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Reproductive biology of *Strelitzia nicolai* and *Strelitzia reginae* in the conditions of a greenhouse.

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ABSTRACT

The performed study of the reproductive biology of *Strelitzia nicolai* and *S. reginae* has shown that in the conditions of a greenhouse, the flowering of inflorescences and the anthesis of flowers considerably increases. Both species feature high reproductive potential: in the flowers of *S. nicolai*, 30-40 ovules are formed, each anther produces about 60,000 pollen grains, the fertility of the pollen is 78%; in the flowers of *S. reginae*, 60-80 ovules are formed, each anther produces about 20,000 pollen grains, and the fertility of the pollen is 93%. The high reproductive potential of both species in the conditions of a greenhouse is not materialized due to high specialization of the flowers, which hinders self-pollination. Self-pollination occurs in 2% of *S. nicolai* flowers, and only one seed is formed in the fruit. In *S. reginae*, self-pollination occurs more frequently, in 7% of flowers, with only 5% of the ovules developing into proper seeds. It is assumed that flowers are randomly geitonogamously pollinated by the pollen from other flowers in the same inflorescence. Performing one-time artificial pollination of flowers increased seed production by both species. The paper also shows the data about germination of fresh seeds and dry-stored seeds after 12 months storage.

Keywords: *Strelitzia nicolai*, *Strelitzia reginae*, flowering phenology, reproductive biology, pollen fertility and germination, pollen-ovule ratio, seed productivity.

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INTRODUCTION

Nowadays, botanic gardens play an important role in preserving plants biodiversity *ex situ*. The Siberian Botanic Garden of the Tomsk State University was founded in 1880; it is the first botanic garden in the Asian part of Russia. During 135 years of its existence, the botanic garden has collected a unique gene pool of the world's flora – over 8,000 species and varieties of plants, with about 3,000 being tropical and subtropical plants grown in greenhouses (Astafurova et al., 2015). Each year, the botanic garden exchanges seeds with 150 botanic gardens in 40 countries around the world.

Plants cultivation in botanic gardens in the climatic conditions that differ from the natural habitat can significantly affect the seasonal development rhythm and the reproductive biology of plants. Particularly often, problems are observed in the reproductive sphere for the plants cultivated in greenhouses, where many factors (temperature, humidity, daylight duration, light intensity, lack of pollinators, etc.) may affect flowering, pollination and seed development. Seed production, being a component of the reproductive process, is one of important indicators of species viability in particular conditions (Vaynagiy, 1974). Maintaining collections of greenhouse plants often requires seed reproduction for obtaining a new seed progeny that is better adapted to the cultivation conditions, and for exchanging seeds with other botanic gardens.

The *Strelitziaceae* Hutch. family in the greenhouses of the Siberian Botanic Garden of the Tomsk State University is represented by four species: *Strelitzia alba* (L. f.) Skeels, *S. nicolai* Regel et K. Koch, *S. reginae* Banks, and *Ravenala madagascariensis* Sonn. The reproductive status was reached by two species: *S. nicolai* and *S. reginae*. The habitat of these plants is South Africa, where specialized pollination systems are widely used (Johnson et al., 2009). The species of the *Strelitzia* Aiton genus are characterized by the presence of ornithophily, i.e., the flowers are pollinated by birds. In the course of evolution, the plants pollinated by birds have developed a variety of mechanisms for interaction with pollinators (Cronk and Ojeda, 2008). For example, in the *Strelitzia* genus, part of flower petals form a special arrow-shaped body that carries androecium and gynoecium stigma, and plays the role of a perch for pollinating birds to sit on for drinking nectar (Scott-Elliot, 1980; Frost and Frost, 1981; Kronstedt and Walles, 1986; Coombs et al., 2007). The existence of such mutualism and the specific pollination mechanism causes problems with seed reproduction in cultivating species of the *Strelitzia* genus in the regions where natural pollinators are unavailable (Wang et al., 2001). In some regions though, other species of birds may become pollinators (Hoffmann et al., 2011). In the continental climate with negative temperatures in the winter, the species of the *Strelitzia* genus are cultivated in the conditions of a greenhouse, where difficulties with seed reproduction are observed as well.

This work is aimed at studying the seasonal development rhythm and reproductive biology of *S. nicolai* and *S. reginae* for increasing seed yield in the greenhouse conditions.

OBJECTS AND METHODS

S. nicolai has been cultivated for about 120 years in the greenhouse of the Siberian Botanic Garden of the Tomsk State University. The sample probably comes from the plant that was used for description in 1858 by Regel and Körniker (1858) at the Imperial Botanic Garden (Russia, St. Petersburg). *S. reginae* has been cultivated in the greenhouse for about 50 years; its seeds had been obtained in 1963 from the Botanic Garden of Jena (Germany). Currently both species grow in a subtropical greenhouse, where the temperature in the period between November and March is $+10\pm 2^{\circ}\text{C}$, between April and May and between September and October is $+18\pm 2^{\circ}\text{C}$, and in June and August - $+25\pm 3^{\circ}\text{C}$. The study has been performed for 3 years (2013 through 2015) on the plants that had reached their generative state.

Phenological monitoring was performed every day during flowering. The total of 100 flowers of *S. nicolai* and 60 flowers of *S. reginae* were observed. All flowers had been numbered with a marker, which allowed to trace duration flowering of inflorescences and anthesis of individual flowers, and to establish the time of fruit ripening. The duration of flowers anthesis was determined by functioning of the arrow-shaped body that consisted of blue petals of the inner perianth, and bearing the stamens and the style. The beginning of anthesis was the moment when blue petals emerged from the bract, and the end of anthesis was the beginning of blue petals' wilting and exudate drying on the style. The time of anthers burst and style receptivity was observed in more details on 20 flowers of each of the species. The style was considered receptive from the moment when exudation occurred, until it dried off. Artificial pollination was performed on

80 flowers of *S. nicolai* and on 50 flowers of *S. reginae*. Pollination of *S. nicolai* flowers was performed with the pollen from inflorescences of other stems of the same clone (geitonogamous pollination), since only 6 stems (ramets) of the same clone in the collection had reached their reproductive status. The flowers of *S. reginae* were pollinated with pollen from other plant specimens (xenogamous pollination). Artificial pollination was performed on the day when the style became receptive and covered with sticky exudate.

Pollen fertility was determined in a histochemical test by staining with acetoorcein (Barykina et al., 2004). Pollen viability was determined by germinating on glass slides covered with a nutrient medium (1% agar-agar, 20% sucrose). Sucrose concentration was chosen experimentally, after assessing the amount of germinated pollen at concentrations of 1, 5, 10, 15 and 20%. The slides with pollen were placed into Petri dishes, and germinated in an incubator at +25 °C. Viable were considered the pollen grains that formed a pollen tube with the length of at least 2 diameters of the pollen grain. The number of pollen grains in an anther was counted on a hemacytometer (Godini, 1981) according to the modified method (Yamburov et al., 2014), and since the pollen was large, the number of pollen grains was counted on the entire area of the hemacytometer mesh. The pollen-ovule ratio was calculated for 1 flower (Cruden, 1977).

In the fruit, the number of ovules and the number of developed seeds were counted, and their size and weight were measured. The germinative power was determined for fresh seeds and for the seeds that had been stored for 1 year in dry conditions at +23±2°C. In both variants of the experiment, 50 seeds of *S. nicolai* and 100 seeds of *S. reginae* were sown into pots with 3:1 mixture of peat and sand. Before sowing, the seeds were wounded with sandpaper, and soaked in water for 12 hours. The seeds were then germinated at +20±22°C for 5 months.

RESULTS

The seasonal development rhythm of *S. nicolai* and *S. reginae* in a greenhouse does not have a pronounced dormant period. The highest intensity of new leaves development was observed in the spring and in the summer, and the lowest - in the autumn, in the period between the end of fruit ripening and the beginning of stem growing. Specimens of *S. nicolai* can grow in the soil of a greenhouse and in pots, but potted plants do not bloom. Regular flowering occurred only on the stems that grow in the ground, and had reached the height of 6-10 m. The specimens of *S. reginae* were also grown in greenhouses in the ground and in pots. The specimens that grew in the ground were 4-5 times more powerful and bloomed more abundantly, as compared to the specimens grown in pots. The height of plants (leaves) was 1-1.5 m.



Figure 1. Flowering of *Strelitzia nicolai*

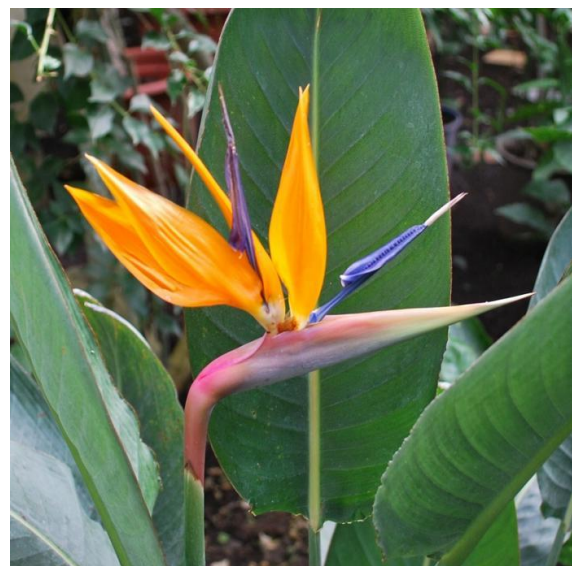


Figure 2. Flowering of *Strelitzia reginae*

Strelitzia Nicolai

Flowering started in February-March. Inflorescences on the stem developed acropetally. Flowering of each stem lasted for 4-5 months. During the year, 2-3 inflorescences were formed on the stems. An inflorescence had 3, rarely 4 bracts, where flowers developed. The total of 20-30 flowers per inflorescence were formed (Table 1). The greatest number of flowers (10-12) was formed in the lower bract, and in each following bract, the number of flowers was lower by 20-30%. The flowers in the 4th bract and the last flowers in the 3d bract often remained hidden in the bract, and did not burst.

Flowering of the inflorescence lasted for 60-90 days, and depended on the temperature in the greenhouse. The longest flowering of inflorescences was observed in February and March, and a shorter was observed for the inflorescences that started flowering in April and May. Local inflorescences on peduncle and flowers in local inflorescences developed acropetally. The flowers emerged from the bract one by one, with the interval of 3-5 days. The duration of flower anthesis, from the moment when blue petals exited the bract until they withered, lasted 5-6 days, while the white petals did not wither for up to 20 days. The blue petals with anthers and styles became almost horizontal within 2 days, and placed themselves at the angle of 10-20° to the bract. During this time, the style remained completely hidden in the bract. On the 3-4th day, the angle increased to 45°, and the style fully emerged from the bract. On the 5-6th day of anthesis, blue petals withered, and took vertical position (90° to the bract). The style became receptive, and was covered with sticky exudate on the 1st day after blue petals emergence from the bract. However, apparently to prevent self-pollination, the style remained hidden in the bract on the first day. Figure 1 shows that on day 2, the styles of young flowers emerged from the bracts only by half. The exudate dried up and the style lost receptivity after 4-5 days.

The anthers burst on the first day after blue petals emerged from the bract. Each anther produced about 60,000 pollen grains. Histochemical test showed that the pollen fertility was 78%. Determination of pollen viability by the method of sprouting on a nutrient substrate showed the highest results on the medium with 20% sucrose, where development of pollen tubes occurred in 50-60% of pollen grains. Exact calculation of viability was difficult, since the pollen was highly aggregated, i.e., bound by multicellular filaments, and, when sown, fell on the nutrient medium in large lumps, containing several dozens of pollen grains.

In the ovary of the flower, 30-40 ovules were formed. There were no pollinators in the greenhouse; however, about 2% of the flowers formed fruit from self-pollination. In case of self-pollination, only 1 seed developed in the fruit. In case of one-time artificial geitonogamous pollination, 43% of *S. nicolai* flowers formed fruit, however, seed productivity was low – only 12% of the ovules developed into proper seeds, and 3% of the ovules developed into underdeveloped seeds.

Fruit ripening lasted 3-4 months and usually ended in August. After ripening and drying, the fruit dehisced and opened by 1/3-1/2 of its length. Independent dissemination did not occur, since the seeds were tightly fixed in the fruit, and did not fall out even when the peduncle completely dried up and wilted.

The seeds were oval, about 10 mm long and 6 mm thick, with bright orange arillus fibers 7-9 mm long. The weight of 1 seed was about 300 mg, and 20% of the seed weight was arillus. Fresh seeds did not germinate for 5 months. After storing the seeds for 12 months, the germination rate was 14%. Seedlings appeared on the 7-8th week after sowing.

Strelitzia reginae

Flowering started in different years varied from December to February. Flowering of each specimen lasted 3-4 (sometimes 5) months, since inflorescences did not develop simultaneously, but within 2-3 months. An inflorescence formed 3-5 flowers, however, in 10% of cases, the last flower did not develop normally, and remained folded in the bract.

Flowering of the inflorescence lasted 50-70 days, and depended on the temperature in the greenhouse. The flowering duration of inflorescence was the longest in December-January, shorter was observed in inflorescences that bloomed in March-April. The flowers emerged from the bract one by one, with the interval of 5-7 (sometimes 14) days. The duration of flower anthesis, from the moment when blue petals

exited the bract until they withered, lasted 10-15 days, while the orange petals did not wither for up to 40 days. The blue petals with anthers and the style became almost horizontal within 2 days, and placed themselves at the angle of 10-20° to the bract. Then the angle increased to 45-50° on the 5-7th day, and on the 10-15th day of the anthesis, blue petals took vertical position (Figure 2). The style became receptive and covered with sticky exudate after 1-2 days after the emergence of blue petals from the bract. Drying of style exudate occurred 10-12 days later, after which blue petals started withering.

Table 1 – Reproductive features of *Strelitzia nicolai* and *Strelitzia reginae* in the conditions of a greenhouse.

Features	<i>Strelitzia nicolai</i>	<i>Strelitzia reginae</i>
The number of flowers per inflorescence	26.1 ± 7.4	3.7 ± 0.8
Percentage of flowers that form fruit by self-pollination	2.2	6.8
Percentage of flowers that form fruit by artificial pollination	42.5	46.2
The number of ovules in a flower	33.8 ± 6.5	69.0 ± 11.0
The number of seeds in the fruit in case of self-pollination	1	3.7 ± 0.6
The number of seeds in the fruit in case of artificial pollination	3.9 ± 1.1	48.9 ± 13.4
The number of pollen grains per anther	61,590.4 ± 11,180.6	19,645.0 ± 7,176.4
The pollen-ovule ratio	9,111.0 ± 1,607.3	1,458.1 ± 382.5
Pollen fertility, %	78	93
Seed length, mm	10.1 ± 0.6	-*
Seed thickness, mm	6.2 ± 0.5	6.1 ± 0.5
Seed weight with arillus, mg	306.1 ± 38.1	139.8 ± 20.4
Germination of fresh seeds, %	0	34
Germination of dry-stored seeds after 12 months of storage, %	14	26

Note: *- the seeds are round, the diameter was measured (thickness)

The anthers usually burst on the first day, but in 20% of cases they burst on the 2nd-3rd day, when the style was already covered with exudate, and was receptive. Each anther produced about 20,000 pollen grains. The histochemical test showed high (93%) pollen fertility. The pollen of *S. reginae* and *S. nicolai* grew best in the medium with 20% sucrose, however, viability of *S. reginae* pollen was higher, i.e., 80-90% of pollen grains formed pollen tubes.

In the ovary of the flower, 60-80 ovules were formed. Self-pollination occurred in 7% of flowers, where only 5% of ovules developed into proper seeds. In case of one-time artificial xenogamous pollination, 46% of flowers formed fruit, where 71% of ovules developed into proper seeds. Underdeveloped seeds resulted from 2% of ovules.

Fruit ripening lasted 5-7 months and usually ended in August or September. After ripening and drying, the fruit dehisced and opened by 1/2-2/3 of their length. Same as in the case with *S. nicolai* fruit, independent dissemination did not occur. The seeds were round, about 6 mm in diameter, with bright orange arillus fibers 3-4 mm long. The weight of 1 seed was about 150 mg, and 10% of the seed weight was arillus. The germinating power of fresh seeds was 34%. After storing the seeds for 12 months; the germination rate was 26%. In both variants of the experiment (fresh and dry-stored seeds), seedlings appeared on the 7-8th week after sowing.

DISCUSSION

Among the species of the *Strelitzia* genus, the seasonal development rhythm and reproductive biology in protected ground (greenhouses, hothouses) have been more studied for *S. reginae*, while *S. nicolai* remains less studied. This is due to higher popularity of *S. reginae*, which is used as an ornamental flowering plant, inflorescences of which are widely used in floristry because of their high decorative effect, long duration of flowering and good endurance of transportation. Also important is the duration of pre-regenerative development in case of growing plants from the seeds - with *S. reginae*, flowering starts after 4-6 years, and with *S. nicolai*, it starts much later. For example, in the Botanic Garden of the Kharkov University (Ukraine), flowering of *S. nicolai* started at 20 years of age (Alyokhin, 2013).

Earlier, researchers found that changing environmental conditions during transferring *S. reginae* to a different climate zone had resulted in increasing the duration of the main ontogenesis stages, and in inhibition of the natural reproduction function (Zhudrik, 2011; Zhudrik et al., 2013 and references therein). Studying seasonal development rhythm of *S. reginae* in various regions (Australia, Hawaii, California, South Africa) showed that the duration of leaf development and the flowering depended on the temperature: at temperatures below +13°C, development was slowed down, and at temperatures above +17°C – development was much faster (Halevy et al., 1987). Our study also showed that in greenhouses, flowering of inflorescences and anthesis of flowers both *S. nicolai* and *S. reginae* considerably increased. The longest flowering of inflorescence was observed between December and March, because in this period the daytime temperature in the greenhouse was +10 to 12°C. Shorter flowering was observed in inflorescences that start flowering in April and May, since the temperature in the greenhouse rose to +18 - 20°C. Besides, in the conditions of the greenhouse, the duration of style receptivity and development of the arrow-shaped body formed by blue petals and bearing androecium and gynoecium stigma increases. Development of the arrow-shaped body from the horizontal to the vertical position for *S. nicolai* takes 5 to 6 days, while for *S. reginae* it takes 10 to 15 days. No difference in the anthesis of pollinated and non-pollinated flowers has been found. In the habitat of *S. nicolai* in South Africa, development of the arrow-shaped body up to the vertical position occurs 2 times faster (Frost and Frost, 1981). Species of the *Strelitzia* genus have specialized mechanisms of flowers pollination by birds, which complicates self-pollination in the conditions of the greenhouse. Despite high pollen to ovules ratio, and good pollen quality, seed production in case of self-fertilization remains very low. This is because the anthers are covered with blue petals and in the natural environment they burst only when birds sit on the arrow-shaped body; however, in the greenhouses the fact that the anthers remain closed prevents occasional transfer of pollen to the style of the flower. Furthermore, for better adhesion to the legs of birds, pollen grains are aggregated with multicellular filaments (Kronstedt-Robards, 1996), which also prevents accidental transfer of pollen in greenhouses. There are 2 possible types of self-pollination of *S. nicolai* and *S. reginae* flowers in greenhouses without pollinators: autogamy or geitonogamy. In our opinion, geitonogamous self-pollination of a flower by the pollen from other flowers in the same inflorescence is more likely. When the arrow-shaped body takes vertical position and its blue petals start withering, a slit between the petals is formed, which exposes the anthers, and the pollen is accidentally transferred to a young flower with a receptive style through this slit.

The use of artificial pollination allows to significantly improve the reproductive ability of *S. nicolai* and *S. reginae* in greenhouse conditions. In our experiment, the percentage of *S. nicolai* flowers that formed fruit increased almost 20-fold, and percentage of *S. reginae* flowers increased almost 7-fold. The problem of finding the reason for low seed productivity of *S. nicolai* fruit remains unresolved, even after artificial pollination (only 12% of the ovules develop into seeds). This may be caused by the fact that we used geitonogamous pollination with the pollen of the flowers of the same clone, or pollination was not performed at the most optimal time. However, even this increased reproductive capacity has allowed to obtain a sufficient number of *S. nicolai* seeds for exchanging with other botanic gardens through *Index Seminum*. The time of pollination has great influence on the reproductive performance. Studies of Zhudrik E. V. (2013) and co-authors have shown that the optimal time for artificial pollination of *S. reginae* is 2nd or 3rd day of the anthesis, then 79% and 56% of the plant form fruit, respectively, while pollination at later dates significantly reduces the percentage of fruit formation. The same authors have established dependence of seed productivity of a fruit on the number of pollinated flowers in an inflorescence: with increasing number of pollinated flowers of *S. reginae*, the number and the average weight of seeds in the fruit decreases. The time of seeds ripening and the number of undeveloped seeds increase simultaneously (Zhudrik et al., 2013).

The pollen-ovule ratio in the flower may be used as a conservative indicator of the reproductive system (Cruden, 1977). There is a positive linear relationship between the seed size and the pollen to ovules ratio, which has been defined for many groups of flowering plants (Cruden and Miller-Ward, 1981; Götzenberger et al., 2006). Our study confirms this hypothesis: compared to the seeds of *S. reginae*, seeds of *S. nicolai* are twice larger, and the pollen to ovules ratio is 6 times higher.

The seeds of species of the *Strelitzia* genus are dispersed in the nature by birds attracted by the bright orange nutritional arillus. The orange color of arillus is ensured by the bilirubin animal pigment (Pirone et al., 2009, 2010). The seeds of *S. nicolai* and *S. reginae* have a dense impervious shell that prevents, if undamaged, germination of fresh seeds; they retain high germination capability when stored in dry conditions during the year (Deno, 1996, 1998). In our study, fresh seeds of *S. nicolai* did not germinate during the 5-month

observation period even after wounding with sandpaper and preliminary soaking in the water; they germinated after dry storage, however, germination was low. On the contrary, fresh seeds of *S. reginae* germinate within 1-2 months, but germination rate is not too high, and decreases slightly after dry storage.

CONCLUSION

The study has shown that in greenhouses *S. nicolai* and *S. reginae* feature prolonged flowering of inflorescences and anthesis of flowers, and increased receptivity of styles. Both species have high reproductive potential, which is not materialized in the conditions of a greenhouse, due to flowers specialization to being pollinated by birds, which prevents self-pollination. For increasing seed productivity and the quality of seeds, xenogamous artificial pollination of flowers is required. Further research is needed for finding the reasons of poor reproductive capacity of *S. nicolai* in the conditions of a greenhouse.

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