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Research article

Pollen germination, early pollen tube growth and ovary development of *Dendrobium* orchid: Dependence on auxin and ethylene

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Pollination

Abstract

Importance of the work: Placing pollinia of some *Dendrobium* cultivars on the stigma of different cultivars may result in no growth of pollen or the ovary.

Objectives: To clarify the requirements of auxin and ethylene for growth of pollen and ovary of *Dendrobium* after pollination.

Materials & Methods: Pollinia of *Dendrobium* 'Pompadour' were placed on the stigma surface of 'Sonia Bom #28' flowers with and without inhibitors of auxin and ethylene. Pollen germination and growth of pollen tube and ovary were monitored. The auxin (indole acetic acid; IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC) contents in the pollinia of 'Pompadour' and 'Sonia Bom #28' were determined.

Results: Pollen germination started within 1 d of placement and the pollen tubes and ovary then grew rapidly. Free auxin (IAA) content in the pollinia of 'Pompadour' was significantly higher than that in the pollinia of 'Sonia Bom #28', while the concentrations of ACC in the pollinia of both cultivars were not significantly different. Pollen germination, pollen tube growth and ovary growth were all inhibited after applications of aminooxyacetic acid (AOA) and 1-methylcyclopropene (1-MCP). Similarly, pollen germination, pollen tube growth and ovary growth were inhibited by alpha-(*p*-chlorophenoxy) isobutyric acid (CPIBA). AOA and CPIBA also inhibited naphthaleneacetic acid (NAA)-induced ovary growth and delayed post-pollination development induced by NAA.

Main finding: Auxin in the pollinia was required for normal pollen germination, the growth of the pollen tube and the growth of the ovary of *Dendrobium* orchid after pollination.

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Introduction

While *Dendrobium* species are native to tropical and subtropical Asia, many Pacific islands, and Australia. The with the the genus occurs in diverse habitats throughout much of south, east and southeast Asia, including China, Japan, India, the Philippines, Indonesia, Australia, New Guinea, Vietnam and many of the islands of the Pacific (Dockrill, 1992). *Dendrobium* is the second largest genus of the Orchidaceae family and was established by Olof Swartz in 1799; today it contains about 1,500 species (Lavarack et al., 2006). *Dendrobium* plants are usually epiphytic (Arditti, 1992). *Dendrobium* species have an important role in the floral industry as a cut flower in ASEAN countries (De et al., 2019). New *Dendrobium* hybrids produce easily from crossings between cultivars of *Dendrobium* resulting in various colourful flowers of new hybrids that are very attractive to consumers (Kamemoto et al., 1999; Kuehnle, 2006). However, some crosses are more successful than others and the underlying mechanisms behind such variation are poorly understood, which hinders some breeding efforts.

Orchid pollinia of many species usually contain detectable amounts of both auxin and 1-aminocyclopropane-1-carboxylic acid (ACC), the direct precursor of ethylene. An apparent exception is *Phalaenopis*, whose pollinia contained ACC but the auxin level was below the detection limit (Strauss and Arditti, 1982; Zhang and O'Neill, 1993; Porat et al., 1998; Ketsa et al., 2001). Shortly after pollination, the orchid pollen germinates and the pollen tubes elongate during the next few days. However, *Dendrobium* is unusual in that it takes more than 7 d before the first pollen tube enters the stigma (Luangsuwalai et al., 2008). The role of several growth-regulating substances on pollen germination and pollen tube elongation has been reported in several plant species. For example, indole acetic acid (IAA) and indole-3-butyric acid (IBA) were found to effectively increase pollen germination for both *Bauhinia purperia* and *B. racemosa*, whereas gibberellic acid (GA_3) and kinetin were found suitable for *Spathodea campanulata* (Kumar et al., 2016). Wu et al. (2008a) found that IAA, GA and zeatin increased pollen tube growth of *Torenia fournieri* with IAA being the most effective. Similarly, IAA, GA_3 and ethylene increased pollen germination and pollen tube growth of groundnut (Malik and Chhabra, 1976), bulbous plants (Piskornik, 1986) and almond (Radović et al., 2016). *p*-Chlorophenoxyisobutyric acid (PCIBA) is known to reduce the effects of auxins, probably by activating an auxin oxidase (Frenkel and Haard, 1973).

The current study of *Dendrobium* applied: 1) AOA, an inhibitor of ACC synthase (Baker et al., 1982); 2) 1-MCP, an inhibitor of ethylene action (Blankenship and Dole, 2003); 3) PCIBA, an auxin antagonist (Frenkel and Haard, 1973); or 4) naphthaleneacetic acid (NAA), a synthetic auxin, on the stigma, prior to pollination to investigate the roles of auxin and ethylene in pollen germination and the growth of the pollen tube and the ovary.

Materials and Methods

Plant material

Inflorescence samples of *Dendrobium* 'Sonia Bom #28' and 'Pompadour' were purchased from a local commercial grower in Bangkok suburban, Thailand. The samples were harvested in the morning when they had 5–6 open flowers and 5–7 flower buds. They were transported to the laboratory within 2 hr after harvest. The stem and attached flower buds were cut off and only the first-five open flowers were used in the various experiments. Each inflorescence stem was cut at an angle 12 cm from the basal end of the first open flower and the stem was then placed in a 15 mL centrifuge tube containing 10 mL of distilled water. The inflorescence samples were held under natural light conditions at room temperature (25 ± 1°C, 79 ± 2% relative humidity, RH).

Pollination

Based on information reported by Wisutiamonkul (2001), pollinia from flowers of 'Sonia Bom #28' when placed onto the stigma of the same flowers of 'Sonia Bom #28' (self-pollinated) showed no post-pollination development processes, while placing pollinia of 'Pompadour' onto the stigma of 'Sonia Bom #28' (cross-pollinated), showed post-pollination development processes. Therefore, in the current study, these two cultivars were chosen for study. Open flowers were hand-pollinated using forceps to remove four pollinia from individual open flowers of 'Sonia Bom #28', of other inflorescences or 'Pompadour' (without removing the anther cap and pollinia to avoid generating any wound ethylene) and placed on the stigma of 'Sonia Bom #28'. The non-pollinated flowers (control) were left without pollinia on the stigma.

Auxin-related treatments applied to the stigma

Whole inflorescence samples bearing only open flowers were either left unpalliated or were pollinated and treated with either distilled water as a control or with solutions of either naphthaleneacetic acid (NAA, 40 µg per flower), aminooxyacetic acid (AOA, 0.3 µmol per flower) or the auxin antagonist alpha-(*p*-chlorophenoxy) isobutyric acid (PCIBA, 10 µg per flower) using a micropipette to apply each solution to the stigma surface. For experiments involving the use of NAA, flowers were pollinated prior to the application of the solution. With the AOA and PCIBA treatments, flowers were pretreated with AOA or CPIB for 24 hr to ensure penetration of the inhibitors prior to pollination. Combinations of NAA followed by either AOA or PCIBA were included.

Inhibition of ethylene: 1-methylcyclopropene treatment

Inflorescence samples bearing only open flowers were held in water in centrifuge tubes and placed in a sealed plastic chamber (37 cm × 47 cm × 35 cm) at 25°C. Then, 1-MCP (EthylBlock, powder), calculated to result in 500 nL/L, was placed in a small glass bottle taped to the inside of the chamber wall. The chamber lid was sealed; then, 1 mL of water was injected into the glass bottle containing the EthylBlock powder, using a hypodermic needle through a septum in the lid. A substantial percentage of the 1-MCP was released immediately after the addition of the water. The chambers remained sealed for 4 hr. Control inflorescence samples were sealed in identical chambers. The inflorescence samples were removed from the chambers and placed individually in centrifuge tubes containing distilled water in a temperature-controlled room at 25°C.

Pollen germination, growth of pollen tube, and ovary development

To determine pollen germination, flowers were collected at intervals of 2 d, at the same time (10.00 AMs) each day. The tepals were cut off and the remaining material was placed in a solution of 37% formaldehyde-acetic acid-ethanol (1:1:9 volume per volume, v/v; FAA) for subsequent analysis. All four pollinia were removed from each individual flower kept in FAA and were placed on a microscope slide. A few drops of 0.1% safranin were placed on the sample which was then squeezed using a glass rod. The specimen was further squeezed under a cover glass. The preparation was examined under a light microscope at 80× magnification. The number of germinated pollen grains was assessed using one field of view and considering the 20 pollen closest to the position of the scale on the ocular micrometer.

The materials used for microscopic analysis of pollen tube growth and ovary development were collected at daily intervals, at the same time of day used for the pollen sampling. To determine the maximum pollen tube length of the germinated pollen, the field of view was divided into 10 segments with equal width, at both sides of the ocular micrometer (the segments were located between 0 and 10, 10 and 20, etc. on the 100 units micrometer scale, which was placed in the middle of the field of view). The length of the longest pollen tubes in each of these segments in the field of view was determined. If no germinated pollen grains were found in a segment, no length was noted and in such a case, the total number of 20 measured was reduced by 1. The angle of the ocular micrometer usually had to be adjusted to the axis of the pollen tube to read the pollen tube length. Fluorescence microscopy of the pollen tubes used the method described by Kho and Bear (1968).

Pollen germination and pollen tube length determinations were carried out on five flowers per treatment, using a randomly chosen field of view for each flower.

Symptoms of senescence

Changes were recorded daily in the physical appearance of the pollinated flowers and the time to the onset of senescence, as indicated by either epinasty (the pollinated flowers turn upside down), venation, labellum yellowing or petal water soaking of individual open flowers.

Extraction and analysis of unconjugated indole acetic acid

The free auxin (IAA) content in fresh pollinia was extracted and purified using two different methods: cold methanol as the extraction medium, following Abdel-Rahman et al. (1975) or using water as the extraction medium, following Porat et al. (1998). Free (unconjugated) IAA in the purified extract was determined using high-performance liquid chromatography (HPLC), with pure IAA as a standard in a Shimadzu series LC-VP chromatograph (Kyoto, Japan) with an ultraviolet detector (SPD-10Avp). Separation was carried out on an Alltima C18 column 5 µm inner diameter (250 mm × 4.6 mm; Alltech; Lexington, KT, USA) with a guard column (CLC-ODS4, 10 mm × 4 mm inner diameter) using 30% methanol and 0.8% acetic acid as the mobile phase. The flow rate was 1.5 mL/min

and the detection wavelength was 280 nm. The retention time for the IAA peak was 12.8 min. At least three biological replications were used. The pollinia fresh weight in each replication was 300 mg. Since one pollinium weighs about 0.263 mg, each replication contained about 1,140 pollinia. Internal standards for IAA were used to check the effect of the extract on IAA measurement.

Extraction and analysis of 1-aminocyclopropane-1-carboxylic acid (ACC)

ACC in fresh pollinia was extracted and analyzed according to Lizada and Yang (1979), as modified by Hoffman and Yang (1982). Pollinia were weighed (0.03 g) and ground in 5 mL of 9% trichloroacetic acid (TCA) using a mortar and pestle. After holding for 12 hr at 4°C, the extract was centrifuged at 12000×*g* (Jouan KR22i Centrifuge; Curis-au-Mont-d'Or, France) for 20 min to remove insoluble cellular debris. The supernatant volume was measured and the ACC content in the pollinia (or floral tissue) was analyzed as follows. A 0.5 mL sample of pollinia extract was placed in a 6 mL tube and 0.1 mL of $HgCl_2$ and 0.3 mL of distilled water were added. Then, the tube was sealed using a serum cap. Approximately 0.1 mL of an ice-cold solution of bleach base (two parts of 5.25% sodium hypochlorite and one part of a saturated solution of sodium hydroxide) was injected into the tube through the seal. The tube was vortexed briefly and placed on ice. After 5 min, the ethylene levels were measured in 1.0 mL of the headspace. To measure the efficiency of conversion of ACC to ethylene in the extracts, 0.5 mL of extract was spiked with 0.4 nmol of ACC to give a volume of 0.9 mL after the addition of the $HgCl_2$. Ethylene production was measured after the addition of the bleach base solution, as described above. The conversion efficiency in freshly made floral extracts was close to 100%. The ACC content was expressed as nanomoles of ACC formed per gram fresh weight (FW).

Statistical analysis

Five replicate inflorescence samples were used in each treatment, with five open flowers per sample. Where possible, analysis of variance was performed and then followed by mean comparisons using Duncan's multiple range test (DMRT) and a t test as necessary using the SPSS Statistics version 23.0 software (IBM Corp.; New York, NY, USA). Each experiment was repeated at least twice. The tests were considered significant at $p < 0.05$.

Results

Pollen germination and growth of the pollen tube and the ovary

Pollen germination was low after self-pollination of 'Sonia Bom #28' flowers with pollinia from 'Sonia Bom #28'. In contrast, pollination of 'Sonia Bom #28' flowers with pollinia from 'Pompadour' resulted in a high rate of pollen germination (Table 1). Pollen tube growth of both 'Sonia Bom #28' and 'Pompadour' did not enter the stigma until day 3. Pollen tube growth of 'Pompadour' started to enter the stigma on day 3 and grew slowly from day 3 to day 4, then increased rapidly until day 7, whereas pollen tube growth of 'Sonia Bom #28' remained unchanged until day 5, then increased slowly thereafter (Fig. 1).

1-MCP and PCIBA inhibited pollen tube growth of 'Pompadour' until day 4 and pollen tube growth then increased rapidly from day 4 to day 5 and subsequently increased gradually until day 7 (Fig. 1). Treatment of the stigma of 'Sonia Bom #28' with AOA considerably reduced the growth of 'Pompadour' pollen with pollen tube growth increasing from day 5 to day 7. By day 7 (Fig. 1), the magnitude of reduction of pollen tube growth by 1-MCP, PCIBA and AOA was not significantly different, but germination and tube growth of the 'Pompadour' pollen were reduced significantly (Table 1).

Table 1 Pollen germination and pollen tube growth at 7 d after pollination in *Dendrobium* 'Sonia Bom #28' flowers pollinated either with 'Sonia Bom #28' pollinia or with pollinia of 'Pompadour', with and without prior treatment of stigma with auxin inhibitor chemicals or after treatment of the flowers with 1-MCP

Treatment	Pollen germination (%)	Pollen tube length (mm)
Pollinated with 'Sonia Bom #28' pollinia (control)	10±0.9^{d}	0.11±0.03^{c}
Pollinated with 'Pompadour' pollinia	81±1.2^{a}	0.53±0.02^{a}
Pollinated with 'Pompadour' pollinia, after prior treatment of flowers with 500 nL/L 1-MCP	57±1.1^{b}	0.26±0.02^{b}
Pollinated with 'Pompadour' pollinia, after 0.3 µmol AOA placed on stigma	50±1.5^{bc}	0.29±0.02^{b}
Pollinated with 'Pompadour' pollinia, after 10 µg PCIBA placed on stigma	50±1.4^{bc}	0.27±0.03^{b}

1-MCP = 1-methylcyclopropene; AOA = aminooxyacetic acid;
PCIBA = alpha-(*p*-chlorophenoxy) isobutyric acid.
Mean±SD within a column superscripted with different lowercase letters are highly significantly ($p < 0.01$) different

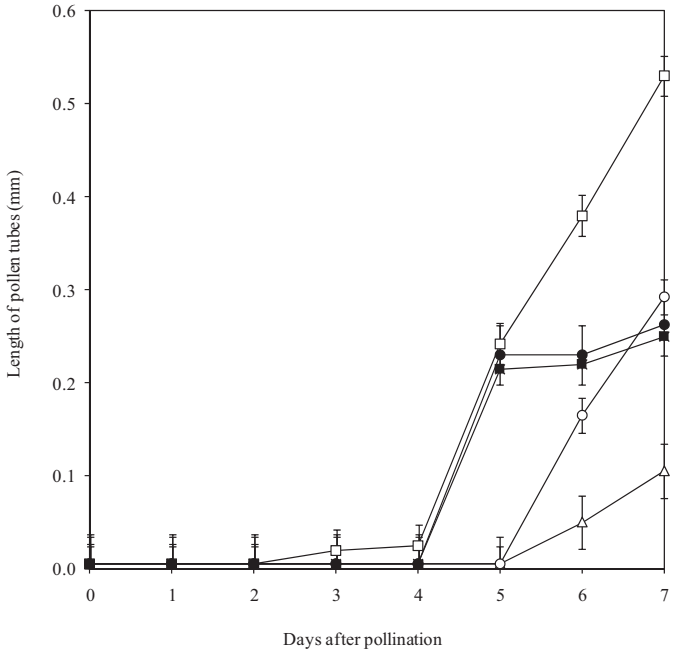

Fig. 1 Pollen tube growth of pollinia from 'Sonia Bom #28' (△) and 'Pompadour' (□) on stigma surface of *Dendrobium* 'Sonia Bom #28' with either 0.3 µmol aminooxyacetic acid (○), 500 nL/L 1-methylcyclopropene (■) or 10 µg alpha-(*p*-chlorophenoxy) isobutyric acid (●) applied prior to pollination with pollinia from 'Pompadour' and results are means of 3–5 pollen tubes ± SD

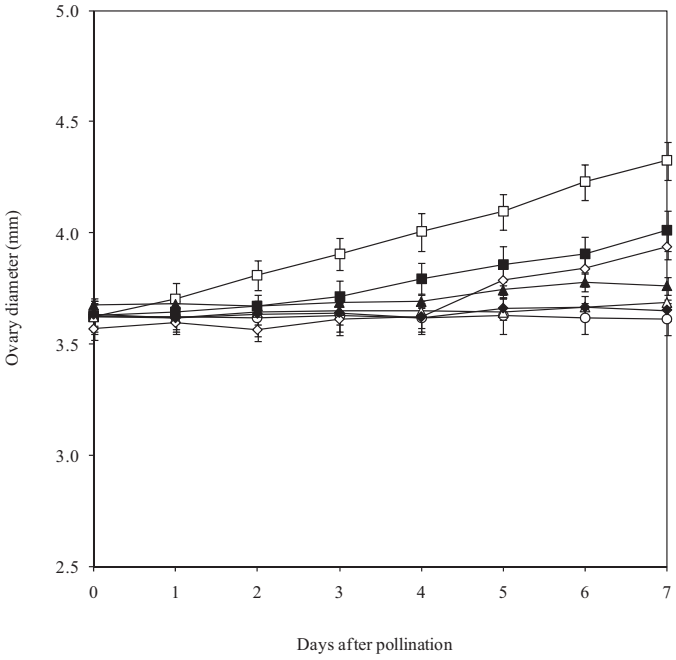

Fig. 2 Ovary growth of *Dendrobium* 'Sonia Bom #28' without pollination (○), pollinated with 'Pompadour' pollinia (□), or with and without chemicals prior to pollination, for non-pollination with 40 µg naphthaleneacetic acid (NAA, ■), 0.3 µmol aminooxyacetic acid + pollination (△), 0.3 µmol aminooxyacetic acid + 40 µg NAA (▲), 10 µg alpha-(*p*-chlorophenoxy) isobutyric acid (CPIBA) + pollination (◊) and 10 µg PCIBA + 40 µg NAA (◆) and results are means of 25 flowers ± SD

The ovary diameter of flowers of 'Sonia #28' without pollination (control) remained unchanged over the experimental period. In contrast, the ovary diameter of the flowers of 'Sonia #28' pollinated with pollinia from 'Pompadour' resulted in a steady increase in the diameter of the ovary over the 7-d period of the experiment (Fig. 2). Treatment of the non-pollinated stigma of 'Sonia Bom #28' with NAA also increased ovary diameter throughout the 7-d period of the experiment but by a lesser amount than occurred with pollination alone. Both AOA and PCIBA, when applied with pollination, prevented both the effect of NAA and the pollination-induced ovary growth (Fig. 2). Treatments of the stigma of 'Sonia Bom #28' with either AOA or PCIBA, before the application of NAA, also significantly reduced ovary growth induced by NAA (Table 2).

Post-pollination response

Post-pollination symptoms of pollinated flowers including epinasty, color of labellum, and ovary growth) were apparent significantly earlier compared with those of non-pollinated (control) flowers (Fig. 3). Similarly, NAA treated flowers rapidly developed post-pollination symptoms similar to those from pollination. Pollinated *Dendrobium* 'Sonia Bom #28' flowers that had been treated with either PCIBA or AOA before pollination or in combination with NAA treatment but no pollination, had significantly delayed development of visible post-pollination symptoms (Table 3).

Levels of auxin and ACC in the pollinia

The IAA concentrations in the pollinia of 'Sonia Bom #28' and 'Pompadour' were 64.7 and 139.8 µg/g FW, respectively, while the ACC concentrations were 585.5 and 575.4 µmol/g FW, respectively. The IAA concentration in the pollinia of 'Pompadour' was significantly higher than that of 'Sonia Bom #28', while differences in ACC concentration between the two cultivars were non-significant (Fig. 4).

Table 2 Ovary growth based on diameter in *Dendrobium* 'Sonia Bom #28' either cross-pollinated with pollinia of 'Sonia Bom #28' or pollinia of 'Pompadour', with and without prior treatment of stigma with auxin and ethylene inhibitor chemicals

Treatment	Ovary diameter (mm)	
	Day 0	Day 7
Non-pollinated (control)	3.62±0.07	3.61±0.07^{d}
Pollinated with pollinia from 'Pompadour'	3.63±0.08	4.33±0.09^{a}
Non-pollinated + 40 µg NAA	3.63±0.05	4.01±0.09^{b}
Cross-pollinated + 0.3 µg AOA	3.62±0.06	3.69±0.07^{d}
Non-pollinated + 0.3 µg AOA + NAA 40 µg	3.68±0.08	3.76±0.08^{cd}
Cross-pollinated + 10 µg PCIBA	3.57±0.05	3.94±0.06^{bc}
Non-pollinated + 10 µg PCIBA + 40 µg NAA	3.63±0.08	3.65±0.07^{d}
F-test	ns	**

NAA = naphthaleneacetic acid; AOA = aminooxyacetic acid;
PCIBA = alpha-(*p*-chlorophenoxy) isobutyric acid.
Mean±SD within a column superscripted with different lowercase letters are highly significantly ($p < 0.01$) different

Table 3 Visible post-pollination symptoms in *Dendrobium* 'Sonia Bom #28' flowers following pollination with 'Pompadour' pollinia and treatment with various auxin and ethylene inhibitor compounds

Treatment	Days after pollination to			
	Epinasty	Yellowish labellum	Venation	Water soaking
Non-pollinated (control)	9.32±0.8^{a}	-	13.60±0.7^{a}	-
Pollinated with pollinia from 'Pompadour'	2.20±0.6^{c}	4.00±0.4	4.00±0.6^{c}	8.00±0.7^{b}
Non-pollinated + 40 µg NAA	3.76±0.7^{bc}	4.00±0.3	4.40±0.5^{c}	8.00±0.7^{b}
Cross-pollinated + 0.3 µg AOA	5.32±0.7^{b}	–	9.56±0.9^{b}	11.40±0.9^{a}
Non-pollinated + 0.3 µg AOA + NAA 40 µg	8.72±0.8^{a}	–	10.00±0.8^{b}	12.00±0.8^{a}
Cross-pollinated + 10 µg PCIBA	2.12±0.8^{c}	4.00±0.3	4.00±0.5^{c}	7.56±0.7^{b}
Non-pollinated + 10 µg PCIBA + 40 µg NAA	3.72±0.6^{bc}	4.00±0.4	4.00±0.5^{c}	8.00±0.8^{b}
F test	**	ns	**	**

Water soaking = first symptoms of visible senescence; NAA = naphthaleneacetic acid, applied after pollination; AOA = aminooxyacetic acid; PCIBA = alpha-(*p*-chlorophenoxy) isobutyric acid, with both applied for 24 hr before pollination.
Mean±SD within a column superscripted with different lowercase letters are highly significantly ($p < 0.01$) different.

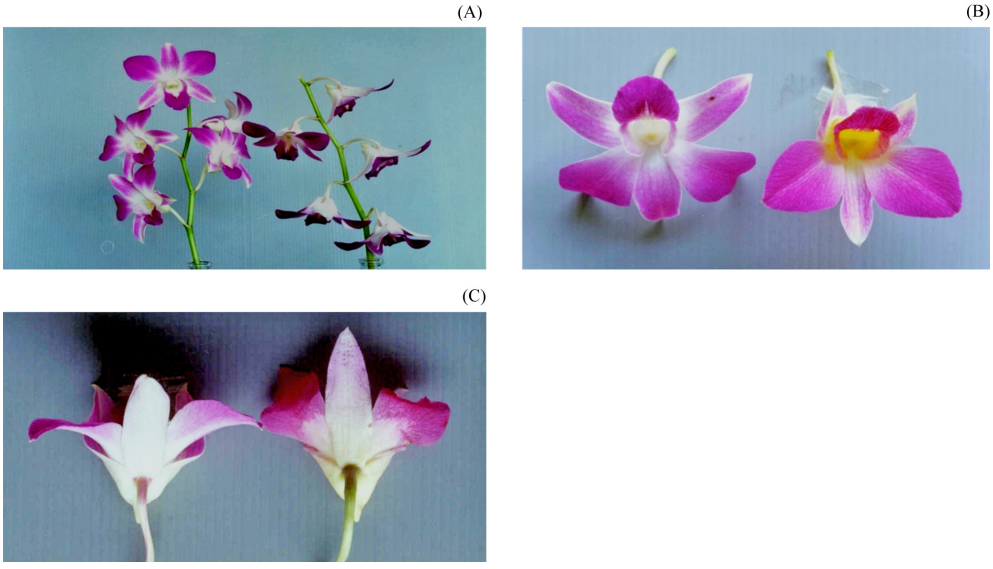

Fig. 3 Post pollination changes in *Dendrobium* 'Sonia Bom #28' flowers: epinasty (A), labellum color (B), ovary growth (C), where left image is non-pollinated and right image is pollinated with pollinia of 'Pompadour' flowers

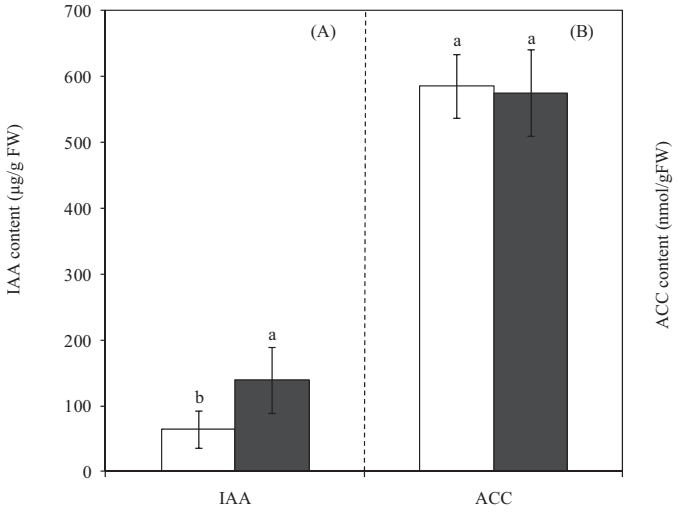

Fig. 4 Contents in the pollinia of *Dendrobium* 'Sonia Bom #28' (□) and 'Pompadour' (■): (A) indole acetic acid (IAA); (B) 1-aminocyclopropane-1-carboxylic acid (ACC), where FW = fresh weight, results are means of three replications ± SD and means not sharing the same lowercase letter are significantly different (using a t test) at $p = 0.01$

Discussion

In cross-pollination, germination of pollen grains within the pollinia started within one day of placement of the pollinia on the stigma surface. Then, the pollen tubes and the ovary grew rapidly. By the second day following pollination, the first pollen tubes had penetrated the pollinium envelope; by day 7, the first pollen tubes had entered the stigma concomitant with the development of post-pollination processes (Figs. 1–3, Tables 1–3) such as drooping, epinasty, labellum yellowing, venation, and ovary growth (Ketsa and Rugkong, 1999; Ketsa et al., 2001; van Doorn and Ketsa, 2021). While the self-pollinated samples, pollen germination and pollen tube growth were less significantly than for the cross-pollination samples (Table 1). The lower ACC concentration could explain, at least in part, the lack of post-pollination effects by these pollinia. However, ACC exogenously applied to the stigma of 'Pompadour' did not stimulate the ovary growth of pollinated flowers, although there have been reported that the ACC induced more ethylene production than for the pollinated orchid flowers and that eventually led to premature senescence of petals and sepals (Ketsa and Luangsuwali, 1996; Ketsa and Rugkong, 2000). This result showed that the enhancement of ovary growth by ethylene is dependent on the simultaneous presence, or action, of other factors (Zhang and O' Neill, 1993). Hormones play a major role in both pollen germination and pollen tube growth (Malik and Chhabra, 1976; Piskornik, 1986; Chauhan and Katiyar, 1998; Wu et al., 2008a, 2008b; Radović et al., 2016). The IAA concentration in the pollinia of 'Pompadour' was significantly higher (over two-fold) than in pollinia of 'Sonia Bom #28', while the ACC concentration in both of the cultivars was similar (Fig. 4). Pollen germination and pollen tube growth were markedly greater where 'Pompadour' pollen was used to pollinate 'Sonia Bom #28' (Table 1). This suggested that IAA rather than ACC in pollinia had an important role in promoting pollen germination and pollen tube growth in *Dendrobium* flowers. Treatments with the auxin antagonist (PCIBA) inhibited germination and tube growth of 'Pompadour' pollen, indicating that IAA in the pollinia was directly involved in the stimulation of pollen germination and pollen tube growth. Treatments with AOA, which inhibits ACC synthesis (Amrhein and Wenker, 1979; Baker et al., 1982) or 1-MCP, which blocks the ethylene receptor (Blankenship and Dole, 2003), also reduced pollen germination and pollen tube growth (Table 1). Similarly, pollen tube growth in petunia was inhibited by the ethylene-antagonists, 2, 5-norbornadiene (NBD) and 1-MCP, which were applied after compatible pollination (Holden et al., 2003). These current results indicated that IAA in the pollinia stimulated 'Pompadour' pollen germination and pollen tube growth via ethylene action. Pollen tube growth has been shown to be regulated by ethylene in a number of species (Dhawan and Malik, 1981; Zhang and O'Neill, 1993; Song et al., 1998; Latha and Jayasree, 2002) and both auxin and ethylene have been shown to be required for pollen germination and pollen tube growth. Pollen has been shown to contain both auxin and ACC (Barendse et al., 1970; Whitehead et al., 1983; Lindstrom et al., 1999; Ketsa et al., 2001) and ACC and ethylene have been shown to stimulate in vitro pollen germination in many species (Song et al., 1998; Yildiz and Yilmaz, 2002). Therefore, it was likely that the auxin and ACC contents in the pollinia of 'Pompadour' reflected the involvement of auxin and ethylene in the germination process, tube growth and ovary development in pollinated flowers of the *Dendrobium* orchid. Ethylene was also found to be important for pollen tube growth in *Phaleanopsis* (Zhang and O'Neill, 1993) and *Sphathoglottis* (Latha and Jayasree, 2002) orchids while, in contrast, it was shown that ethylene did not affect the rate of pollen tube growth of lily (Sfakiotakis et al., 1972) or petunia (Hoekstra and van Roekel, 1988). However, carbon dioxide with or without ethylene increased pollen tube length in lily (Sfakiotakis et al., 1972) and significantly increased pollen germination of cocoa, while ethylene was apparently not involved (Aneja et al., 1992).

The anti-auxin, 2, 3, 5-triiodobenzoic acid (TIBA), when applied to the stigma before pollination, reduced the effect of pollination on ovary growth (Ketsa et al., 2006). TIBA also prevented column growth and other post-pollination phenomena in pollinated flowers of *Dendrobium* (Luangsuwalai, 2007). In contrast, application of TIBA to the ovary of zucchini squash after anthesis, induced fruit set and early fruit growth (Martínez et al., 2013). Apparently, TIBA inhibited auxin efflux carrier activity (Al-Hammadi et al., 2003).

Overall, these results appeared to indicate that both auxin and ethylene were required for pollen germination and growth of the pollen tube and ovary in *Dendrobium*.

Following pollination, there is a rapid acceleration of senescence in the different floral parts, including epinasty, fading of the petals, wilting and water soaking of the tissues (Porat et al., 1995; Ketsa and Rugkong, 1999; Luangsuwalai et al., 2008). In the current study, NAA was able to produce most of the same effects as those of pollination on these post-pollination responses when 'Sonia Bom #24' was pollinated with 'Pompadour' pollinia, similar to that reported previously (Ketsa and Rugkong, 2000; Ketsa et al., 2001). PCIBA, an auxin antagonist, and 1-MCP and AOA, both ethylene inhibitors, were able to counteract the effects of pollination and of NAA application on the post-pollination responses that were recorded in the current study. This agreed with the hypothesis that auxin is required to act through ethylene in order to promote ovary growth (Ketsa and Rugkong, 2000; Ketsa et al., 2006). The unconjugated IAA and ACC in the pollinia may act in a coordinated manner and be the cause of the various physiological responses associated with senescence of the different flower parts, as suggested by Zhang and O'Neill (1993).

Taken together, the following can be concluded: the phases of gametogenesis (pollen germination and pollen tube growth) and ovary growth were partially inhibited by compounds that block ethylene synthesis and ethylene action, and by an auxin antagonist. This indicated that these developmental processes depended at least on unconjugated IAA located in the pollinia, which may act through ethylene or work in a coordinated manner. Epinasty and early petal senescence were induced by auxin application and blocked by an inhibitor of ethylene synthesis and action. Therefore, both epinasty and early senescence may, be induced by auxin, which acts through ethylene.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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