Self-pollination and Pollen Germination in Japanese Morning Glories (*Ipomoea nil*)Daiichiro Miyajima¹, Ikumi Karito² and Rieko Fujisawa²¹ Faculty of Agriculture, Miyazaki University, Miyazaki 889–2192² Faculty of Agriculture, Shinshu University, Kamiinagun, Nagano 399–4511

Summary

Self-pollination and pollen germination in Japanese morning glories (*Ipomoea nil* Roth.) were investigated. Within the flower buds, anthers were situated under stigmas; 90% of these anthers became situated over stigmas as the filaments elongated during anthesis. The flowers were thus selfed with approximately 40 pollen grains adhering to the stigmatic surface; 17% of pollen germinated. The pollen to ovule ratio was 180 and there were no significant differences in seed set between flowers which were hand-pollinated with an abundance of pollen grains and those which were autonomously selfed in bags. Thus we consider the Japanese morning glories to have evolved to produce seeds primarily by selfing.

Key Words: autonomous selfing, *Ipomoea nil*, pollen germination, seed production.

Introduction

Japanese morning glory (JMG) plants produce seeds over a long period following a successive opening of new flowers. Therefore, to harvest seeds of JMG requires close attention and much hand labor which lead to their high prices. To improve seed production in JMG, the seed producing mechanisms, need to be elucidated. Hagiwara (1930) concluded that seeds of JMG are produced primarily by selfing, based on the outcrossing rates and an autonomous self-pollination mechanism in this species. In this study, self pollination and pollen germination on stigmatic surfaces in JMG were investigated.

Materials and Methods

This study was done at the farm of the Faculty of Agriculture of Shinshu University, Nagano Prefecture, Japan, in 1998. On May 16, seeds of 'Akatsukinomurasaki' (Sakata Seed Corp., Kanagawa), which were raised from *retracted*, *dragonfly*, and *Globose* strains, were directly seeded in a plastic film greenhouse in rows 1.5 m apart; the distance between future seedlings was adjusted to 1.0 m by thinning seedlings after their emergence. Two liters per plant of 14N–2.2P–5.8K liquid fertilizer (5g·liter⁻¹) were applied to plants weekly. Seedlings were allowed to climb freely on 2 meter poles; no lateral shoots were removed.

Distance between stigmas and anthers, and pollen load on stigmas at flowering

Distances between the centers of stigmas and those of anthers were measured at 17:00 on the day before

anthesis and at 10:00 at anthesis. Five flowers from 5 separate plants were sampled for measurement on each sampling day. The pollen grains adhering to the stigmatic surfaces of two flowers from each of five plants were counted using a binocular microscope at 10:00 at anthesis.

Pollen to ovule ratio and pollen germination on stigmatic surface

The number of pollen grains per flower was counted, using a binocular microscope immediately after the anthers dehisced. Pollen to ovule ratio was determined. Germination of pollen grains on stigmatic surfaces was determined as follows: petals and stamens were removed from buds ready to flower within days, covered with paper bags (23.5 × 25 cm). At 07:00 on the day of flowering, 10 bagged flowers were cut at the bases of peduncles. Flowers were placed in a plastic case with their cut ends placed in distilled water; 10 pollen grains from another flower on the same plant were placed on the summits of cauliflower-shaped stigmas at a distance more than 5 times their diameters using a binocular microscope. Four hours after pollination, 10 flowers were immersed in FAA. Three days later, the pollen grains remaining on the stigmas were counted. If the number of pollen grains on a stigma was under 10, pollen grains were considered to have separated on account of FAA. A block of the stigmatic surface loaded with a pollen grain was excised, using a pair of tweezers with sharp edges, placed on their slides, and stained with a drop of acetocarmine. The block was then softly squashed under a cover slip and the status of the pollen grains assessed under a microscope (Nikon, Optiphot2; × 100). A pollen grain was considered to have germinated when the length of the pollen tube was equal to or

longer than the diameter of the grain.

Breeding system

In August, 20 flower buds which were ready to flower within a few days were covered with paper bags, whereas 20 were emasculated before bagging. Flowers with and without bagging were treated as follows : 1) hand-outcrossed; 2) hand-selfed; 3) open-pollinated; and 4) covered with bags throughout flowering period. An equal number of flowers to be open-pollinated were not bagged. Emasculated flowers were outcrossed or selfed by hand in the early morning at anthesis. The number of mature seeds in each fruit was counted 50 days after treatment; the number of seeds per fruit and percentage seed set were calculated. The data were analysed by analysis of variance.

Results and Discussion

Distance between stigmas and anthers, and pollen load on stigmas at flowering

The number of stamens in a flower was 5 to 7; 5 was the dominant. Stamens and pistils were visible in flower buds in the afternoon, a day before flowering. All anthers were situated below stigmas at this stage (Fig. 1). Flowering of JMG is controlled by an circadian rhythm that responds to light and temperature; it usually begins at midnight (Kaiharu and Takimoto, 1979, 1980, 1981). Stamens elongated as the flowering progressed; some anthers became situated over the stigmas. Unlike

Fig. 2. Photomicrograph of a germinating and a non-germinating pollen grains on a stigma. A white arrow indicates a pollen tube.

Fig. 1. Distances between the centers of stigmas and those of anthers in flowers of Japanese morning glory 'Akatsukinomurasaki' at 17:00 on the previous day and at 10:00 on the day of opening of flowers. 'A' and 'S' denote the length of anthers and the height of stigmas, respectively.

the results of Hagiwara (1930), some anthers remained below the stigmas. At anthesis, 89% of the anthers were superior to the stigmas at 10:00. Hagiwara (1930) reported that flowers of JMG could produce seeds in flower buds by autonomous selfing at 21:00 on the day before opening. In our experiment, an identical autonomous self-pollination mechanism occurred, whereby the stigmatic surfaces of bagged flowers had pollen grains on them at 10:00 at full bloom. The number of pollen grains per stigma was 40 ± 12 . Thus, self-pollination occurred autonomously, independent of pollinators.

Pollen to ovule ratio and pollen germination on stigmatic surface

The number of ovules in a flower ranged from 6 to 8. The number of pollen grains per flower was 1290 ± 170 , and the pollen to ovule ratio was approximately 180. According to Cruden (1977), this plant species is classified as facultative autogamy.

A pollen tube from a germinating pollen grain was clearly observed in a crushed block of a stigma (Fig. 2); 17% of the pollen germinated on stigmatic surfaces. Therefore, the pollen load on the stigmatic surface must be necessarily high for seed set in JMG that had several ovules in each flower.

Breeding system

There were no significant differences in seed production among treatments. The number of seeds per fruit and percentage of seed among all treatments were 3.5

and 52%, respectively. Because there were no significant differences in seed production between selfed flowers in bags and hand-pollinated ones with a large number of pollen grains, pollen load by selfing and low germination of pollen were not the primary causes for low seed production in Japanese morning glories. Hagiwara (1930) determined that the degree of natural crossing of JMG is under 5%. Because there was no significant difference in seed production among selfed, outcrossed and bagged flowers, JMG are facultatively autogamous plants.

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アサガオ (*Ipomoea nil*) における自家受粉と花粉発芽

宮島大一郎¹・狩戸郁美²・藤澤利枝子²

¹ 宮崎大学農学部 889-2192 宮崎市学園木花台西

² 信州大学農学部 399-4511 上伊那郡南箕輪村

摘 要

アサガオ (*Ipomoea nil* Roth.) '暁の紫' を用いて自家受粉機構と柱頭上での花粉発芽について調べた。つぼみ内でやくは柱頭より下位に位置していたが、そのうち 90% のものは開花後柱頭の上位に位置した。アサガオは開花の過程で自動的に自家受粉を行い、約 40 粒の花粉が柱頭に付着した。柱頭上での花粉発芽率は 17% であった。花粉胚珠比は 180 で、十分量の花粉での人工授粉と袋かけ状態での自発的な自家受粉に任せた花の間に種子稔実の有意な差は見られなかったことから、アサガオは主に自殖により種子を生産していると考えられた。