



## Effects of silicon on seed setting rate of rice intersubspecific hybrids

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**ABSTRACT.** The present study found semi-sterility in rice intersubspecific hybrids of ‘Taichung 65’ x ‘Guangluai 4’ and ‘Ludao’ x ‘Qiuguang’. Embryo sac fertility was evaluated using the overall staining transparent method. The results showed that the embryo sac contained a normal egg cell, normal synergid cells, polar nuclei cells, and antipodal cells, indicating that semi-sterility was caused mainly by pollen semi-sterility. In the pot experiment, the effects of silicon on the seed-setting rate of the two intersubspecific hybrids were examined. The results showed that the rate of anther dehiscence, number of pollen per stigma of F1 plants, potential of pollen grain germination, and fertility of the spikelet were significantly improved by the utilization of silicon fertilizer.

**Key words:** Embryo sac fertility; Intersubspecific hybrid; Silicon; Seed setting rate; Semi-sterility

## INTRODUCTION

The F1 hybrid between indica and japonica varieties of rice shows strong heterosis, but the use of heterosis is limited by its ubiquitous semi-sterility. Hybrid semi-sterility can be manifested as female infertility, male sterility, anther-unslit, and dichogamy (Liu et al., 1997).

In hybrids with male semi-infertility and normal pistils, pollen fertility is approximately 50%. Theoretically, a small amount of pollen is required for fertilization, but the spikelet is semi-infertile. Kitamura (1962) first reported that anther-unslit decreased the seed-setting rate of F1 hybrid rice. Ekanayake et al. (1990) found that at least 20 pollens per stigma are required to ensure normal fertilization; otherwise, the flowers will be infertile. In addition, rice is a typical siliconphile plant. Previous experiments showed that the plant had the greatest demand for silicon during the reproductive growth phase, and the number of fertile florets after the utilization of silicon fertilizer during the floret-phase was 1.5-fold higher than that in the control group (Ma et al., 1989). A large number of field experiments demonstrated that application of silicon fertilizer could increase rice yield by 10-26% (Epstein, 1999; Shang et al., 2009). However, whether the application of silicon can improve the seed-setting rate of F1 between rice subspecies has not been reported previously. In this study, we examined the impact of silicon on pollen dehiscence, pollen viability, and seed-setting rate of spikelets in an intersubspecific F1 hybrid to provide a theoretical basis for the silicon-enhancement effect on the seed-setting rates of intersubspecific F1.

## MATERIAL AND METHODS

### Plant material and process

Japonica ('Taichung 65' and 'Guangluai 4') and indica ('Ludao' and 'Qiuguang') varieties of rice were used in this study. The hybrids used were the F1 generation of 'Taichung 65' x 'Guangluai 4' and 'Ludao' x 'Qiuguang'.

In rice growth masasue, seedlings were washed with quartz sand at 30°C for 14 days before being transplanted in a 2-L porcelain culture tank. For both the control group (without silicon) and silicon group, a porcelain culture tank was lined with plastic wrap to prevent the container from interacting with the silicon in the nutrient solutions. The nutrient solution was prepared based on the formulation of the International Rice Institute (Yoshida et al., 1976). Silicon was added in the form of silicic acid at a concentration of 1.5 mM. Silicic acid was exchanged from sodium styrene by using a hyperacidity cinnamic cationic switching resin (Shanghai Chemical Reagent Company of China Pharmaceutical Group, Shanghai, China). A month after transplantation, the rice was treated with silicon or remained untreated, and the plants began heading during the 4th month.

### Methods

The pollen fertility survey was conducted as described by Li et al. (2007). Soon before plant blossoming, 3 glumous flowers were removed from the main spike of each plant and placed in a solution of ethanol:acetic acid (3:1), which was stored at 4°C. The samples were stained with 1% I<sub>2</sub>-KI and observed under a 10X (10-fold) microscope, and the fertility was determined based on pollen form and color. For the spikelet fertility survey, at the mature stage, 3 spikes per plant were collected to survey spike fertility, and the average value was calculated as spikelet fertility per plant.

Embryo sac fertility was examined using the Ehrlich hematoxylin overall staining-transparent method, and samples of flowering F1 of 'Taichung 65' x 'Guangluai 4' and 'Ludao' x 'Qiuguang' were selected. Samples were collected from a mature embryo sac. A total of 300 embryo sacs from different parts of different spikelets were randomly selected and fixed in the formalin-acetic acid-alcohol fixative for 24 h. After rehydration, the samples were stained by Ehrlich hematoxylin overall staining with love's hematoxylin stain for 20-40 min, and then washed with distilled water for 2 to 3 times (24 h), followed by washing with water 3 to 4 times (1 to 2 days) until the color of materials changed from purple to gray-blue. The samples were dehydrated with ethanol before treatment with methyl salicylate to make the tissues transparent. After 24 h, the samples were observed under the Olympus BH-2 microscope (Olympus, Tokyo, Japan).

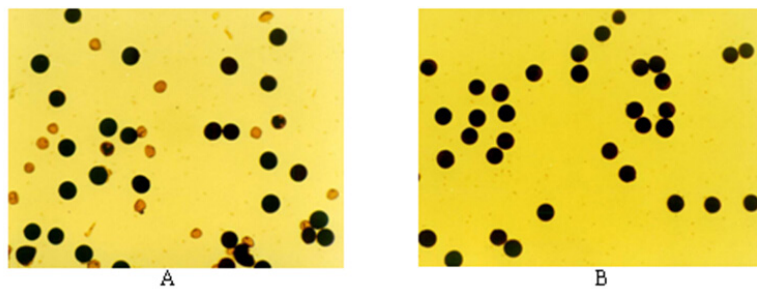
To measure pollen on the stigma, 10 opened flowers were collected and fixed in formalin-acetic acid-alcohol fixative, and then stained with aniline blue. The number of pollens on the stigma was observed by fluorescence microscopy (Matsui et al., 2000).

Pollen germination *in vitro* was performed using the method described by Wang et al. (2000), with some modifications. Pollen germination in the control and treated groups *in vitro* was observed using liquid medium. Pollen germination was observed under a microscope, with pollen tube length greater than or equal to half the diameter of pollen was defined as germination. The experiment was repeated 5 times, examining at least 500 pollens each time.

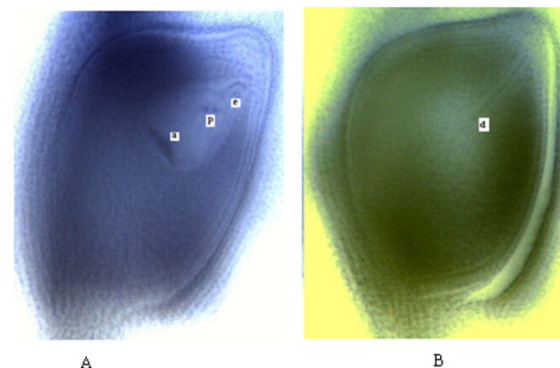
## RESULTS

### Fertility observation

We examined pollen fertility, spikelet fertility, and embryo sac fertility of the parents ('Taichung 65', 'Guangluai 4', 'Ludao', and 'Qiuguang') and F1 hybrids ('Taichung 65' x 'Guangluai 4' and 'Ludao' x 'Qiuguang') in the control group (without silicon treatment). The fertility of the 4 parents was normal (Figure 1B); pollen and spikelet fertility of 'Taichung 65' x 'Guangluai 4' were 40.1 and 25.8% respectively, and that of 'Ludao' x 'Qiuguang' were 50.3 (Figure 1A) and 40.3%, respectively. The embryo sacs of the 2 F1 hybrids were observed. The normal embryo sac contained 1 egg apparatus (including 1 egg cell and 2 synergid cells), 1 central cell consisting of 2 nuclei (polar nuclei), and antipodal cells (Figure 2A), while the abortive embryo sac only had traces of cell degeneration (Figure 2B) in the normal embryo sac location. A total of 150 embryo sacs were observed in the 2 hybrids. The rates of embryo sac abortion were 5.33% in 'Taichung 65' x 'Guangluai 4' (8) and 3.33% 'Ludao' x 'Qiuguang' (5). Therefore, the semi-sterility of intersubspecific F1 hybrids was caused by male semi-sterility.



**Figure 1.** Pollen grain fertility. **A.** Semi-sterility pollen. **B.** Fertility pollen.



**Figure 2.** Embryo sac observation using whole stain-clearing technique. **A.** Fertile embryo sac of Nipponbare with visible antipodal cells (a), polar nucleus (p), and egg organ (e). **B.** Sterile embryo sac with degenerated cells (d).

### Effects of silicon on the number of pollen on the stigma

Rice seeding rate depends on the fertilization rate, while normal fertilization primarily depends on the number of pollen grains on the stigma. A low number of pollen grains cannot properly fertilize and seed, and pollen germination on the stigma can also affect the setting rate of the spikelet. Pollen fertility and spikelet fertility of the 4 parents were normal, but values for the F1 hybrids were abnormal between indica and japonica rice. Thus, only the F1 hybrids were studied.

After flowering for 1 h, the number of pollens scattered on the stigma and the number of pollens germinated in the 2 F1 hybrids were observed under natural conditions. Two hundred florets of the 2 F1 hybrids of the silicon group and control group were observed under a fluorescence microscope. Statistical analysis showed that the stigma of the 2 F1 hybrids in the silicon group was similar and could normally accept pollen, with a larger number of pollen grains on the stigma. About 90% of florets had a pollen number greater than 25 per stigma (Figure 3A), with at least 16 pollen per stigma, while the control group showed a lower number of pollen on the stigma. Approximately 8% of the florets had more than 20 pollen per stigma, whereas 75% florets had lesser than 20 pollen per stigma (Figure 3B), and 17% of florets did not have any pollen on the stigma (Figure 3C).

Figure 3 shows a higher number of pollens germinated on the stigma of F1 hybrids, and there was a significant difference in the number of pollens germinating in the control group, with no germination observed in most florets.



**Figure 3.** Pollen number and pollen germination on stigma. Stigma of plant with application of silicon. **A.** About 90% of the plants with pollen number greater than 25 per stigma. **B.** Stigma of plant with non-application of silicon. **C.** Approximately 17% of the plants with no pollen on the stigma.



### Effect of silicon on the number, viability, and seed-setting rate of pollen

*In vitro* germination culture was conducted using F1 pollen with and without silicon treatment for the 2 intersubspecies hybrids. The *in vitro* germination rate of pollen after silicon processing was  $75.15 \pm 1.28\%$  and  $76.23 \pm 1.34\%$  (Figure 4B), in treatment and non-treatment group, respectively, whereas the *in vitro* germination rate without silicon processing was  $46.7 \pm 1.5\%$  and  $48.13 \pm 1.49\%$ , in treatment and non-treatment group respectively (Figure 4A). A significant difference was observed between the 2 groups. Furthermore, pollen germination *in vitro* showed a more obvious group effect. Plants with a larger number of pollen grains germinated more easily. After silicon treatment of F1 of 'Taichung 65' x 'Guangluai 4' and 'Ludao' x 'Qiuguang', the seed-setting rates were  $65.5 \pm 1.2\%$  and  $68.7 \pm 1.5\%$ , respectively. Thus, our findings demonstrated that the rate of anther dehiscence, the number of pollen per stigma of F1 plants, the potential of pollen grain germination, and the fertility of spikelets could be significantly improved by application of a silicon fertilizer.

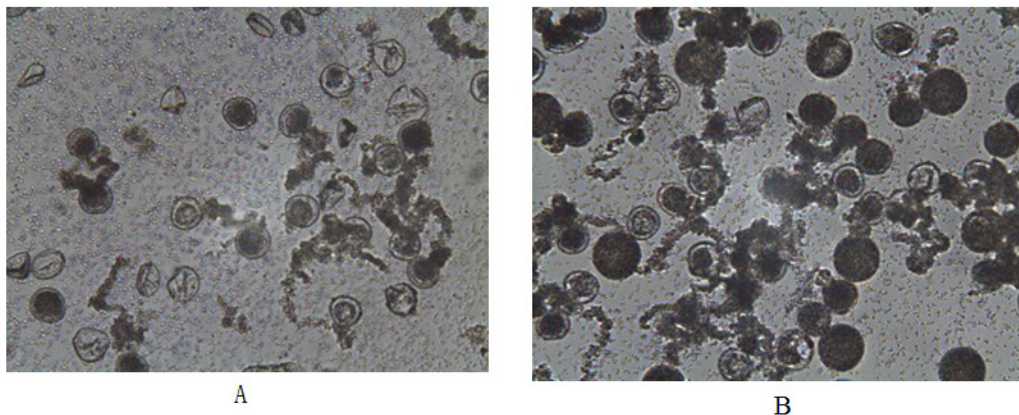


Figure 4. *In vitro* germination of rice pollen.

### DISCUSSION

Lu rice is a unique rice germplasm with the weed habit in the downstream areas of the Yangtze River. It is mainly located in the Lianyungang, Jiangsu Province ( $34^{\circ}33'$  to  $34^{\circ}46'N$ ,  $119^{\circ}13'$  to  $119^{\circ}30'E$ ). Its taxonomic status in the genus *Oryza* is controversial. In this study, by using Hybrids subspecies and Lu rice with subspecies japonica hybrids, these two types of sub-species hybrids were studied. We found that semi-sterility existed in both subspecies. The largest limitation to the effective use of subspecies resources are the semi-sterility in subspecies between hybrids. However, we also found that the fertility sac of the hybrids between the 2 subspecies hybrids was normal, with the male showing half-infertility. Theoretically, even if this type of sub-species hybrid accounted for only 10% of normal pollen and the stigma of all plants received sufficient amounts of pollen, fertilization could occur. The present study further analyzed these 2 types of subspecies hybrids.

Paddy rice is a typical siliconophile, with silica content *in vivo* up to 10 to 20%, which is 10-fold higher than that of nitrogen and 20-fold, that of phosphorus. Silicon fertilizer shows

positive effects on the growth and development of the crop, and the effect is more significant in gramineous crops. Numerous studies have indicated that after rational application of silicon fertilizer to rice, yield and resistance to lodging are very significantly increased (Ma et al., 1989; Epstein, 1999; Shang et al., 2009). Studies have shown that at high temperatures stress, silicon can promote the dehiscence rate of rice anther and the pollination of stigma (Li et al., 2005). Therefore, we examined whether increasing silicon would increase the seed-setting rate of the subspecies. The results showed that increased silicon not only promoted cracking of the rice anther but also increased the number of pollen on the stigma. Additionally, silicon increased the pollen germination rate, therefore improving the seed-setting rate of subspecies hybrids. Silicon has an important impact on the mechanical strength of the cell wall. Thus, we predict that the addition of silicon is likely to affect the mechanical strength and the thickness of the anther wall, affecting cracking of the anther and increase in the number of pollen grains on the stigma. The fertility sac of the hybrids between the 2 subspecies hybrids was normal, increasing the rate of seed setting.

### Conflicts of interest

The authors declare no conflict of interest.

### ACKNOWLEDGMENTS

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