosis—formation of microspore, takes place under the ground, within the bud. Pollen grain of the flower, emerging on the ground surface, is at the mononuclear stage. At this stage, embryo sacs are not formed yet in either of these two species.

Flowering in *S. lutea* starts in autumn at the end of September, 25-30 d; mass flowering starts in 8-10 d; average life-span of an individual flower is 5-7 d. Flowering in *S. fischeriana* starts 2-3 months later (January-February) than that in *S. lutea*. In conditions of cold winter, mass flowering starts in March and lasts 1.5-2 months. Life span of an individual flower is 15 d. Both species are entomophilic.

Some disturbances are observed in the process of microsporogenesis (Figs. 3b and 3c). Mature pollen is bicellular, elliptical and monoporous. It looks like fertile when stained with acetocarmine [14]. Fertility rate is 76%-78% for *S. lutea* and 44%-46% for *S. fischeriana*.

On artificial medium 10%-15% saccharose, germination rate of mature pollen is quite low, 14%-20% for *S. lutea* and 10%-15% for *S. fischeriana*.

Germination rate of pollen grains, occurring on stigma as a result of artificial pollination, is low at 2%-3%.

Protandry is strictly manifested in the studied species, which along with other reasons, unknown so far, favours the infertility of the species.

In *S. lutea* 14-32 ovules are set and 42-50 ovules in *S. fischeriana*.

Blooming in the studied species *S. lutea* and *S. fischeriana* proceeds well in spring, but seeds are not

Fig. 3 Flowers and generative structures of *S. fischeriana*.

Fig. 4 Flowers and generative structures of *S. colchiciflora*.

produced either in conditions of open pollination, or as a result of intraspecific artificial pollination. Both species are infertile in Azerbaijan as well, as reported by Kapinos [18]. During five years, more than 3,000 flowers were artificially pollinated and no fruit-set

was registered. Only single facts of formation of bicellular embryo from the zygote were described in the process of these experiments.

Only once a single fruit, with three well developed seeds, has been found in *S. lutea* as a result of open

pollination, but it failed to germinate.

The picture is different in case of *S. colchiciflora* (Figs. 4a-4d), which resembles *S. lutea*, though its plants are smaller as compared with *S. lutea*, and flowering also takes place in autumn. Great part of flowers does not emerge on the ground surface. Formation of generative structures and pollination-fertilization proceed under the ground and early in the spring fertile seeds emerge above ground.

In conditions of NBGG, *S. lutea* and *S. fischeriana* have revealed complete infertility. The collections maintained here are obtained by means of vegetative propagation by bulblets, while *S. colchiciflora* revealed the ability for propagation by seeds.

3.1 Ex-situ Conservation

A seed bank was established in collaboration with colleagues from the Royal Botanic Gardens, Kew,

within the scope of the Millennium Seed Bank Project. After processing in the laboratory, 500 seeds of *S. colchiciflora* were deposited in the CRSB at NBGG (Fig. 4e), and herbarium vouchers in the National Herbarium of Georgia (TBI). Duplicate samples of the seed collections were sent to the Millennium Seed Bank and herbarium vouchers placed in Kew Herbarium (K).

3.2 Living Collections

Also stock of living plants of *S. colchiciflora* was established (Fig. 4f). It had been determined that the freshly harvested seeds (May) sown in the same month germinate quickly. Being sown in pots, they gave sprouts in 40-45 d. Different is the germination time required for seeds harvested in the previous year and sown in spring of the next year. 210 d and more were required for those seeds to germinate. Germination percent is lower as well.

As concerns as *S. lutea* and *S. fischeriana*, their

Fig. 5 Scheme of *ex-situ* conservation activities at the Department of Plant Conservation of NBGG.

living collections, obtained as a result of vegetative propagation by division of bulbs, are established on the collection plot of the Department of Plant Conservation of NBGG. The mentioned reserve of seedlings (seeds and living collections) is created for the aim of reintroduction works in the nature in case of need, and as initial material to be supplied for pharmacological companies in case of interest.

A summary scheme, illustrating content of *ex-situ* conservation activities, in progress in the CRSB of NBGG is shown in Fig. 5.

4. Conclusions

S. lutea and *S. fischeriana*, the species native to the Caucasus region and introduced to NBGG decades ago, have been found completely infertile and vegetatively propagating by bulblets, while *S. colchiciflora* revealed capacity for propagation by seeds.

It has been found that the freshly harvested seeds of *S. colchiciflora* require 30-45 d to germinate, though the time required for germination was longer and germination percentage was lower for seed collections in the previous year.

Long-term conservation of seeds of the studied species *S. colchiciflora* in CRSB and establishing of living collections of this species at the experimental plot were chosen as the method for safeguarding this critically endangered species.

For the higher safety, seed collections of *S. colchiciflora* are duplicated to the Millennium Seed Bank of the Royal Botanic Gardens, Kew (UK) within the scope of the Millennium Seed Bank Partnership.

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