Role of Ethylene on Flower Senescence of *Torenia*

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Summary

Flower senescence of *Torenia fournieri* was investigated in relation to ethylene production and sensitivity. Ethylene production of flowers increased with flower senescence; pistil contributed to climacteric increase of ethylene production whereas other organs such as the petal, stamen, and calyx did not. Flowers were not sensitive to ethylene on the day of anthesis, but developed sensitivity as the flower senesced. Pollination and wounding of stigma markedly accelerated abscission of flowers, which was accompanied by climacteric ethylene production. Climacteric ethylene, following pollination and stigma wounding, was mainly produced by the pistil, particularly the style with stigma. The abscission of the intact, pollinated and stigma-wounded flowers was significantly inhibited by treatment with silver thiosulfate complex (STS), an ethylene action inhibitor. These results clearly show that the flower senescence of *Torenia* is mainly regulated by ethylene.

Key Words: ethylene, pollination, silver thiosulfate, stigma wounding, *Torenia fournieri*.

Introduction

*Torenia fournieri* Lind., commonly known as torenia or wishbone flower, belongs to the family Scrophulariaceae; it is now widely used as bedding ornamental plants in summer because of its tolerance of high temperature. *Torenia* flowers normally abscise about 1 week after anthesis, but some flowers often abscise within a few days after anthesis. This phenomenon reduces the ornamental value of *Torenia* plants. What factor accelerates flower abscission remains unclear.

Flowers are classified into ethylene sensitive and insensitive flowers (Woltering and van Doorn, 1988). Senescence of flowers, sensitive to ethylene, is mainly regulated by ethylene. In some ethylene sensitive flowers such as orchids (Burg and Dijkman, 1967; Zhang and O’Neill, 1993; Porat et al., 1995), petunia (Gilissen and Hoekstra, 1984; Whitehead et al., 1984; Hoekstra and Weges, 1986), and cyclamen (Halevy et al., 1984), pollination greatly stimulates flower senescence. Similarly, in *Digitalis*, which belongs to the Scrophulariaceae, flower abscission is promoted by pollination (Stead and Moore, 1979, 1983). In *Petunia*, the flower senescence was stimulated by wounding the stigma (Gilissen and Hoekstra, 1984; Whitehead et al., 1984; Nichols and Frost, 1985; Hoekstra and Weges, 1986; Lovell et al., 1987). In these examples, the acceleration of flower senescence is considered to be regulated by an increase in ethylene sensitivity and the subsequent climacteric ethylene production (Halevy et al., 1984; Whitehead and Halevy, 1989; Porat et al., 1995). From these findings, we speculate that ethylene is involved in flower senescence of *Torenia*. However, little is known about the relationship between flower senescence and ethylene in *Torenia*.

In this paper, we describe how flower senescence of *Torenia* is regulated by ethylene. Furthermore, pollination and stigma-wounding accelerated flower abscission, a phenomenon which is also closely related to ethylene evolution.

Materials and Methods

Plant materials

Potted plants of *Torenia fournieri* cv. Murasakibana (Takii & Co., Ltd, Kyoto) were grown in a growth chamber at 25 / 20 °C (day and night). A 12–hr light photoperiod (8:00–20:00) was provided by a mixture of metal halide lamps and high pressure sodium lamps with a light intensity of 150 μmol m⁻² s⁻¹ at plant height. Unless otherwise stated, the potted plants were kept as above throughout the experimental periods. Anthesis is considered terminated when the petals have abscised or lost turgor.

Pollination and stigma wounding

Flowers, 1 day after anthesis, were self–pollinated with freshly collected pollen. To wound the stigma, it including the upper part of the style, was crushed 5 times with a small pair of tweezers. After these treat-
ments, flowers on potted plants were kept as above until the petals abscised.

**Measurement of ethylene production**

The whole flower or its various parts collected from 5 flowers were placed in test tubes (14.8 ml), which were then sealed and kept at 25 °C. Two hours later, a 1-ml gas sample was withdrawn into a syringe and its ethylene concentration determined by a Shimadzu gas chromatograph model GC-7A, equipped with an alumina column and a flame ionization detector.

**Evaluation of ethylene sensitivity**

Five potted plants were placed in a transparent acryl box with a septum (53 liter). A designated amount of ethylene was introduced into the vessel through the septum. After keeping the box at 25 °C under 150 μmol m⁻² s⁻¹ for 6 hr, the plants were removed, kept as described in “Plant materials” and observed until their sensitivity to ethylene was determined.

**STS treatment**

STS solutions were prepared freshly by mixing equal volumes of AgNO₃ and Na₂S₂O₅· 5H₂O in a molar ratio of 1 to 8, respectively. Flowers on the day of anthesis were sprayed to run-off with different concentrations of STS solution. Distilled water served as the control. The next day, flowers were pollinated or stigma-wounded and their longevity determined as the time from anthesis to petal fall.

**Results**

**Changes in ethylene production**

Longevity of Torenia flower was 8.1 ± 0.4 days (mean ± standard errors). The corresponding changes in ethylene production by flowers during this period was very low until 6 days after anthesis; it sharply increased thereafter (Fig.1).

Our data on the changes in ethylene production by various organs with time reveal that a climacteric ethylene production occurred in the pistil but not in the other organs (Fig.2). To determine the contribution of each organ to ethylene production, we examined ethylene production of each organ and found that the petal produced the most ethylene, followed by calyx on the day of anthesis; whereas more than 80% of ethylene was produced by a pistil 8 days after anthesis (Fig.3).

**Ethylene sensitivity of flower**

Fig.4 shows the effect of ethylene concentration on the abscission of flowers at different ages. When flowers at anthesis were exposed to 2 or 20 μl·liter⁻¹ ethylene, flower longevity was affected little but in flowers 1 day after anthesis, 20 μl·liter⁻¹ ethylene promoted their abscission. Two days after anthesis, 2 μl·liter⁻¹ ethylene was also effective in promoting abscission of flowers. In flowers 4 days after anthesis, more than 50% of flowers abscised immediately after exposure to 2 and 20 μl·liter⁻¹ ethylene. These results indicate that ethylene sensitivity increases with the age of the flower.

**Effect of pollination and wounding on the longevity and ethylene production**

Pollination and wounding of stigma markedly promoted abscission of flowers (Table 1), accompanied by ethylene evolution (Fig.5). On examining the ethylene production of style with stigma, ovary and petal 3 days after treatments, we found that regardless of the treatments, the style produced the most ethylene, followed by the ovary, on both organ and fresh weight bases. On the contrary, petals produced very little ethylene after these treatments (Fig.6).

**Effect of STS on the longevity of pollinated and stigma-wounded flowers**

With increasing concentration of STS, the longevity of control flowers was correspondingly extended. STS at 0.02 mM markedly extended longevity of pollinated flowers, but not that of stigma-wounded flowers, whereas at 2 mM, the longevity of both pollinated and stigma-wounded flowers equaled that of the control flowers (Table 2).
Fig. 2. Changes in ethylene production by petal, stamen, pistil and calyx on fresh weight basis. Values are means of 4 replications ± standard errors.

**Discussion**

Woltering and van Doorn (1988) classified flowers into 4 groups based on their ethylene sensitivity, and showed that flowers in most of the Scrophulariaceae plants are sensitive to ethylene. According to their criterion, ethylene sensitivity of *Torenia* flowers on the day of anthesis is judged to be relatively low because the abscission of flowers was affected little by exposure to 20 μl / liter⁻¹ ethylene. However, with aging, abscission of flowers was accelerated by exposure to ethylene (Fig.4), indicating that ethylene sensitivity increased as the flower senesced. In carnation, a typical ethylene sensitive flower, ethylene sensitivity, likewise, increased with flower senescence (Woodson and Lawton, 1988).

In carnation, the climacteric increase of ethylene is caused by an autocatalytic reaction, which is involved in the increase in sensitivity to ethylene (Mayak et al., 1977; Bulfer et al., 1980; Jiang et al., 1994). In this study, the flower of *Torenia* showed a climacteric increase in ethylene (Figs.1 and 2), which is accompanied with an increase of sensitivity to ethylene (Fig.4). Furthermore, treatment with STS, which inhibits ethylene production by binding to the ethylene binding site, extended flower longevity (Table 2). These findings suggest that flower abscission of *Torenia* is also regulated by autocatalytic ethylene production.

Pollination and stigma wounding markedly promoted flower abscission, a phenomenon accompanied by a climacteric increase in ethylene production (Table 1,
Fig. 4. Effects of exposure to ethylene on flower senescence. Flowers 0 (A), 1 (B), 2 (C) and 4 (D) days after anthesis were exposed to 0, 2 and 20 μl·liter⁻¹ ethylene, respectively. Parentheses in each figure represent mean values of days to flower abscission after ethylene treatment ± their standard errors (n ≥ 10). Longevity was determined from ethylene treatment to flower abscission.

Table 1. Effect of pollination and stigma wounding on the longevity of flowers. Values are means of 7 replications ± standard errors. Flowers 1 day after anthesis were pollinated or stigma-wounded.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Longevity after treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td>Pollination</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Stigma wounding</td>
<td>3.4 ± 0.3</td>
</tr>
</tbody>
</table>

Fig. 5. Changes in ethylene production of flowers after pollination and stigma wounding. Values are means of 4 replications ± standard errors.

Fig. 6. Effects of pollination and stigma wounding on ethylene production from petals, style with stigma, and ovary on fresh weight (A) and on organ (B) basis. Values are means of 4 replications ± standard errors.

Fig. 5) whereas treatment with 2 mM STS completely nullified acceleration of abscission induced by these treatments. This reaction confirms that acceleration of flower abscission induced by these treatments is regulated by ethylene. In many plants, such as orchids and petunia, acceleration of flower senescence by pollination is also regulated by ethylene (Burg and Dijkman, 1967; Whitehead et al., 1984; Zhang and O'Neill, 1993). In these examples, ethylene sensitivity is found to increase before climacteric ethylene production (Whitehead and
Table 2. Effect of STS on the senescence of pollinated and stigma - wounded flowers. Values are means of 10 replications ± standard errors.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>STS concentration (mM)</th>
<th>Longevity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>8.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>9.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>10.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.8 ± 0.4</td>
</tr>
<tr>
<td>Pollination</td>
<td>0</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>8.8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>8.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13.2 ± 0.9</td>
</tr>
<tr>
<td>Stigma wounding</td>
<td>0</td>
<td>4.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13.5 ± 0.5</td>
</tr>
</tbody>
</table>

Halevy, 1989; Porat et al., 1995). Whether pollination and stigma wounding increase sensitivity to ethylene in Torenia flowers remains unsolved.

In petunia, pollination and stigma wounding promote senescence of flowers (Gilissen and Hoekstra, 1984; Whitehead et al., 1984; Hoekstra and Weges, 1986; Lovell et al., 1987) as we found with Torenia flowers (Table 1). However, treatment with 0.02 mM STS extended longevity of pollinated flowers, but not that of wounded flowers, which indicates that stigma wounding increases ethylene sensitivity more than pollination. Woltering et al. (1997) recently found that with petunia, ethylene production from petals is increased within a few hours by stigma wounding, but not by pollination. From this finding, they concluded that pollination and stigma wounding were different signals in petunia. In Torenia, pollination and stigma wounding may also induce different signals, leading to different sensitivity to ethylene.

Petals did not show climacteric ethylene production with flower senescence (Figs. 2 and 3). Furthermore, pollination and stigma wounding increased ethylene production of pistils, but did not increase that of petals (Fig. 6). These results are consistent with those reported with Digitalis (Stead and Moore, 1983). Woltering et al. (1995) reported that ethylene diffuses through the pistil from the petal after pollination in orchids. They also showed that ethylene diffuses after stigma wounding in Petunia (Woltering et al., 1997). These findings suggest that ethylene produced by ovary diffuses and acts on the abscission zone, leading to flower abscission.

Contributions of various organs to ethylene production differ among plant species. In carnation, the petal produces the most ethylene on each organ basis, but the style produces the most ethylene on a fresh weight basis (Nichols, 1977). In Phalaenopsis, the labellum produces the most ethylene, followed by the petal (O’Neill et al., 1993). The stamen and petal produce ethylene at relatively high levels, whereas the pistil of the sweet pea produces little ethylene (Ishihara et al., 1991). In Torenia, the pistil produced the most on both an organ and a fresh weight basis (Fig. 2) which is attributed to gene expression and activities of various enzymes involved in ethylene biosynthesis. In Phalaenopsis, 1-amino-cyclo-propane-1-carboxylic acid (ACC) is deduced to be transported from the other organs because gene expression of ACC synthase is absent in the petal (O’Neill et al., 1993).

Torenia can be easily propagated and raised in a growth chamber. Furthermore, many uniform flowers open every day and their abscission is markedly accelerated by pollination and stigma wounding. Because this plant is a suitable material to investigate flower senescence, Aida and Shibata (1995) transforms Torenia by using Agrobacterium. We isolated a gene that codes ACC oxidase. This enzyme catalyzes the final step of ethylene biosynthesis; thus we are now trying to introduce the isolated gene into Torenia. Since flower abscission of Torenia is mainly regulated by ethylene as described above, flower longevity of the transgenic Torenia should be longer than that of the wild type.

Literature Cited


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