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Effect of pre-bloom GA application on pollen tube growth in cv. Delaware grape pistils

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Summary

Pre-bloom application of 100 ppm gibberellin A_3 (GA) to Delaware grape clusters (*Vitis labrusca* Bailey) induces the set of seedless berries when treated about 14 d before full bloom (FB). By contrast, GA application less than 10 d before FB results in the set of both seeded and seedless berries. In order to clarify the mechanism underlying the production of seedless berries by GA treatments, pollen tube transmitting tissue (TT) development and pollen tube growth in pistils were investigated in 2003 and 2004. Clusters were treated with GA 14 d before FB (normal GA treatment) or 8 and 7 d before FB (late GA treatment), and were hand-pollinated with Muscat of Alexandria pollen at FB. Untreated clusters (control) were also pollinated. No significant difference in TT development in various parts of style and ovary was found among the two treatments and control. On the other hand, at the upper part of the ovary, pollen tube growth in normal GA treatment was significantly inhibited 8 and 24 h after pollination compared with those in late GA treatment and control. Pollen tubes reached the micropyle 24 h after pollination in control and 72 h after pollination in late GA treatment, whereas no pollen tube reached the micropyle after a normal GA treatment. Most pollen tube tips in GA-treated ovary tissues were found to be coiled up. These results indicate that inhibition of pollen tube growth in pistils after normal GA treatment may be due to biosynthesis of pollen tube inhibitor(s), leading to unfertilized ovules. By contrast, late GA treatment allows pollen tube penetration into the lower ovary and, in rare cases, into the micropyle which leads to seed formation.

K e y w o r d s : Delaware grape, GA treatment, pollen tube growth, seedlessness, transmitting tissue.

A b b r e v i a t i o n s : ECM = extra-cellular matrix, FB = full bloom, GA = gibberellin A_3, PGI = pollen tube growth inhibitor, TT = transmitting tissue.

Introduction

Delaware grapevines (*Vitis labruscana* Bailey) produce seedless berries when clusters are treated with 100 ppm gibberellin A_3 (GA) solution 12-17 d before full bloom (FB) (Sugiura and Inaba 1966, 1968). A second application of GA two weeks after FB is necessary to increase the size of seedless berries relative to that of seeded ones. The mechanism underlying the production of Delaware seedless berries by pre-bloom GA treatment has not been entirely elucidated. Sugiura and Inaba (1968) proposed that insufficient ovule development at bloom and poor germinability of pollen grains in GA-treated clusters may cause ovule fertilization failure. Pollen tube growth in GA-treated pistils was, however, not examined in their investigations. Okamoto *et al*. (1989) reported that grape pistils contain various levels of pollen tube growth inhibitor (PGI) that significantly affect the percentage and/or number of seeded berries per cluster. They also indicated that transmitting tissue (TT) development in grape pistils is cultivar-dependent and significantly affected by cultivation condition, resulting in different ovule fertilization rates (Okamoto *et al.* 2001a, b). In this work, we investigated the effect of pre-bloom GA application to Delaware clusters on TT development in pistils as well as pollen tube penetration into ovules.

Material and Methods

The experiments were conducted in 2003 and 2004. The 2003 test was carried out in a commercial vineyard, located in the Kumenan district, Okayama Prefecture, using three mature (8-year-old) Delaware grapevines showing standard vine vigor. The vines were grafted on 5 C rootstock, trained to a bi-lateral double cordon system on a horizontal trellis, and developed 40-50 bearing shoots in each 6-m-long cordon. Sixty clusters of average size and similar developmental stage were selected. On May 11, the first 20 clusters were dipped into a 100 ppm GA solution (normal GA treatment). The second 20 clusters were treated 6 d later (late GA treatment). Another 20 clusters were dipped into distilled water (control). Clusters of both, normal and late GA-treatment were at full bloom on May 24 and those of the control on May 26. All the clusters were hand-pollinated at full bloom with pollen collected from cv. Muscat of Alexandria. The number of set berries was counted 20 d after FB. The percentage of seedless berries in each cluster was calculated at harvest.

Thirty freshly bloomed pistils were collected from each treatment and fixed with FAA solution (50 % ethanol:acetic acid:formalin = 90:5:5 v/v). Another 30 pistils per treatment were sampled three d after pollination and fixed with FAA solution. The samples were dehydrated with an ethanol-

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butanol series, embedded in paraffin blocks, and sliced into 14-16-μm-thick cross sections with a microtome. The sectioned pistils sampled on the day of blooming were stained with Schiff's reagent and alcian blue to observe TT development. Sections of pistils three d after pollination were stained with aniline blue to count the number of pollen tubes at various parts of pistil under a fluorescent microscope with time lapse after pollination.

In 2004, 4-year-old Dealware vines planted in the Experimental Vineyard of the Okayama University were used. Fifteen vines were divided into 3 groups, each of which was used for the normal GA treatment on May 3, the late GA treatment on May 10, and the untreated control. Each cluster was pollinated with Muscat of Alexandria pollen at full bloom. Thirty pistils were collected from each treatment three d after pollination and fixed with FAA solution. After being rinsed in running water, pistils were torn longitudinally separating both septa in an ovary using forceps under a binocular. After staining with aniline blue, morphology of pollen tube tips, found on the septum surface, were photographed under a fluorescence microscope. The percentage of seedless berries was calculated for 10 clusters per treatment at harvest.

Results and Discussion

Seedless berry set: The number of total berries per cluster was larger in normal GA treatment (average 102.2 berries) than in late treatment (86.2) or control (51.3). The percentage of seedless berries in normal GA-treated clusters were higher than 94 % in both seasons, indicating that such clusters are acceptable as 'seedless clusters' on most Japanese markets. In the late GA treatment, however, the seedless percentage was as low as about 60-74 %, indicating that this treatment was insufficient for inducing seedlessness.

Such differences in GA efficacy in terms of treatment time have been reported for Delaware (ITAKURA *et al.* 1965) and for Muscat Bailey A (MIURA and OKAMOTO 2004) grapes.

TT development: 2-6 layers of TT cells were found in upper and middle ovary and only one to three layers in lower ovary in each treatment. No significant difference in the TT cell layer number was detected among GA-treated and control pistils in various parts of ovaries.

The anatomy of TT development in grape pistils has been studied by OKAMOTO *et al.* (2001 a) for 11 cultivars grown in Japan. The numbers of TT cell layers in Delaware ovaries, counted in this study, are similar to those of several diploid cultivars that were investigated, indicating that TT development in Delaware grape pistils is obviously normal. OKAMOTO *et al.* (2001 b) reported that both TT development and pollen tube growth in cv. Pione pistils were improved when the shoot was less vigorous. This finding indicates that TT development in grape pistils is possibly modified by cultivation conditions. However, pre-bloom GA treatment of Delaware grape clusters did not affect TT development in the present study, suggesting that the seedless berry production in GA-treated Delaware clusters is not directly related to TT development.

Pollen tube growth in pistil: As shown in the Table, pollen tube numbers in middle style were sig-

Table

Effect of pre-bloom GA treatment on pollen tube growth with time lapse after pollination in Delaware grape pistils*)

Pistil part and treatment	Hours of polination 8	16	24	48	72
Middle style					
Normal GA	6.2 b	10.2 b	9.8 c	11.1 b	22.0
Late GA	7.1 b	12.4 b	17.8 b	15.9 b	20.0
Control	11.8 a	17.6 a	31.1 a	30.9 a	28.3
Upper ovary					
Normal GA	0.3 b	1.9 c	0.9 b	3.7 b	6.0 b
Late GA	2.9 ab	9.0 b	9.2 a	9.7 a	10.6 ab
Control	5.1 a	11.0 a	12.1 a	9.1 a	12.3 a
Middle ovary					
Normal GA	0.0	0.1 c	0.4 c	1.5 b	2.5 b
Late GA	0.1	1.8 b	1.8 b	2.8 ab	4.3 ab
Control	0.6	2.9 a	3.1 a	3.2 a	5.1 a
Micropyle					
Normal GA	0.00	0.00	0.00	0.00 b	0.00 c
Late GA	0.00	0.00	0.02	0.04 b	0.08 b
Control	0.00	0.04	0.08	0.28 a	0.32 a

*) Presented as the average number of pollen tubes in cross sections of various parts of pistils. 20-25 pistils were examined. Values having same alphabets in each part and each hour are not significantly different (by T-test, $p < 0.05$).

nificantly smaller in normal and late GA treatments than those of the control 8 h after pollination. The numbers were increased thereafter but the increase was retarded in normal and late GA treatments 16-48 h after pollination. At the upper and middle parts of ovary, pollen tube number in normal GA treatment was small 16 and 24 h after pollination, thereafter the number increased to some extent. Pollen tube growth into micropyles was severely inhibited by both normal and late GA treatments and no tubes reached the micropyle even 72 h after pollination in normal GA treatment. It is true that the number of pollen tubes reaching the middle ovaries and the micropyle were increasing continuously even at final pistil sampling, 72 h after pollination. We examined again pollen tube growth until 5 d after pollination in 2004 and no tube penetrating into micropyle was found in normal treatment pistils (data not shown). We suppose that pollen tube penetration into micropyles in GA-treated pistils may not be restored even 72 h after pollination.

The finding that a very small number of pollen tubes reached the lower ovary and no pollen tube penetrated the micropyle in normal GA treatment are proof that the GA treatment directly inhibited pollen tube growth in pistils.

Morphology of the pollen tube tip: Most pollen tubes in style grew down straightly through TT in each treatment. In ovarian tissue, pollen tubes in untreated control pistils elongated almost normally along the surface of TT. By contrast, in GA-treated pistils, we observed at high percentages of abnormal pollen tube tips, coiled up and apparantly ceasing elongation (Figure). In upper, middle and lower parts of ovary, 71-82 % of pollen tube tips were coiled up in the normal GA treatment and 50-70 % in the late GA treatment. By contrast, only 8-16 % of total tips were coiling in control pistils. This indicates that pre-bloom GA treatments had caused some biochemical changes inside and/or outside TT in ovaries, which results in abnormal coiling of pollen tube tips and stop of pollen tube elongation. Okamoto *et al.* (2002) extracted the TT extra-cellular matrix (TT-ECM) from cv. Pione grape pistils using an apoplast extraction method and detected inhibitory activity for pollen tube growth. Further investigations should be conducted to elucidate the effect of pre-bloom GA treatments on pollen tube growth inhibitors in TT-ECM of Delaware pistils.

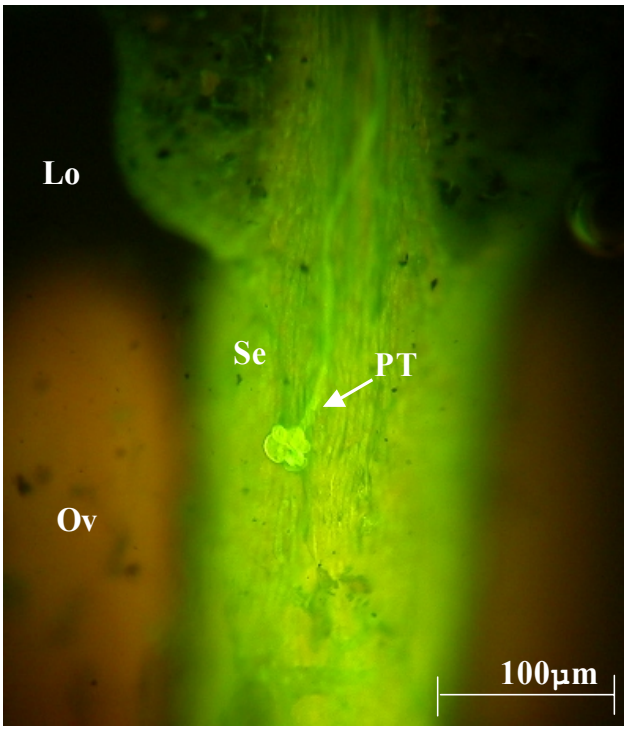

Figure: Coiled pollen tube tip in GA-treated Delaware pistil. The pistil was sampled 3 d after pollination, torn longitudinally to separate both septa, and stained with aniline blue. Lo: locule, Se: septum, PT: pollen tube, Ov: ovule (x 40).

Conclusions

The seedless berry set in Delaware grape clusters can be induced by a pre-bloom GA treatment 14 d before FB. GA treatment does not negatively affect TT development. In Delaware grapes, most pollen tubes were found at the surface of septum and not in TT, even in untreated pistils. This suggests that TT in Delaware pistils originally have only weak function for pollen tube growth. On the other hand, the pre-bloom GA treatment caused significant inhibition of pollen tube penetration into ovarian tissues even if the stigmas were pollinated with potent pollen grains with high germinability. The GA treatment may produce PGI(s) that cause coiling of pollen tube tips resulting in the entire inhibition of pollen tube growth. Further analytical works to identify the PGI should be conducted.

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