Inhibition of Seed Formation by Anomalous Ovule in ‘Kyo-temari’, a Parthenocarpic Tomato (Solanum lycopersicum L.) Cultivar

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‘Kyo-temari’, a parthenocarpic tomato cultivar with the pat-2 gene, produces only a few seeds. To elucidate the cause of seed formation restriction in ‘Kyo-temari’, we observed pollen tube elongation and ovule morphology in some parthenocarpic tomatoes. In ‘Renaissance’, a parthenocarpic tomato cultivar with the pat-2 gene, pollen tube elongation was inhibited at the style base; however, in ‘Kyo-temari’, the pollen tubes elongated in the style normally but did not enter the ovules. The percentage of ovules in which a pollen tube entered was 70.4% in ‘Louis 60’, 0.8% in ‘Kyo-temari’, and 5.1% in ‘Renaissance’. From observing transverse sections of ‘Kyo-temari’ ovules, there were many ovules with an abnormal micropyle. Because these anomalous ovules were not observed in ‘Renaissance’, a peculiar factor is considered as the cause of anomalous ovule formation and seed formation restriction in ‘Kyo-temari’.

Key Words: anomalous ovule, parthenocarpic tomato, pat-2, pollen tube elongation, seed formation.

Introduction

Fruit set and development generally depends on pollination and fertilization, which are very sensitive to environmental conditions. For example, high or low temperature inhibits these processes. Parthenocarpy is the phenomenon in which fruit set and development occur without pollination and fertilization. Parthenocarpic tomatoes can produce normal fruit under unfavorable conditions for pollination and fertilization. Three parthenocarpic genes, pat, pat-2, and pat3/pat-4, are well known in tomatoes (Gouguet et al., 2005). ‘Severianin’, a pat-2 parthenocarpic tomato cultivar, can produce normal fruit at high or low temperature (Lin et al., 1983a; Philouze and Maisonneuve, 1978a; Vardy et al., 1989) and parthenocarpic fruit of this cultivar are not puffy (Philouze and Maisonneuve, 1978b). Despite these advantages, there has been little development and use of parthenocarpic tomato cultivars.

The parthenocarpy induced by the pat-2 gene does not cause male or female sterility (Lin et al., 1983a), and normal seeds can be obtained by pollination; however, in ‘Renaissance’, there are only a few seeds (Ohkawa et al., 2008). Johkan et al. (2010) reported that a high indoleacetic acid (IAA) concentration inhibits the elongation of pollen tubes at the style base in ‘Renaissance’, resulting in poor fertilization and failure of ovule development, and that seed development could be induced by p-chlorophenoxyisobutyric acid (PCIB), which inhibits auxin action.

‘Kyo-temari’ is the commercial name for the ‘MPK-1’ tomato, which was developed in F2 plants between a non-parthenocarpic commercial cultivar and an offspring of ‘Severianin’, which exhibited strong parthenocarpy (Hosokawa et al., 2004; Kataoka et al., 2004). ‘Kyo-temari’ produces only a few seeds and is propagated vegetatively. PCIB treatment had no effect on seed formation in ‘Kyo-temari’ (unpublished data), unlike ‘Renaissance’.

To elucidate what restricts seed formation in ‘Kyo-temari’, we observed pollen tube elongation in the style and ovary and examined the morphology of ovules. We found that the anomalous ovules restrict seed formation in ‘Kyo-temari’.

Materials and Methods

Plant materials

Parthenocarpic tomato cultivars ‘Kyo-temari’ and ‘Renaissance’ (Sakata Seed Co., Ltd., Kanagawa, Japan), and a non-parthenocarpic tomato cultivar ‘Louis 60’ (Takii Seed Co., Ltd., Kyoto, Japan) were used. Cuttings...
from ‘Kyo-temari’ plants, which has been maintained by vegetative propagation, were planted in a 72-cell tray filled with vermiculite and placed at 25°C in a growth chamber illuminated for 12 hours a day. ‘Renaissance’ and ‘Louis 60’ seeds were sown in a 72-cell tray filled with a mixture of peat moss and vermiculite (2:1, v/v). Seedlings were transplanted into a hydroponic rock wool bed or containers filled with a mixture of bark compost, sand, and chaff (6:3:1, v/v), in a greenhouse. The distance between plants was 20 cm in hydroponic beds and containers. Each plant was fertilized daily with Otsuka-A nutrient solution (Otsuka Chemica Co., Ltd., Osaka, Japan) adjusted to an EC of 1.2–1.5 and a pH of 5.5–6.5.

**Seed productivity**

Five plants per row were grown in soil culture with fertigation in a greenhouse without heating from April 14, 2010. At anthesis, the flowers of each cultivar were pollinated with ‘Louis 60’ pollen. We sampled 12 young fruit about 2 weeks after anthesis and 32 mature fruit of each cultivar. The pericarp of each young fruit was removed and ovules visible to the naked eye were counted. Mature fruit were weighed and cut into halves in the equatorial plane followed by counting the seeds in each part.

**Pollen tube elongation in style and ovary**

‘Louis 60’ and ‘Kyo-temari’ were grown in soil culture with fertigation from September 7 and 8, 2009, and ‘Renaissance’ was grown in hydroponic rock wool culture from September 28, 2009, in the same greenhouse without heating. Flowers were pollinated with ‘Louis 60’ pollen at anthesis, and the pistils were sampled 24 h after pollination. The styles were embedded in a 4% agar block for sectioning. Transverse sections 30 μm thick were prepared with a plant microtome, stained with 0.1% aniline blue (0.1N K$_3$PO$_4$), and observed with fluorescence microscopy. The pollen tube area in five styles for each cultivar was digitized with imaging software (Image-Pro Plus v.5.0, Media Cybernetics, Silver Spring, MD, USA) and the entire area of the pollen tubes (pixel) was measured. The ovaries were fixed and stored in FAA containing ethanol, water, formalin, and glacial acetic acid (9:9:1:1, v/v) until observation. They were washed with running tap water, stained with 0.1% aniline blue (0.1N K$_3$PO$_4$), and observed with fluorescence microscopy. The ovules were excised from the placenta under a stereo microscope and the percentage of ovules into which the pollen tube entered was obtained for 10 ovaries.

**Ovule structure**

All tomato plants were cultivated in soil culture with fertigation in a greenhouse without heating from April 14, 2010. At anthesis, the ovaries of each cultivar were sampled. After fixation with FAA, the ovaries were washed with running tap water and dehydrated with ethanol. After substituting ethanol with Technovit 7100 resin (Heruaeus Kulzer, Wehrheim, Germany), the materials were embedded in resin and coagulated in a mold. Transverse sections 2 μm thick were prepared with a rotary microtome, stained with toluidine blue O, and observed with light microscopy.

**Seed viability and morphology**

Mature seeds were obtained from a cross between ‘Kyo-temari’ and ‘Louis 60’ and self-pollination of ‘Louis 60’ in 2009 and 2010, and stored in a desiccator at room temperature until the germination test, which was conducted with three replicates of 100 seeds. The seeds were sown in a laboratory dish with two filter papers absorbed with 4 mL distilled water. The laboratory dish was put in a growth chamber at 25°C. The percentage of seed germination was measured 12 days after sowing. Seed morphology was examined after incubation at 25°C for one day within a wrap of wet filter paper. These were cut longitudinally, stained with lactophenol cotton blue (20 g phenol crystal, 20 mL lactic acid, 40 mL glycerol, 20 mL of distilled water, and 50 mg cotton blue) and observed with stereo microscopy.

**Statistical analysis**

The difference between two independent means was tested with Student’s t test at the 0.01 significance level. Steel-Dwass tests were used to test the difference between more than two means at the 0.05 significance level using software (MEPHAS, Osaka University, Suita, Japan).

**Results**

**Seed productivity**

The daily average temperature varied between 15 and 30°C. ‘Renaissance’ produces larger fruit than ‘Kyo-temari’ and ‘Louis 60’, which produce medium-sized fruit. Hence, the number of ovules per fruit was more in ‘Renaissance’ than in either ‘Louis 60’ or ‘Kyo-temari’ (Table 1). The number of seeds per fruit was significantly lower in parthenocarpic tomatoes than in the non-parthenocarpic tomato and the number of ‘Kyo-temari’ and ‘Renaissance’ seeds was 3.0 and 29.6 per fruit, respectively. In ‘Renaissance’, the number of seeds in the upper half of the fruit was more than that in the lower half. On the other hand, in ‘Louis 60’ and ‘Kyo-temari’, the number of seeds in the upper half was not different than that in the lower half. The percentage of normal seed formation was 83.0% in ‘Louis 60’, 1.6% in ‘Kyo-temari’, and 7.9% in ‘Renaissance’.

**Pollen tube elongation in style and ovary**

The daily average temperature varied between 15 and 28°C. Many pollen tubes reached the style base in ‘Louis 60’ (Fig. 1A), ‘Kyo-temari’ (Fig. 1B), and ‘Renaissance’ 24 h after pollination (Fig. 1C). Although the stigma was
covered with enough pollen, the digitized pollen tube area did not differ among cultivars ($P < 0.05$, Fig. 2).

Pollen tubes elongated from upper to lower halves of the ovary of 'Louis 60' (Fig. 1D) and 'Kyo-temari' (Fig. 1E), whereas they stopped at the upper half of the ovary of 'Renaissance' (Fig. 1F). In 'Louis 60', pollen tubes entered most of the ovules (Fig. 1G), whereas pollen tubes entered few ovules in 'Kyo-temari' (Fig. 1H) and some of the ovules in 'Renaissance' (Fig. 1I). The percentage of ovules in which a pollen tube entered was 70.4% in 'Louis 60', 0.8% in 'Kyo-temari', and 5.1% in 'Renaissance' ($P < 0.05$, Fig. 3).

The morphology of the pollen tubes that entered the ovules was normal in 'Louis 60' (Fig. 4A) and 'Renaissance' (Fig. 4C), whereas it was abnormal in 'Kyo-temari' (Fig. 4B).

**Ovule structure**

The daily average temperature varied between 15 and 30°C. All ovules had a normal micropyle in 'Renaissance' and 'Louis 60' (Fig. 4D, F), whereas they had an abnormal structure near their micropyle, for example, without the location where synergid cells should exist, in 'Kyo-temari' (Fig. 4E). We distinguished abnormal ovules from normal ovules by the structure of the micropyle.
Seed viability and morphology

The percentage of seed germination was 99.7% in ‘Louis 60’ and 5.7% in ‘Kyo-temari’ (P < 0.01, Fig. 5). The morphology of ‘Louis 60’ seeds was normal (Fig. 4G), whereas the morphology of some ‘Kyo-temari’ seeds was abnormal; they collapsed inside or had an abnormal embryo (Fig. 4H, I).

Discussion

The number of seeds and the percentage of ovules which became seeds were much lower in parthenocarpic tomatoes, ‘Renaissance’ and ‘Kyo-temari’, than in the non-parthenocarpic tomato, ‘Louis 60’. This result corresponds with reports that the number of seeds in a parthenocarpic tomato is lower than that in a non-parthenocarpic tomato (Kataoka et al., 2004; Ohkawa et al., 2008).

Johkan et al. (2010) reported that pollen tube elongation was inhibited at the base of the style in ‘Renaissance’. Although the pollen tube area at the base of the style did not differ among cultivars in this experiment, the stigma was covered with enough pollen and the style of ‘Renaissance’ was thicker than those of ‘Louis 60’ and ‘Kyo-temari’, so it seemed that pollen tube elongation was inhibited at the base of the style in ‘Renaissance’. Furthermore, in ‘Renaissance’, pollen tubes stopped elongating in the upper half of the ovary and did not reach the lower half, which seemed to be the cause of fewer seeds in the lower half than in the upper half. These results indicate that the restriction of seed formation in ‘Renaissance’ is caused by the inhibition of pollen tube elongation not only at the base of the style but also in the ovary.

The percentage of ovules which became seeds is lower in ‘Kyo-temari’ than in ‘Renaissance’, but in ‘Kyo-temari’, the pollen tubes did not stop growing at the base of the style and grew from the upper half to the lower half of the ovary; however, few pollen tubes entered the ovules. In this experiment, the formation of pseudo-embryos was not observed until 3 days after anthesis (data not shown), so this inhibition of pollen tube elongation was not caused by the existence of a pseudoembryo.

In a few ovules that a pollen tube entered, the pollen tube meandered or bent. From observing the transverse section of ovules, many anomalous ovules that collapsed near the micropyle were observed. These results indicated that anomalous ovules may restrict normal seed formation in ‘Kyo-temari’.

The fertilization of angiosperm is generally composed of several stages. First, pollen germinates on the stigma and pollen tubes grow through the style into the ovary. A pollen tube that reaches the ovary enters an ovule through the micropyle. The tip of a pollen tube bursts in synergid cells and releases two sperm cells. One sperm cell unites with an egg cell, the other unites with the central cell, and double fertilization occurs. The mechanism controlling these phenomena is not completely clear.

However, in *Torenia fournieri*, it has recently been shown that the attractant of a pollen tube is in synergid cells (Higashiyama et al., 2001), and the protein secreted from synergid cells guides the pollen tubes to the ovules (Okuda et al., 2009). Synergid cells also may play an important role in guiding pollen tubes to ovules in tomatoes. In this experiment, ovules which lost the location of synergid cells were observed in ‘Kyo-temari’, although it was not clear whether synergid cells existed or not. The low percentage of ovules in which a pollen...
tube entered and many anomalous ovules observed in ‘Kyo-temari’ suggest that these two phenomena are closely related. Furthermore, the percentage of hybrid seed germination between ‘Kyo-temari’ and ‘Louis 60’ was low and the internal structure of the seeds was abnormal. This may suggest that anomalous ovules inhibit normal double fertilization.

Anomalous ovules were not observed in ‘Renaissance’ and ‘Severianin’ (data not shown), so a factor other than the pat-2 gene was related to this phenomenon. The restriction of seed formation by anomalous ovules was reported in a parthenocarpic grape, ‘Himrod Seedless’ (Wang and Horiuchi, 1990). Many ovules of ‘Himrod Seedless’ have a long inner integument and large micropyle. The embryo sac has a central cell but lacks an egg cell or synergid cells. This degeneration of the egg apparatus causes the formation of seedless fruits.

The formation of seedless fruit is composed of two processes, not producing seeds and growing fruits without seed formation. In the ‘Delaware’ grape, gibberellin (GA) treatment before anthesis causes the formation of an imperfect embryo sac that does not have an egg cell or a central cell, and GA treatment 10 days after anthesis promotes fruit growth (Muranishi, 1968), indicating that GA cause not only fruit growth but also abnormal formation of the embryo sac.

In the pat-2 parthenocarpic tomato, the concentration of GA$_{20}$ in the ovary is higher than that in non-parthenocarpic tomatoes and it is thought that the high concentration of GA$_{20}$ induces parthenocarpy (Fos et al., 2000; Hazra et al., 2010). Furthermore, the GA biosynthesis inhibitor reduces parthenocarpic expression and restricts parthenocarpic fruit development (Kataoka et al., 2008). These results indicate that the fruit set and development of the pat-2 parthenocarpic tomato is closely related to that of endogenous GA.

Hazra and Dutta (2010) suggested that an independent minor factor affects a major factor, the pat-2 gene in ‘Oregon Pride’, a pat-2 parthenocarpic tomato cultivar. Vardy et al. (1989) hypothesized a model composed of two recessive alleles, a major effect (pat-2) and a minor effect (mp). These reports suggest the existence of a minor factor that affects parthenocarpy expression. It is possible that the minor factor affects the ability of GA biosynthesis or sensitivity to GA and changes parthenocarpy expression.

‘Kyo-temari’ was selected from F$_2$ plants between a non-parthenocarpic commercial cultivar and an offspring of ‘Severianin’ that exhibited strong parthenocarpy (Hosokawa et al., 2004; Kataoka et al., 2004) and expressed very strong parthenocarpy. The strong parthenocarpic of ‘Kyo-temari’ might be caused by the strong ability of endogenous GA biosynthesis or high sensitivity to endogenous GA and they might be related to the abnormal morphology of ‘Kyo-temari’ ovules.

In conclusion, we showed that the pat-2 parthenocarpic tomato, ‘Kyo-temari’, has anomalous ovules, which restrict seed formation by inhibiting pollen tubes from entering ovules.

It is not clear whether the anomalous ovules of ‘Kyo-temari’ are related to the expression of parthenocarpy; therefore, further studies are needed. If the cause of anomalous ovules is independent of parthenocarpic expression, it is possible to breed a parthenocarpic tomato cultivar that can produce many normal seeds.

**Literature Cited**


