Seed Formation Promoted by Paclobutrazol, a Gibberellin Biosynthesis Inhibitor, in \textit{pat-2} Parthenocarpic Tomatoes

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Seed production in \textit{pat-2} parthenocarpic tomatoes is sufficiently low for the propagation of parthenocarpic tomato cultivars. To establish the technology to promote seed formation in \textit{pat-2} parthenocarpic tomato plants, we examined the effects of paclobutrazol, a gibberellin biosynthesis inhibitor, on seed formation. The parthenocarpic \textit{F\textsubscript{1}} cultivar ‘Renaissance’, that has the homozygote recessive gene \textit{pat-2}, was treated with paclobutrazol at 0 (control), 0.2, 1, 5, or 25 mg per pot (5.1 L) by irrigation to the culture medium. With increasing application of paclobutrazol, stem diameter increased, while stem length, leaf length, leaf width, and fruit fresh weight decreased. The percentage of fruits with seeds increased with increasing levels of paclobutrazol, and it reached 100% with 1 mg/pot and higher. Seed number per fruit increased from 12 at 0 mg/pot of paclobutrazol to 52 and 74 at 1 and 5 mg/pot, respectively. To confirm the practicality of this technology, the parthenocarpic purebred strain ‘PASK-1’, the seed parent of ‘Renaissance’, was treated with 0 (control), 1, or 5 mg/pot of paclobutrazol. All flowers of ‘PASK-1’ were emasculated before flowering and crossed at flowering with pollen of the parthenocarpic purebred strain ‘PF81IK’, the pollen parent of ‘Renaissance’. The percentage of fruits with seeds increased from 59% at 0 mg/pot of paclobutrazol to higher than 95% at 1 and 5 mg/pot. Seed number per fruit increased from 21 at 0 mg/pot of paclobutrazol to 45 and 46 at 1 and 5 mg/pot, respectively. From these results, we concluded that paclobutrazol promotes seed formation in \textit{pat-2} parthenocarpic tomato plants.

Key Words: \textit{F\textsubscript{1}} cultivar ‘Renaissance’, fruit fresh weight, seeded fruit, seed number, seed production.

Introduction

Genetically parthenocarpic tomato cultivars enable the production of normal sized fruits without either pollination or synthetic auxin treatment (Lukyanenko, 1991). In 2000, the parthenocarpic tomato ‘Renaissance’ was released (Sugahara et al., 2002) by using the parthenocarpic gene \textit{pat-2}, which originated in the Russian tomato ‘Severianin’ (Philouze and Maisonneuve, 1978a, b). ‘Renaissance’ showed a reliably high fruit setting and normal fruit growth in greenhouses year-round because of its parthenocarpy (Ohkawa et al., 2006). At both high and low temperatures, the fruit setting percentage of ‘Renaissance’ was very high, and the fruits developed normally without treatment with 4-chlorophenoxy acetic acid, an auxin (Ohkawa et al., 2007). Even under 70% shading during a particularly hot period, the normal flower-buds of ‘Renaissance’ expressed parthenocarpic characteristics (Ohkawa et al., 2010). Because of these characteristics, parthenocarpic tomato cultivars could have higher productivity than non-parthenocarpic cultivars that were either pollinated or treated with synthetic auxin (Gouguet et al., 2005). However, seed production is sufficiently low in parthenocarpic tomatoes (Kataoka et al., 2008; Ohkawa et al., 2008) as to prevent successful propagation. Efficient seed production is necessary to increase the availability of parthenocarpic tomato cultivars.

In ‘Renaissance’, ovary development before flowering might suppress seed development when crossing at 2 days after flower opening (Ohkawa et al., 2008). In the highly parthenocarpic tomato ‘MPK-1’, which was found in \textit{F\textsubscript{2}} plants between a non-parthenocarpic commercial cultivar and an offspring of ‘Severianin’ that exhibited strong parthenocarpy (Hosokawa and Yazawa, 2004), the ovary developed from the stage before flowering, and a pseudoembryo simultaneously developed in the embryo sac cavity (Kataoka et al., 2004a).
Kataoka et al. (2008) suggested that this pseudoembryo development, which filled up the embryo sac cavity, prevented fertilization. Because gibberellin activity increased from flower opening in parthenocarpic tomatoes (Kataoka et al., 2004b; Koshioka et al., 1994; Mapelli et al., 1978), endogenous gibberellin was considered to play an important role in the early stages of fruit growth and development (Kataoka et al., 2004b). Uniconazole-P, a gibberellin biosynthesis inhibitor, completely inhibited fruit growth and suppressed pseudoembryo development (Kataoka et al., 2003), supporting the importance of gibberellin in fruiting in parthenocarpic tomatoes. Kataoka et al. (2008) indicated that a positive correlation between fruit fresh weight and seed number per fruit was observed by application of uniconazole-P to the culture solution in the parthenocarpic tomato ‘Severianin’. Therefore, they showed that this method could increase seed formation efficiency by choosing large fruits at harvest (Kataoka et al., 2008). However, there were no differences in fruit weight and seed number between the different concentrations of uniconazole-P (Kataoka et al., 2008), and the cause was not evident. Thus techniques are needed to increase seed number per fruit in parthenocarpic tomatoes.

In this study, we attempted to increase seed production in pat-2 parthenocarpic tomatoes by application of paclobutrazol, a gibberellin biosynthesis inhibitor like uniconazole-P, to the culture medium. ‘Renaissance’, that has the homozygote recessive gene pat-2, was used as a model of parthenocarpic tomato plants and confirmed the increase in F1 seeds by crossing the seed and pollen parents of ‘Renaissance’.

Materials and Methods

1. Seed formation promoted by paclobutrazol treatment in the parthenocarpic tomato ‘Renaissance’ with homozygous pat-2

We used a parthenocarpic F1 tomato ‘Renaissance’, which was homozygous for the recessive parthenocarpic gene pat-2 like its seed parent ‘PASK-1’ and its pollen parent ‘PF811K’ (Sugahara et al., 2002).

In a greenhouse, the seeds of ‘Renaissance’ were sown on February 6. The seedlings were transplanted to 10.5 cm diameter pots (570 mL) on February 15, and to 21 cm diameter pots (5.1 L) on March 23. They were fertigated with liquid fertilizer (OK-F-1, Otsuka Chemical Co., Ltd., Japan). On March 25, plants were irrigated with 47 mL/pot Bounty Flowable (21.5% paclobutrazol, Syngenta Japan Co., Ltd., Japan) diluted 50,000-, 10,000-, 2,000-, or 400-fold with water, resulting in paclobutrazol concentrations of 0 (control), 0.2, 1, 5, and 25 mg/pot, respectively (hereafter referred to as the control, 0.2 mg treatment, etc.). All flowers in each treatment were artificially self-pollinated at flowering, when the petals fully opened and had turned yellow. The greenhouse was ventilated and heated to higher than 28°C and lower than 12°C, respectively. All plants were pinched above the second truss leaving two leaves. The number of fruits on each truss was reduced to three. Five plants were used for each treatment and 30 fruits were sampled from each treatment.

At the end of cultivation, we evaluated the following growth parameters: percentage of normal and undergrown fruits, percentage of fruits with seeds, average fruit fresh weight, and average number of seeds per fruit. Undergrown fruit was defined as those with poor development, a lusterless pericarp, and delayed coloring compared with normal fruit. Undergrown fruits and fruits with blossom-end rot were excluded from calculations of average fruit fresh weight and average seed number per fruit.

2. F1 seed formation promoted by paclobutrazol treatment to the parthenocarpic purebred strain ‘PASK-1’, the seed parent of ‘Renaissance’

Parthenocarpic purebred strains ‘PASK-1’ and ‘PF811K’ are the seed and pollen parents of ‘Renaissance’, respectively. Seeds of ‘PF811K’ were sown on February 2 in a greenhouse. The seedlings were transplanted to 10.5 and 21 cm diameter pots on February 15 and March 18, respectively. Seeds of ‘PASK-1’ were sown on February 10, and the seedlings were transplanted to 10.5 and 21 cm diameter pots on February 23 and March 24, respectively. Paclobutrazol was added to the culture medium of the ‘PASK-1’ plants at 0 (control), 1, or 5 mg/pot on March 25. All flowers of ‘PASK-1’ were emasculated before flowering and were crossed with the pollen of ‘PF811K’ when the flowers opened. Plants were treated as described above in Experiment 1, except that we used seven plants instead of five plants for each treatment and we sampled 42 fruits per treatment instead of 30.

3. Statistical analysis

Mean comparisons were performed using the Tukey-Kramer test to examine differences among treatments at the 5% level of significance.

Results

1. Seed formation promoted by paclobutrazol treatment in the parthenocarpic tomato ‘Renaissance’ with homozygous pat-2

Table 1 shows the effects of paclobutrazol application on the growth of ‘Renaissance’. The greater the paclobutrazol concentration, the thicker the stem diameter under the first and second trusses, and the shorter the stem length. Although leaf length and width both decreased with increased levels of paclobutrazol, there were no significant differences in leaf fresh weight except in the 25 mg treatment, which had significantly lower weight. Numbers of leaves under the first and second trusses were statistically indistinguishable among different paclobutrazol treatments.

The effects of paclobutrazol treatment on the fruit
characteristics of ‘Renaissance’ are shown in Table 2. The fruit setting was 100% irrespective of paclobutrazol concentration. There were no undergrown fruit in any of the treatments. The percentage of fruit with seeds was 67% at 0 mg and increased steadily to 100% at levels of 1 mg paclobutrazol and higher. However, average fruit fresh weight decreased with increasing application of paclobutrazol. Average seed number per fruit increased from 12 in the control to 25 at 0.2 mg, 52 at 1 mg, and 74 at 5 mg; the 1 mg and 5 mg treatments yielded significantly more seeds than the control. Seed number per fruit in the 25 mg treatment declined to 40, which was significantly more than the control, but significantly less than the 5 mg treatment. A significant positive correlation was not observed between fruit fresh weight and seed number per fruit in the 0 and 0.2 mg treatments, but was observed in the 1, 5, and 25 mg treatments (Fig. 1).

2. F1 seed formation promoted by paclobutrazol treatment to the parthenocarpic purebred strain ‘PASK-1’, the seed parent of ‘Renaissance’

Table 2 shows the effects of paclobutrazol on the growth of the parthenocarpic purebred strain ‘PASK-1’. Average stem diameter under the first and second trusses grew thicker, and average stem length decreased with increasing levels of paclobutrazol. Although average leaf length and width both significantly decreased with increasing levels of paclobutrazol, there were no significant differences in average leaf fresh weight, or in the numbers of leaves under the first and second trusses, among different paclobutrazol concentrations.

The effects of paclobutrazol on fruit characteristics of ‘PASK-1’ are shown in Table 4. The fruit setting was 100% irrespective of paclobutrazol treatment; however, 2% of the fruits in the 5 mg treatment were undergrown. The percentage of fruits with seeds increased from 59% at 0 mg to more than 95% at the 1 and 5 mg treatment. Fruit fresh weight decreased with increasing application of paclobutrazol, and was statistically lower than the control in both the 1 mg and 5 mg treatments. Seed number per fruit was 45 and 46 at 1 and 5 mg, respectively; both were significantly greater than 21 seeds per fruit in the control.

Table 2. Effect of paclobutrazol on the fruit characteristics of the parthenocarpic tomato ‘Renaissance’.

<table>
<thead>
<tr>
<th>Paclobutrazol application rate (mg/pot)</th>
<th>Percent fruit setting</th>
<th>Percent seeded fruit</th>
<th>Fruit fresh weight per fruit (g)</th>
<th>Seed number per fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal fruita</td>
<td>Undergrown fruita</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>67</td>
<td>225 a’ 12 d</td>
</tr>
<tr>
<td>0.2</td>
<td>100</td>
<td>0</td>
<td>87</td>
<td>194 ab 25 cd</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>166 b 52 ab</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>132 c 74 a</td>
</tr>
<tr>
<td>25</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>88 d 40 bc</td>
</tr>
</tbody>
</table>

Table 3. Effect of paclobutrazol on the growth parameters of the parthenocarpic tomato ‘Renaissance’.

<table>
<thead>
<tr>
<th>Paclobutrazol application rate (mg/pot)</th>
<th>Stem diameter (mm)</th>
<th>Stem length (cm)</th>
<th>Stem fresh weight per plant (g)</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
<th>Leaf fresh weight per plant (g)</th>
<th>No. of leaves under the truss</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.1 d’</td>
<td>70.0 a</td>
<td>44.7 a</td>
<td>58.4 a</td>
<td>426 a</td>
<td>8.0 a</td>
<td>12.0 a</td>
</tr>
<tr>
<td>0.2</td>
<td>16.3 c</td>
<td>49.3 b</td>
<td>41.6 b</td>
<td>45.4 b</td>
<td>434 a</td>
<td>8.0 a</td>
<td>12.4 a</td>
</tr>
<tr>
<td>1</td>
<td>18.5 bc</td>
<td>46.3 b</td>
<td>39.8 ab</td>
<td>43.6 b</td>
<td>448 a</td>
<td>8.0 a</td>
<td>12.2 a</td>
</tr>
<tr>
<td>5</td>
<td>21.2 a</td>
<td>41.9 c</td>
<td>37.9 b</td>
<td>41.2 b</td>
<td>438 a</td>
<td>7.8 a</td>
<td>12.0 a</td>
</tr>
<tr>
<td>25</td>
<td>20.1 ab</td>
<td>35.3 d</td>
<td>29.9 c</td>
<td>33.6 c</td>
<td>305 b</td>
<td>7.8 a</td>
<td>11.8 a</td>
</tr>
</tbody>
</table>

* Capacity of 5.1 L.

* Leaf under the 2nd truss.

* Different letters within columns indicate significant difference by the Tukey-Kramer test at 5% level.

Discussion

In tomato, normal fruits can be induced by treatment with synthetic auxin even in environmental conditions unsuitable for pollination and fertilization. In non-parthenocarpic tomatoes, synthetic auxin treatment results in the formation of pseudoembryos from the innermost layer of integument in the embryo sac cavity of the ovary (Asahira et al., 1967; Kataoka et al., 2003; Serrani et al., 2007). In ‘Severianin’, homozygous for...
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the recessive parthenocarpic gene *pat-2* as well, the pseudoembryos formed similarly to those of non-parthenocarpic tomatoes induced by synthetic auxin (Lin et al., 1983). They developed in the embryo sac cavity where seeds were originally formed. Because of that, pseudoembryo development was stable in not only...
unsuitable conditions for fertilization, but also suitable conditions in parthenocarpic tomatoes, such that seed formation was inhibited. If parthenocarpic tomatoes are fertilized under conditions where pseudoembryo development is restricted, seeds might develop (Kataoka et al., 2008). Kataoka et al. (2003) reported that pseudoembryo development was controlled with uniconazole-P, a gibberellin biosynthesis inhibitor. Paclobutrazol, another gibberellin biosynthesis inhibitor, also inhibited the fruit setting and development in pat-2 parthenocarpic tomatoes, while the simultaneous application of GA₃ fully reverted this inhibition (Fos et al., 2000).

In this study, paclobutrazol was added to the culture medium in a 5.1 L pot of ‘Renaissance’, and all flowers were artificially self-pollinated at flowering. Paclobutrazol prevents the activity of cytochrome P450, which catalyzes the formation of ent-kaurenoic acid from ent-kaurene in the gibberellin biosynthetic pathway (Hedden and Graebe, 1985). Paclobutrazol retards vegetative growth of plants, and makes them dwarfish (Ueno, 1989). In our study, we saw evidence of dwarfing at all paclobutrazol treatment levels, suggesting that application to the roots was effective.

Paclobutrazol successfully increased the percentage of fruits with seeds in the parthenocarpic tomato ‘Renaissance’ at all levels of treatment, reaching 100% at 1 mg/pot and higher. This indicates that 1 mg/pot of paclobutrazol was sufficient to inhibit pseudoembryo development and promote seed formation, although 0.2 mg/pot was not. It was suggested that paclobutrazol at 1 mg per pot suppressed pseudoembryo development in the embryo sac cavity as well as uniconazole-P (Kataoka et al., 2003), and promoted fertilization in parthenocarpic tomatoes.

In prior studies of non-parthenocarpic tomatoes, fruit weight increased in proportion to the number of seeds, because developing seeds enhanced the sink activity of fruits (Varga and Bruinsma, 1976). In this study, we did not observe a significant positive correlation between fruit fresh weight and seed number per fruit at the 0 and 0.2 mg treatments, but the correlation was significant at 1, 5, and 25 mg (Fig. 1). We suspected that the correlation at the 0 and 0.2 mg treatments was weakened by the inclusion of heavy seedless fruits. Kataoka et al. (2008) similarly showed a positive correlation between fruit fresh weight and seed number per fruit in the parthenocarpic tomato ‘Severianin’ by application of uniconazole-P, a gibberellin biosynthesis inhibitor, to the culture solution about 2 weeks before flowering. As a result, they recommended selecting well-grown fruits at harvest to increase seed production of ‘Severianin’. In our study, seed number per fruit was largest at 5 and 1 mg/pot of paclobutrazol application. Although we also observed a significant positive correlation between fruit fresh weight and seed number per fruit at the 25 mg treatment, both parameters were lower than those of the 5 and 1 mg treatments. Because a source-sink balance of tomato was observed between leaf area and fruit fresh weight (Yoshioka et al., 2001), we suspected that the decrease in leaf area at 25 mg might have inhibited fruit development. Thus, it was suggested that there was a proper application rate when we applied paclobutrazol to the culture medium.

To confirm the practicality of this technology, the parthenocarpic purebred strain ‘PASK-1’, the seed parent of ‘Renaissance’, was also treated with paclobutrazol at 0, 1, and 5 mg/pot. All flowers of ‘PASK-1’ were emasculated before flowering and crossed at flowering with the pollen of the parthenocarpic purebred strain ‘PF811K’, the pollen parent of ‘Renaissance’. Dwarfing was observed in the 1 and 5 mg treatments, indicating paclobutrazol activity. The percentage of fruits with seeds in both the 1 and 5 mg treatments was higher than that of the control. Seed number per fruit in both the 1 and 5 mg treatments was significantly larger than that of the control. Moreover, because the germination rates of obtained seeds were higher than 90% irrespective of paclobutrazol treatment levels (data not shown), it was conceivable that there was no problem to treat with paclobutrazol practically. Therefore, we concluded that paclobutrazol addition to the culture medium effectively increased seed number in the F₁ cultivar of pat-2 parthenocarpic tomatoes.

In our study, the most effective treatment level, 5 mg/pot, yielded an average of only 74 seeds per fruit in this pat-2 homozygous cultivar. This number is less than that of Japanese commercial cultivars, which yield about 150 seeds per fruit (Ohkawa et al., 2008). Prior studies have reported that minor genes for phytohormone metabolism and environmental conditions might affect the expression of parthenocarpic gene pat-2 (Kataoka et al., 2008). Therefore, future work should investigate the effects of paclobutrazol treatment on other pat-2 parthenocarpic lines with different genetic backgrounds.

Paclobutrazol application to the culture media just after transplanting is simple and labor-saving compared with dipping flowers or foliar application. However, the residual effects of paclobutrazol treatment on plant growth and development in long-term cultures are not clear. The effects on seed formation of paclobutrazol application to the culture media should be investigated using tomato plants pinched at higher than the third truss.

Auxin plays an important role in fruit development by parthenocarpy (Gouguet et al., 2005); the expression of genetic parthenocarpy is correlated with the accumulation of auxin in the ovary (George et al., 1984; Ikeda et al., 1999; Pandolfini et al., 2002). Johkan et al. (2010) suggested that a high level of indoleacetic acid at the base of the style inhibited pollen tube elongation, and prevented fertilization and ovule development in a pat-2 parthenocarpic tomato. They also reported that treatment with p-chlorophenoxyisobutyric acid (PCIB), an antiauxin, did not decrease either the rate of the fruit setting or fruit fresh weight, and increased seed number.
per fruit. However, they did not refer to the influence of PCIB treatment on pseudoembryonic development in parthenocarpic tomatoes. Moreover, it was not investigated how a gibberellin biosynthesis inhibitor had influence upon pollen tube elongation in the style. In the past, several researchers have reported correlations between gibberellin and auxin (Koshioka et al., 1994; Sastry and Muir, 1963; Serrani et al., 2008). Koshioka et al. (1994) and Serrani et al. (2008) showed that auxin seems to be able to stimulate gibberellin biosynthesis, but in turn, Sastry and Muir (1963) showed that the application of gibberellin could induce an increase in auxin content. Like this, the signal transduction pathways of auxin and gibberellin have not been sufficiently elucidated (de Jong et al., 2009). Further elucidation of the relationship between gibberellin biosynthesis inhibitor and antiauxin is necessary to increase the seed number of pat-2 parthenocarpic tomatoes and to run up to the level of Japanese commercial cultivars.

We conclude that application of paclobutrazol, a gibberellin biosynthesis inhibitor, to the roots of pat-2 parthenocarpic tomatoes via irrigation of the culture medium promotes seed formation, and that this method is effective in practical parthenocarpic F₁ seed production.

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