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Increased percentage of fruit set of F_1 hybrid of *Capsicum chinense* during high-temperature period

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ABSTRACT

Low percentage of fruit set in fruit vegetables cultivated in greenhouses, such as tomato (*Solanum lycopersicum*), eggplant (*S. melongena*), and capsicum (*Capsicum*), in high-temperature seasons has been reported as one of the biggest problems in commercial vegetable production. In this study, *C. chinense* 'Sy-2' and 'No. 3686' were cultivated in greenhouses for a year. Remarkably low percentages of fruit set (3.2% and 0%, respectively) were obtained in the high-temperature season (40 °C/25 °C: day/night). However, the percentage of fruit set was recovered with the decrease in temperature in autumn and winter (30 °C/20 °C). On the other hand, in F_1 ('Sy-2' × 'No. 3686'), the percentage of fruit set was 40.8% even in the high-temperature season. On *in vitro* pollen germination test, the pollen of F_1 was found to have a higher germination rate than that of 'Sy-2' or 'No. 3686' during the high-temperature season. After 'Sy-2' was cross-pollinated with 'Sy-2' and 'No. 3686' pollen, percentages of fruit set were 23.3% and 0%, respectively. It is noteworthy that the cross-pollination of 'Sy-2' with F_1 pollen resulted in a considerably higher percentage of fruit set (73.3%). When F_1 was self-pollinated, the percentage of fruit set (56.7%) was higher than that after when cross-pollinated with 'Sy-2' or 'No. 3686' pollen. Interestingly, no fruit set was obtained when 'No. 3686' was cross-pollinated with 'Sy-2', 'No. 3686', or F_1 pollen. The results for 'No. 3686' indicated that not only does the male factor affect pollen germination ability but also unknown female factors are involved in reducing the percentage of fruit set of *C. chinense* under the high-temperature season. A significant increase in the percentage of fruit set in the high-temperature season was also observed in the reciprocal cross of F_1, 'No. 3686' × 'Sy-2'. Furthermore, 10 plants of ('No. 3686' × 'Sy-2') × 'Sy-2' and 31 plants of 'Sy-2' × ('No. 3686' × 'Sy-2') were cultivated in a greenhouse, but no difference was found in the percentage of fruit set between these two combinations. Therefore, our findings suggest that cytoplasmic factor(s) does not influence the percentage of fruit set.

1. Introduction

In the greenhouse cultivation of fruit vegetables, reduction in the percentage of fruit set because of high temperatures in summer is a major problem. Although reduction in the percentage of fruit set at high temperatures can be alleviated using high-temperature-resistant cultivars (Abdul-Baki, 1991; Abdul-Baki and Stommel, 1995), yield loss in the high-temperature season depends on the influence of a range of various factors, leading to the difficulties in solving this problem (Driedonks et al., 2016; Gruda, 2005).

Hormonal treatments are used to enhance the stability of fruit set in fruit vegetables. For example, Kaur et al. (2017) showed that indole acetic acid treatment improved the fruit-set ability of *Capsicum annuum* even in the high-temperature period. However, the external treatment to promote fruit setting is often a laborious and costly burden to farmers, and in some cases, even the application of external treatment to promote fruit setting is not able to overcome the negative effects of environmental conditions and improve the stability of fruit set (Abad and Monteiro, 1989; Martín-Closas et al., 2009). Parthenocarpic cultivars have also been used in tomato (*Solanum lycopersicum*) (Hawthorn, 1937), eggplant (*S. melongena*) (Acciarri et al., 2002), and cucumber (*Cucumis sativus*) (Pike and Peterson, 1969; De Ponti, 1976) to induce stable fruit set during the high-temperature season. However, much time is required to explore and breed parthenocarpic mutants. To the best of our knowledge, no report has been published on the available genetic resources for the introduction of parthenocarpy to commercial cultivars in hot pepper, sweet pepper, and pimento, which all belong to *Capsicum* spp. There are some cultivars which have a parthenocarpic

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fruit set ability, but are inadequate fruit enlargement (Honda et al., 2012). Therefore, the issue with the low percentage of fruit set cause by high-temperature stress in fruit vegetables is still not completely solved.

Long-term, extensive research on low percentage of fruit set due to high-temperature stress has been conducted in tomato, one of the most popular fruit vegetables worldwide. Studies have reported that the inhibition of the development of the tomato anther and pollen under high-temperature stress was one of the causes of the low percentage of fruit set (Levy et al., 1978; Peet et al., 1998; Xu et al., 2017b). Sato et al. (2000) concluded that the decline in the pollen germination ability and pollen release ability causes low percentage of fruit set of tomato under high-temperature conditions.

Capsicum is a fruit vegetable widely used all over the world and belongs to Solanaceae. The five-domesticated species of this genus are *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, and *C. pubescens*. *C. chinense* has been reported to poor fruit-set ability under high-temperature conditions (Garruña-Hernández et al., 2012). In our examination, *C. chinense* 'Sy-2' and 'No. 3686' (both are inbred cultivars), cultivated in a greenhouse at high temperatures, did not set fruit in the high-temperature season without the application of external treatments that stimulated fruit setting. However, the F_1 hybrid of these two cultivars successfully set many fruits even under high-temperature conditions. Therefore, this F_1 hybrid of *C. chinense* can overcome low percentage of fruit set under high-temperature conditions. To further elucidate the causes of low percentage of fruit set in *C. chinense* and attempt to improve its reproductive abilities in the high-temperature season, we conducted self- or cross-pollination experiments using three plants: 'Sy-2', 'No. 3686', and F_1.

2. Materials and methods

2.1. Plant materials

For all experiments, *C. chinense* plants were grown at the agricultural farm of Kyoto University (Kyoto City, Japan) in 2016 and 2017. In 2016, two plants of *C. chinense* 'Sy-2' (inbred cultivar), three plants of 'No. 3686' (inbred cultivar), six plants of their hybrid 'Sy-2' × 'No. 3686' (F_1), 10 plants of ('No. 3686' × 'Sy-2') × 'Sy-2', and 31 plants of 'Sy-2' × ('No. 3686' × 'Sy-2') were used for experiment. The seeds were sown in commercial soil (Kumiai nippi, Nihon Hiryo Co. Ltd., Tokyo, Japan) and placed in an incubator maintained at a constant temperature 25 °C. Seedlings were transplanted into No. 8 plastic pots (diameter 240 mm, height 210 mm, and capacity 4 L) and placed in a plastic house (made of polycarbonate). Mixed soil composed of Akadama soil, leaf mold, peat moss, and vermiculite at an approximate ratio of 4:4:1:1 (v/v/v/v) was placed in the plastic pots. A volume of 50 mL of slow-release fertilizer, Long 413-270 (NPK = 14-11-13; JCAM AGRI. CO., LTD., Tokyo, Japan) was applied when the experimental plants were transplanted to the No. 8 plastic pots. HYPONeX (NPK = 6-10-5; HYPONEX JAPAN CORP., LTD., Osaka, Japan) was diluted to 1/500 and applied about once a week. During the cultivation period, external treatments to promote fruit setting, such as vibration and hormonal treatment, were not carried out. The plastic house was covered with a net (mesh size 0.25 mm) to prevent invasion of insect pollinators. The temperature inside the plastic house during the cultivation period was recorded using a data logger (TR-52S; T&D Corporation, Nagano, Japan).

In June 2016, a plant of 'Sy-2', a plant of 'No. 3686', and two plants of 'No. 3686' × 'Sy-2', which was a reciprocal cross of F_1, were cultivated in a phytotron within a temperature range of 35 °C/25 °C (max./min.). No insect pollinators were allowed in the phytotron.

In 2017, three plants of 'Sy-2', three plants of 'No. 3686', and five plants of F_1 were used for the experiment. These plants were grown in the same plastic house using the same soil and fertilizers as in 2016. During the growth period, external treatments to promote fruit setting, such as vibration pollination and hormonal agent treatments, were not performed. The temperature inside the house during the growth period was recorded using a data logger (TR-72wf-H; T&D Corporation).

2.2. Measurements of the percentage of fruit set

In 2016, before flowering, the flower buds of 'Sy-2', 'No. 3686', and F_1 were marked using a marker pen. Two weeks later, fruit set or the number of dropped flowers were counted, and the percentages of fruit set of total flowers (fruits and dropped flowers) were calculated. Every week, 10 flowers of each of the two inbred cultivars and F_1 were investigated. The experimental period continued from July 1 to September 28, which is in the high-temperature season in Japan. The percentages of fruit set of 'Sy-2', 'No. 3686', and 'No. 3686' × 'Sy-2' were also investigated in the phytotron in June. In addition, fruit set of each of the 10 plants of ('No. 3686' × 'Sy-2') × 'Sy-2' and each of the 31 plants of 'Sy-2' × ('No. 3686' × 'Sy-2') were counted twice, from June to July and from August to September.

In 2017, the number of flowers that were opened from August 1 to August 31 [high-temperature period; approximately 40 °C/25 °C (max./min.); Supplementary material 1] and the number of retained fruits of 10 branches of each individual were recorded, and the percentage of fruit set was calculated.

2.3. Number of pollens on the stigma

Flowers were collected after anthesis and placed on 1% agar medium in a plastic petri dish of 60-mm diameter. Then, the number of pollens on the stigma was counted using a stereoscopic microscope (SZ61; OLYMPUS CORPORATION, Tokyo, Japan). Data collection was done once a month from June to September 2016.

2.4. Measurements of the percentage of fruit set after artificial self- or cross-pollination

Anthers were emasculated from each 120 flowers of 'Sy-2', 'No. 3686', and F_1 the day before flowering and pollinated with the pollen collected from the anther of the flower on the day of flower opening. Each 30 flowers of 'Sy-2', 'No. 3686', and F_1 were self-pollinated. Each 30 flowers were cross-pollinated with another cultivar or F_1, and each 30 flowers with the other one. In addition, each 30 flowers, which were only emasculated, served as a negative control (NC).

2.5. Assessment of the pollen germination rate

In the high-temperature period, 5–10 flowers were collected on each of the 0, 1, and 2 days after flowering (DAF 0, DAF 1, and DAF 2), from 9 to 10 o'clock in the morning.

To determine the pollen germination rate of plants cultivated under controlled conditions, 'Sy-2', 'No. 3686', and F_1 were grown in an incubator and supplied with tap water by bottom irrigation. The daylight length of the incubator was adjusted to 14 h and temperature to 30 °C/25 °C (light/dark). The pollen germination rate of plants that had been previously acclimatized in the incubator conditions for at least 7 days was investigated. Since the pollen germination rate of 'No. 3686' was exceedingly low, its pollen germination was also examined using plants grown at 30 °C/20 °C.

To conduct the *in vitro* pollen germination test, two anthers from each flower were collected and transferred into a 1.5-mL plastic tube (a total of 10–20 anthers from each cultivar). Next, the pollens were suspended in 1 mL of a solution composed of 5% (w/v) sucrose and 100 ppm (w/v) boric acid. A volume of 0.2-mL pollen suspension was uniformly dispersed on 1% agar medium containing ingredients identical to those in the pollen suspension. The petri dishes with the culture pollen were placed in a dark room at a temperature of approximately 20 °C. After a 24-h incubation, photographs of pollen germination were taken using a biological microscope (BX53; OLYMPUS CORPORATION)