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Gibberellic acid impairs fertilization in Clementine mandarin under cross-pollination conditions

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ABSTRACT

We investigated the effect of gibberellic acid (GA_3) in the fertilization of Clementine mandarin cv. 'Clemenules' (*Citrus clementina* Hort. ex Tan.), a parthenocarpic variety that produces seedless fruit due to its self-incompatible nature, but yields seedy fruits when grown under cross-pollination conditions.

Experiments were conducted with on-tree 'Clemenules' flowers and 'Fortune' mandarin pollen (*C. clementina* Hort. ex Tan. × *C. reticulata* Blanco), which is sexually compatible with the former. Preanthesis treatment at −2 days after anthesis (−2 DAA) enhanced ovule abortion in both unpollinated and cross-pollinated (at +2 DAA) flowers. In the latter, the number of pollen tubes reaching the ovules was significantly reduced although pollen grains were not treated; thus, fertilization was partially avoided and seed set was reduced. When GA_3 was applied at anthesis (0 DAA) at the time of pollination, ovule abortion was again enhanced, and pollen tube growth was completely arrested; thus, fertilization was prevented and seed set was impeded. When GA_3 was applied 24 h after pollination (+1 DAA in flowers pollinated at anthesis), pollen tube growth was impaired but not arrested and ovule abortion was enhanced; therefore, fertilization was not prevented but impaired.

We conclude that, when applied the days around anthesis, GA_3 (10 mg l^{-1}) impairs fertilization by either enhancing ovule abortion or reducing pollen tube growth, in 'Clemenules' flowers under cross-pollination conditions. The intensity of the response depends on the physiological flower state at the moment of treatment.

1. Introduction

Clementine (*Citrus clementina* Hort. ex Tan.) is a parthenocarpic mandarin that produces seedless fruit due to its self-incompatible nature. However, Clementine mandarin may produce seedy fruit if there are compatible pollen sources nearby, and seeds dramatically reduce fruit quality and growers' returns. Seed set under cross-pollination conditions is higher in Clementine mandarin than in other parthenocarpic species such as the male sterile cultivar Satsuma mandarin (*Citrus unshiu* Marc.). This has been directly related to differences in their effective pollination periods (EPPs), which is shorter for Satsuma mandarin given its short ovule receptive period [1].

Satsuma and Clementine mandarins also differ in their parthenocarpic ability. Satsuma mandarin is characterized by a high degree of natural parthenocarpy and fruit set, whereas Clementine mandarin is almost incapable of setting seedless fruit. This has been directly related to levels of endogenous 13-hydroxy-gibberellins (GAs) during anthesis, being higher in Satsuma than in Clementine mandarin [2]. Moreover, Gibberellic acid (GA_3) improves parthenocarpic fruit set in Clementine but has little influence in Satsuma [2].

The question is if these endogenous levels of GAs in the flower during anthesis also influence the EPP and, therefore, determine fertilization and seed set under cross-pollination conditions.

Although no studies have been done yet for *Citrus* specifically, GAs have been related to pollen and ovule development. It has been explained the physiological role of endogenous GAs, promoting pollen germination and tube growth in *Arabidopsis thaliana* [3], tomato (*Lycopersicon esculentum*) [4] and *Petunia hybrida* [5]. Also studied has been the effect of exogenous GAs promoting or inhibiting pollen development *in vitro*, depending on the species, the concentration applied and the germination medium [5,6]. Experiments conducted *in vivo* revealed that GA_3 applied to grape (*Vitis labrusca*) flowers before or during anthesis severely inhibited pollen germination and pollen tube growth [7,8].

Further, GAs are related to ovule degeneration. Ovary levels of GAs in sweet cherry (*Prunus avium*) are increased by high spring

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temperatures inducing early embryo sac degeneration; ovule abortion was also induced by GA_3 applied on bursting buds and counteracted with paclobutrazol [9], a GA synthesis inhibitor. This effect of GA_3 on ovule abortion was also observed in grapes (*Vitis* spp.) [7,8].

In brief, seedless mandarins with high parthenocarpic ability and low seed set under cross-pollination conditions (e.g. Satsuma) are characterized by a high ovary level of GAs during anthesis [2] and a short EPP [1], while seedless mandarins with low parthenocarpic ability and high seed set under cross-pollination conditions (e.g. Clementine) are characterized by low ovary level of GAs during anthesis [2] and a long EPP [1]. Moreover, applied GAs have been proved to control pollen and ovule development in many species, but as of yet, not in *Citrus*. Thus, the main purpose of this study was to determine the effect of GA_3 in the fertilization of Clementine mandarin under cross-pollination conditions.

2. Materials and methods

Experiments were conducted on adult trees (>15 years old) from the self-incompatible Clementine mandarin, cv. 'Clemenules' (*C. clementina* Hort. ex Tan.), grafted onto Carrizo citrange rootstock [*C. sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.], grown in a loamy clay soil with drip irrigation and under standard cultural conditions. Pollen from 12-year old 'Fortune' mandarin trees (*C. clementina* Hort. ex Tan. × *C. reticulata* Blanco), which is sexually compatible with 'Clemenules' mandarin, was used as pollinator.

Trees were placed in the germplasm bank of *Citrus* located in the Instituto Valenciano de Investigaciones Agrarias (I.V.I.A.), Moncada (Valencia), Spain, latitude 39°35′N, longitude 0°23′W and altitude 65 m, and in a commercial orchard located in Kiyú, Uruguay, latitude 34°39′S, longitude 56°45′W and altitude 35 m.

In the experiment I (Uruguay, 2004), unpollinated flowers were treated with 10 mg l^{-1} GA_3 before anthesis (−2 days after anthesis, DAA) to determine the effects of GA_3 on pistil and ovule longevity. In the experiment II (Spain, 2005 and 2006), flowers were treated with 10 mg l^{-1} GA_3 before anthesis (−2 DAA in 2005 and −7 DAA in 2006). To determine the effect of the treatment on fertilization without treating pollen grains, cross-pollination was carried out +2 DAA. Finally, in the experiment III (Spain, 2006), flowers and pollen grains were treated with 10 mg l^{-1} GA_3 together with pollination at anthesis (0 DAA) or 1 day later (+1 DAA) to identify the effect of GA_3 on pollen tube growth, ovule longevity and fertilization. Flowers were always emasculated and bagged to avoid undesirable pollination.

Provided that *Citrus* fruit set is highly dependent on the type of inflorescence [10,11], only single-flowered leafy shoots from 'Clemenules' mandarin trees were randomly selected for the experiments. A minimum of 60 flowers per treatment distributed all around the canopy in three trees per experiment were used. To monitor the rate of ovule abortion in the first experiment, 12 flowers per treatment and collecting dates were fixed in FPA (10% formaldehyde, 10% propionic acid, 80% ethanol at 70%) at 0, 3, 6, 9 and 12 days after pollination. In the second and third experiments, 50 flowers per treatment were allowed to reach fruit maturity for seediness evaluation, and 10 flowers per treatment were fixed in FPA +12 DAA (second experiment) or every 3 days from anthesis to +12 DAA (third experiment) to evaluate pollen grain germination, pollen tube development and ovule abortion and/or fertilization.

The pollen tubes in the stigma, style and ovules were monitored on squash preparations of pistils previously softened in 5% sodium sulphite in a microwave for 1.5 min. They were stained with 0.1% aniline blue in 0.1N PO_4K_3 [12] and observed under an Olympus BX50 (Tokyo, Japan) fluorescence microscope using a U-MWU filter. The frequency of germination on the stigma was evaluated by counting 200–300 pollen grains per flower. In all the experiments, a pollen grain was considered to be germinated when the pollen tube length was larger than the pollen grain diameter [13]. Pollen tube growth in the style was established as the percentage of the style traversed by the longest pollen tube in each flower. The percentage of ovules with pollen tubes reaching the micropyle was calculated from 10–15 ovules per flower. Ovule degeneration was assessed as the percentage of ovules with a callose layer at the chalazal end by counting 10–15 ovules per flower [14–16].

The day before pollination, 'Fortune' mandarin flowers at the balloon stage and just prior to anthesis were randomly selected for pollen collection. Anthers were removed and placed on a piece of paper at room temperature to dry and trigger dehiscence. All pollinations were performed using a small brush.

Treatments were carried out using a hand sprayer and a non-ionic wetting agent (nonylphenylpoly-ethyleneglycol ether, 20%, w/w) at 0.01% was added to the GA_3 (Berelex 1.6% a.m, Syngenta, Italy) solution.

Analysis of variance was performed on the data ($P \leq 0.05$) using the Duncan multiple range test for means separation. Percentages were transformed to arc sin to homogenize the variance.

3. Results

3.1. Ovule abortion and pollen development

When pollination is not accomplished in 'Clemenules' mandarin, the ovules abort and parthenocarpic fruit growth begins. In our first experiment (2004), we studied the effect of GA_3, applied at preanthesis stage (−2 DAA), on ovules from emasculated and non-pollinated flowers. The rate of ovule abortion is given in Fig. 1. Ovules from control flowers did not present symptoms of degeneration until +12 DAA when 10% of ovules per ovary had callose layering at the chalazal end (Fig. 1). When GA_3 (10 mg l^{-1}) was applied −2 DAA, the rate of ovule abortion was significantly accelerated (Fig. 1).

The induction of ovule abortion triggered by preanthesis application of GA_3 was used in a second experiment (2005) to interfere in flower fertilization. GA_3 10 mg l^{-1} was applied −2 DAA, and +2 DAA flowers were cross-pollinated. The rate of ovule abortion was significantly increased with the GA_3 application. Thus, +13 DAA 40% of the ovules from treated flowers showed signs

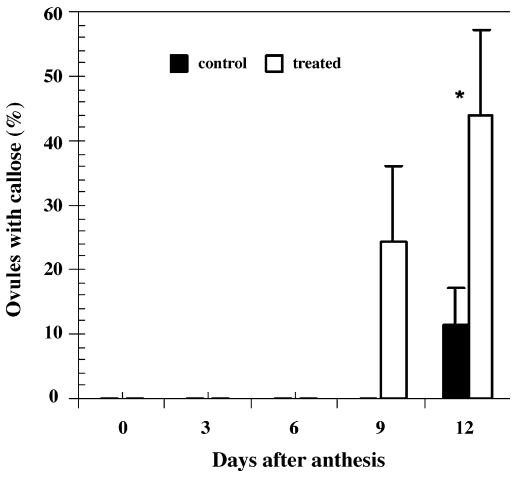

Fig. 1. The rate of ovule abortion is modified by GA_3 (10 mg l^{-1}) in unpollinated flowers of 'Clemenules' mandarin. Treatment was applied −2 DAA. Values are means ± S.E. of 10–15 ovules per flower in 10 flowers per day and treatment, and correspond to experiment I (2004); *Significant differences ($P \leq 0.05$; Duncan test).