Reproductive biology of a rare species Begonia ludwigii in greenhouse conditions of the Siberian Botanical Garden of Tomsk State University

Reproductive biology of a rare species Begonia ludwigii in greenhouse conditions of the Siberian Botanical Garden of Tomsk State University

M S Yamburov, A I Lykhina, S B Romanova, V M Smolina and E Yu Zharnakova

Siberian Botanical Garden, Tomsk State University, Tomsk, Russia

E-mail: sbg-orangery@yandex.ru

Abstract. The paper presents the results of a reproductive biology study and the Begonia ludwigii seasonal development rhythm - a species classified by the International Union for Conservation of Nature as a rarity category EN (endangered species). The studies have shown that when cultivated in the Tomsk State University Siberian Botanical Garden greenhouse conditions, this species has high potential seed productivity (about 75,000 ovules per inflorescence), high fertility and pollen viability, but at the same time low true seed productivity (seed rate not more than 27%). It was established that the Begonia ludwigii seeds, in the greenhouse conditions, are tied to heterogamous pollination due to the absence of pollinating insects. The use of xenogamous artificial pollination increased the coefficient of seed productivity by almost 3 times. The paper also describes the full seasonal development rhythm, flowering biology, biomorphological features of pollen and seeds.

1. Introduction

Currently, anthropogenic pressure is increasing on natural ecosystems, which leads to a reduction in the habitats of many plant species and even to the complete disappearance of some of them. In the modern world, botanical gardens play a large role in preserving plant biodiversity - the botanical gardens scientific activity helps to preserve, and in some cases restore, species that are disappearing or disappeared in nature. Most often in botanical gardens of temperate and northern latitudes, when creating collections of rare and endangered species, the main emphasis is on outdoor plants. Greenhouse plant collections can also contribute to the conservation and reintroduction of endangered species. A vivid example of this is the experiment to return to nature seedlings of (Cycas micholitzii Dyer) grown from seeds obtained in the Botanical Garden greenhouses of the Botanical Institute named after V.L. Komarov RAS [1].

The Siberian Botanical Garden of Tomsk State University (SibBS TSU) is the oldest botanical and introduction centre in the Asian part of Russia. The unique gene pool of the world flora is collected in the outdoor and greenhouse collections at SibBS TSU - over 8,500 species, forms and varieties, of which about 4,000 are cultivated in greenhouses. An important area of SibBS TSU activity is the attraction of rare and endangered species to the collection and study of both local and world flora.

Currently, 90 species are cultivated in the greenhouses of SibBS TSU, classified by the International Union for Conservation of Nature as rare and endangered with high categories of rarity (CR, EN, VU). One of these species is Begonia ludwigii Irmsch., first discovered in 1931 by

E.K. Gray and later, in 1937, described by Edgar Irmscher [2]. B. ludwigii (figure 1) is endemic to the Republic of Ecuador, where only 4 populations of this species are known and the expansion of farmland is the main limiting factor leading to a reduction in natural habitats. B. ludwigii is listed by the International Union for Conservation of Nature in the list of endangered species and has the conservation status EN (endangered species) [3].

Figure 1. Ludwig Begonia (Begonia ludwigii).

There are about 1300 species in the genus Begonia L., 51 of which are listed by the International Union for Conservation of Nature as endangered species [3]. Many species of the genus Begonia are highly decorative and resistant to growing indoors, which has provided them with great popularity in indoor floriculture. Also, many species have high phytoncide activity, which allows them to be recommended for use in medical, educational and other institutions to reduce microbial activity [4–7].

Most species of the Begonia genus have a high rhizogenic ability and is easily rooted by cuttings, as well as by individual leaves and even leaf segments. However, seed reproduction of many species in greenhouse conditions is difficult, as they are entomophilous plants, and pollinators are often absent in greenhouses. Seed productivity, as a component of the reproductive process, is one of the important indicators of the viability of a species in specific conditions [8]. Seed productivity indicators may decline due to various factors (soil and air temperature, humidity, daylight hours, light intensity, etc.) [8, 9]. The likelihood of geytonogamous pollination can also be difficult since most begonias are monoecious plants with dioecious flowers and the phases of male and female flowering in the inflorescence do not always overlap in time.

The purpose of the paper is to study the seasonal rhythm of development and reproductive biology of B. ludwigii in the greenhouse conditions for the successful reproduction of the ex situ species.

2. Methods and materials

In SibBS TSU B. ludwigii cultivated in the greenhouse "moderately humid tropics", where from November to March, the daytime temperature is +20 ... +22 °C, night +18 ... +20 °C; and from April to October, daytime temperature +24 ... +28 °C, night temperature +22 ... +24 °C. Humidity in the winter is 60–70%, in the summer 70–90%. Seeds of B. ludwigii were obtained by delectus exchange from the Botanical garden of Grüningen (Switzerland). The study was conducted on 4-year-old generative individuals for 2 years (2016–2017).

Phenological observations during flowering were carried out daily. As the inflorescences flourished, all the flowers were numbered with a marker, which made it possible to trace the life span of both male and female flowers, as well as quantify all the flowers in the inflorescence. On 5 inflorescences, 10 female flowers each were artificially pollinated with pollen from other specimens

(xenogamous pollination), the remaining female flowers tied to seeds were considered as geitonogamous pollinated (male flower pollen from the same inflorescence).

For each inflorescence, the percentage of fruiting was determined as the percentage of female flowers that formed the boxes of the total number of female flowers [9].

Pollen fertility was determined according to an accelerated procedure by acetoorcein staining [10]. Temporary preparations were prepared from a mixed sample of pollen. Fertile pollen grains were stained with acetoorsein burgundy, sterile remained colourless. At least 500 pollen grains were counted on each preparation. Measurements of morphometric parameters (length, width) of pollen grains were carried out on a Carl Zeiss Axio Lab A1 microscope, using the AxioVision program for image acquisition, processing and analysis. Pollen viability was determined by the method of D.A. Trankovsky [10] - pollen was sown on glass slides coated with a nutrient medium (1% agar-agar, 5% sucrose). The sucrose concentration was selected experimentally, after assessing the amount of sprouted pollen at concentrations of 1, 5, 10, 15, and 20%. Slides with pollen were placed in Petri dishes and germinated in an incubator at a temperature of 25 °C. Pollen grains were considered viable, forming a pollen tube of at least double the length of the pollen grain.

After maturation, fruit capsules were collected and the number of all normally developed seeds in the capsule and unfertilized ovules was collected and counted on an MSP-1 stereoscopic microscope. For ease of counting, the seeds were divided into small portions and poured into a thin line on a folded sheet of paper. Potential seed productivity was estimated as the total number of developed seeds and unfertilized ovules in the box, true seed productivity was estimated as the number of developed seeds, the seed rate was calculated as a percentage of real seed productivity from potential [11].

To determine the weight of the seeds, seeds were taken from 10 capsules, which showed the highest coefficient of seed productivity (75–85%) when counting. The seeds of each box were weighed on an electronic scale with an accuracy of 0.0001 g. Knowing the weight of the sample and the number of seeds developed, such indicators as the weight of 1000 seeds and the number of seeds per 1 g were calculated. Measurements of the morphometric parameters of seeds (length and width) were carried out on an MSP-1 microscope. The number of seeds in the sample was 100 pcs. Laboratory seed germination was determined according to GOST 24933.0-81 [12] with an extension of the germination period to 7 weeks. The change in terms is due to the fact that according to GOST 24933.0-81, the terms for determining the germination energy and germination of seeds Begonia semperflorens Link et Otto and Begonia × tube hybrid Voss, the seeds of which germinate much earlier than of B. ludwii, are regulated.

Statistical data processing was carried out using Microsoft Office Excel, the average values of the characteristic (x) and the error of the average value of the characteristic (s_x) were calculated.

3. Results and discussion

The seasonal rhythm of development of B. ludwigii in the greenhouse conditions is characterized by the presence of a dormancy period beginning in late January - early February and lasting about 3 months (figure 2). B .ludwigii, like many other species of the Begonia genus, is a monoecious with dioecious flowers on a common inflorescence. Floral bud development and inflorescence growth begins in late November - early December and lasts 1-2 weeks. The flowers in the inflorescence bloom basipetally. Flowering begins in early December and lasts 4-6 weeks (30-40 days). The phase of male flowering inflorescence precedes female and lasts 4-5 weeks. The female flowering phase of the inflorescence is approximately 2 times shorter than the male flowering phase and lasts about 2 weeks. The time-based application of the male flowering phase to the female lasts about a week, and in the last week of female flowering, a single flowering of male flowers is observed.

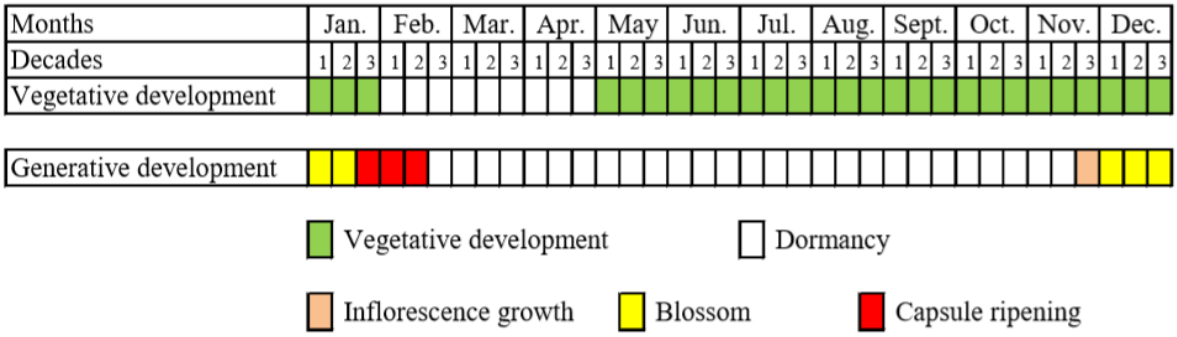

Figure 2. The full rhythm of seasonal development of Begonia ludwigii in the greenhouse conditions of SibBS TSU.

The duration of the blossom of the male flower is 5-7 days, female - 3-4 days. In total, 100-150 flowers are laid on the inflorescence, the ratio of the number of male flowers to female is close to 3: 1. About 10-15 % of male flowers in the inflorescence dry out and fall off at the bud stage. The percentage of fruiting (the percentage of female flowers tying the capsules) is 80–88% since up to 20% of female flowers fall at the bud stage.

The study of the male reproductive sphere showed that in the green house conditions of SibBS TSU B. ludwigii has a high fertility and pollen viability - 96.4 and 87.3%, respectively. The length of pollen grains is 18.3 ± 0.2 μm, and the width is 12.3 ± 0.1 μm (figure 3).

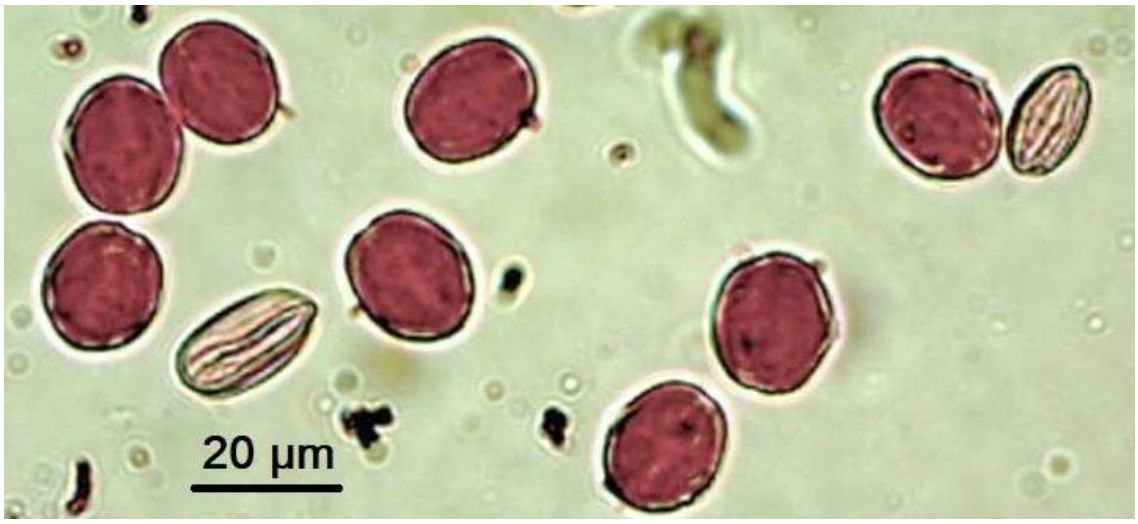

Figure 3. Fertile (burgundy) and sterile (colourless) pollen grains of Begonia ludwigii.

In 4–5 weeks after the corolla of the female flowers withers, the capsules fully ripen and remain on the inflorescence in a dry state. During maturation, the capsules droop, their proximal part becomes upward directed. Dissemination begins after the capsules completely dry and the nests are opened in the proximal part. The dropping of the capsules during ripening and opening them in the proximal part leads to the fact that the seeds do not immediately spill out and can remain in the box for 2-3 weeks until the peduncle completely falls.

B. ludwigi seeds are very small, oblong-oval, 398.4 ± 2,3 μm long and 252.1 ± 2.2 μm wide (figure 4. The hilum is round, located at the tip of the seed. The surface of the seed testa is unevenly cellular, glabrous, matte, dark brown. The weight of 1000 seeds is 0.0094 g, in 1 g about 110 000 seeds. Seed

germination begins 3-4 weeks after sowing. Laboratory germination of seeds is high - 92.8%. Twin seeds with a common testa were encountered singly, probably resulting from the abnormal development of integuments even at the stage of ovule formation.

Figure 4. Begonia ludwigii seeds.

B. ludwigii potential seed productivity is high - on average 2046.5 ± 92.9 ovules per one 3-nest capsule (74903 ± 3401 per inflorescence) (figure 5). However, true seed productivity is low - 544.1 ± 23.6 seeds per capsule (19915 ± 865 per inflorescence, seed rate - 26.6%).

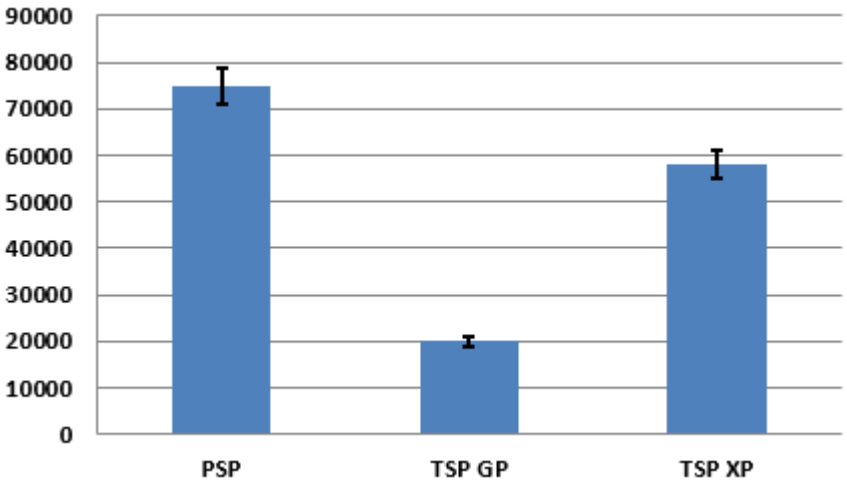

Figure 5. Seed productivity of Begonia ludwigii. PSP - potential seed productivity, TSP GP - true seed productivity of geytonogamous pollination, TSP XP - true seed productivity of xenogamous pollination.

Since there are no pollinating insects in the greenhouses, it seems that the seeds are formed to the geytonogamous pollination of female flowers by male flowers of the same inflorescence. This fact is supported by the fact that higher real seed productivity was found in begonia fruits, which bloomed in

the first week of the female phase of flowering inflorescence, which to a greater extent overlapped with the phase of male flowering. Significantly lower real seed productivity was observed in begonia fruits, which bloomed in 2 weeks when single blooming of male flowers was observed.

To increase seed productivity and seed quality, artificial xenogamous pollination was conducted, as a result of which true seed productivity increased almost 3 times - up to 1587.8 ± 75.8 seeds per capsule (58114 ± 2773 per inflorescence, coefficient of seed productivity - 77.6%).

4. Conclusion

The study of the reproductive biology of a rare species of Begonia ludwigii showed that this species has a high reproductive potential, but for its maximum realization in the green house conditions, it is necessary to carry out artificial xenogamous pollination. The seed productivity increase allowed the inclusion of B. ludwigii in delectus exchange with other botanical gardens of Russia and abroad, which will help to preserve this rare and endangered species ex situ. Currently, to maintain the ex situ genetic polymorphism of B. ludwigii, a collection of specimens of this species from different botanical gardens of the world is being created based on the SibBS TSU. Also, the seeds of B. ludwigii were transferred for storage to the Federal Seed Cryostorage of the Permafrost Institute named after P.I. Melnikov SB RAS (Yakutsk city).

Acknowledgments

This research was supported by the Ministry of Education and Science of the Russian Federation (state registration number of the theme: 37.7810.2017/8.9).

References

[1] Arnautova E M 2010 Rare and endangered plants of world flora in the greenhouses of the Botanical Garden of the Botanical Institute *Studies of the Tomsk State University. Ser. Biological: Botanical gardens. Introduction problems* **274** 62-3
[2] Muller M 2003 *Begonia ludwigii* Irmsch. Sacramento Branch, American Begonia Society URL: http://www.begonias.org
[3] Nature Conservation International Union. List of threatened species URL: www.IUCN.Redlist.org q1658 q0669
[4] Isayeva R Y, Kaspari V M and Yurchak L D 1984 Antifungal properties of some plants of the family begonia *Theses reports of the First Republican Conference on Medical Botany* (Kyiv:Ukraine) 168-9
[5] Cibula N V and Ferchalova T D 2000 *Phytoncidal plants in the interior: air healing with the help of plants* (Novosibirsk:Russia/KN) p 112
[6] Ferchalova T D 2008 Biological features of some types of *Begonia* genus (*Begonia* L.) in the greenhouse culture and interiors *Abstract of the thesis for the degree of Candidate of Biological Sciences* (Novosibirsk:Russia) p 16
[7] Ferchalova T D and Baikova E V 2008 The results of the introduction of the Begonia genus (Begoniaceae) in the Central Siberian Botanical Garden Plant World of Asian Russia 2 89-94
[8] Vainagi I V 1974 On the plants' seed productivity studying method *Botanical J.* **59(6)** 826-31
[9] Levina R E 1981 *Reproductive biology of seed plants* (Moscow:Russia/Nauka) p 96
[10] Barykina R P, Veselova T D, Devyatov A G, Dzhalilova H Kh, Ilyina G M and Chubatova N V 2004 *Handbook of Botanical Microtechnology. Basics and methods* (Moscow:Russia/Publishing house of the Moscow State University) p 312
[11] 1980 *Guidelines for the seed science of introducers*, ed. N.V. Qitsina (Moscow:Nauka) p 64
[12] GOST 24933.0-81. Seeds of flower crops. Acceptance procedure and methods of sampling. Specifications for determining the seeds sowing qualities