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Research article

1-Methylcyclopropene inhibits ethylene production and post-pollination changes of *Dendrobium* flowers

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Abstract

Importance of the work: Stimulation of ethylene production by pollination causes early flower senescence and the initiation of many post-pollination developmental processes.

Objectives: To investigate the effects of 1-methylcyclopropene (1-MCP) on the ethylene production and post-pollination changes of *Dendrobium* cv. Sonia Bom #17 florets following pollination.

Materials & Methods: Open florets were treated with 1-MCP prior to pollination and monitored for ethylene production, the 1-aminocyclopropane-1 carboxylic acid (ACC) content, activities of ACC synthase (ACS) and ACC oxidase (ACO), gene expression, epinasty of florets, yellowing of the labellum, perianth venation and water-soaking of the floret tissues after pollination.

Results: Treatment of florets with 1-MCP prior to pollination delayed epinasty of florets, yellowing of the labellum, perianth venation and water-soaking of florets after pollination. In addition, the 1-MCP treatment reduced ovary growth, ethylene production, ACC content and the activities of ACS and ACO in pollinated florets. The ACC content and ACO activity increased more in the column plus ovary than in the perianth of pollinated florets while ACS activity in the column plus ovary increased less than in the perianth. The *DenACO* gene in the perianth of non-pollinated florets with the 1-MCP treatment was expressed more highly than that of pollinated florets with the 1-MCP treatment. The *DenACO* gene was expressed slightly throughout the study period in the column plus ovary of pollinated florets with the 1-MCP treatment.

Main finding: 1-MCP treatment delayed perianth senescence and decreased ethylene production, ACC. ACS and ACO in pollinated florets. The column was the likely source of ethylene production, while the increased ethylene production might have been partially due to an increase in *DenACO* gene expression.

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Introduction

Pollination of flowering plants involves pollen dispersal, reception and fertilization that leads to embryogenesis and, eventually, to fruit development, while during the post-pollination phase, developmental processes are usually associated with senescence symptoms (Lord and Russell, 2002). In orchids, following flower pollination, symptoms of senescence rapidly occur leading to petal pigmentation changes, epinasty of the florets, venation in the perianth, wilting of the florets, water soaking of perianth tissues and the growth and development of the ovary (van Doorn and Ketsa, 2021).

Cross- or self-pollination with compatible pollinia of *Dendrobium* hybrids immediately produces ethylene and exhibits rapid ovary growth after pollination, whereas cross- or self-pollination with the incompatible pollinia does not (van Doorn and Ketsa, 2021). The symptoms of compatibly pollinated flowers showed premature senescence such as epinasty, drooping, venation, fading, labellum yellowing, petal senescence and ovary growth one day after pollination (Luangsuwalai, 2007). Pollination stimulates an increase in ethylene production via the induction of 1-aminocyclopropane-1 carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) activities (Porat et al., 1994; Hilioti et al., 2000; Ketsa and Rugkong, 2000), similar to ethylene treatment (Lerslerwong and Ketsa, 2008). Pollination has been shown to lead to an increase in ethylene production and the expression of ACS and ACO genes. For example, in pollinated *Phalaenopsis* orchid flowers, ACS activity increased in the stigma, the ovary and in labellum tissues (O'Neill et al., 1993). In addition, the ACS gene expressed at high levels in the gynoecium and labellum tissues approximately 18 h after pollination but, in contrast, was undetectable in the perianth (O'Neill, 1997). Pollination of carnation flowers has been shown to induce ethylene biosynthesis and expression of *DC-ACS1* in petals and *DC-ACS2* and *DC-ACS3* in styles (Jones and Woodson, 1999).

1-Methylcyclopropene (1-MCP) is an effective inhibitor of ethylene action (Blankenship and Dole, 2003) and has been shown to inhibit ethylene production (de Wild et al., 1999; Li et al., 2000) and to delay plant senescence (Serek et al., 1994; Chen et al., 2015; In et al., 2015). The current study investigated the effects of 1-MCP on the regulation of ethylene biosynthesis and on the post-pollination developmental processes of *Dendrobium* cv. Sonia Bom #17 open florets following pollination.

Materials and Methods

Plant material

Inflorescence samples of *Dendrobium* cvs. Sonia Bom #17 and White 5n were purchased from a local grower in Samut Sakhon province, central Thailand. The samples were harvested in the morning and had a combination of 5–8 open florets and 5–6 closed floral buds. The samples arrived at the laboratory within 2 h of harvest. Each inflorescence stem was recut at an angle at the basal end, 12 cm from the first open floret. Open florets from the first up to the fifth were retained and all other open florets and floral buds were removed. Each individual inflorescence with the retained open florets was held in a 15 mL centrifuge tube containing 10 mL of distilled water in natural light and at room temperature (25±1 °C, 75±3% relative humidity; RH).

1-Methylcyclopropene treatment

The 1-MCP was applied as a pre-treatment to both non-pollinated and pollinated florets 4 h prior to pollination. The non-pollinated (control) and pollinated inflorescences were placed separately, prior to pollination, in a sealed plastic chamber (37 cm × 47 cm × 35 cm) at 25 °C for 4 h. 1-MCP was generated by adding water to 1-MCP powder (EthylBloc®; Floralife Inc.; Walterboro, SC, USA), that was placed in a vial. Introducing water into the vial generated 1-MCP gas resulting in a final concentration of 500 μL/L of 1-MCP. Fans were used to maintain air circulation within the sealed chamber. The control inflorescences were held in the sealed plastic chamber for 4 h but were not treated with 1-MCP. Inflorescences were removed from the chambers and placed individually in centrifuge tubes containing distilled water and held in a temperature-controlled room at 25 °C at 75±3% RH and 15 μmol/m^2/s photosynthetic photon flux density from 1700 hours to 1900 hours at which time they were either pollinated or left non-pollinated.

Pollination

Based on a preliminary study, cross-pollination with compatible pollinia from many *Dendrobium* hybrids, including *Dendrobium* cv. White 5n, induced ethylene production and premature senescence. Therefore, pollinia of *Dendrobium* cv. White 5n were chosen for the current study. Open florets were cross-pollinated by hand by placing the pollinia from open florets of *Dendrobium* cv. White 5n onto the stigma of cv. Sonia Bom #17 without removing the anther cap or the pollinia of the receiving open floret. All open florets within an inflorescence were pollinated at the same time.

Ovary diameter and time to epinasty, floret drooping and perianth venation

The ovary diameter of individual florets of both non-pollinated and pollinated cv. Sonia Bom#17 was determined at the proximal end, using calipers. Orchid florets exhibited epinasty following pollination, which was measured as the distance between the lip and the peduncle (Ketsa and Rugkong, 1999). The time to epinasty, and the perianth venation of the petals and sepals in individual open florets were determined based on daily observations. The overall post-treatment life of an inflorescence was determined daily.

Ethylene production

Individually inflorescences with open florets were held in a vial containing distilled water and placed in airtight 4 L glass bottles fitted with gas sampling ports. Five bottles (inflorescences) were used per treatment. At various intervals, the bottles were sealed for 2 h after which a 1 mL gas sample was withdrawn from the headspace for ethylene measurement. A gas chromatograph equipped with a flame ionization detector (Shimadzu; Kyoto, Japan) was used for these measurements. Ethylene production was calculated and expressed as nL C_2H_4/g/h.

Extraction and analysis of 1-aminocyclopropane-1 carboxylic acid content (ACC), ACC synthase and ACC oxidase activity

ACC and ACS in the column plus ovary and in the perianth (petals plus sepals) were separately extracted (following Hoffman and Yang, 1982) from five individual florets on five separate inflorescences and assayed (following the method of Lizada and Yang, 1979). ACO was extracted and assayed following Kato and Hyodo (1999).

DenACO gene

A *DenACO* primer was obtained from BIOTEC, Thailand. A pair of forward (5’ ›ATGGAGCTTCTTGAGGGTTC‹ 3’) and reverse (5’ ›TCAAGCAGTAGGAATCGGCTG‹ 3’) primers for the *DenACO* gene (EF487343.1, *Dendrobium* cv. Karen) was used for semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR).

Semi-quantitative reverse transcriptase polymerase chain reaction

Senescence-related gene expression was performed using a semi-quantitative RT-PCR method, where the 820 bp cDNA fragments encoding ACO genes were amplified. The PCR conditions were: sample preheated at 94°C for 3 min followed by 35 cycles with a denaturing temperature set at 94 °C for 30 sec and an annealing temperature at 55 °C. The reaction was terminated with a final extension at 72 °C for 50 min. The PCR products were separated using electrophoresis at 100 V for 20 min and the gel was stained in 2.5 μg/mL ethidium bromide solution for 15 min. The DNA bands were visualized under an ultraviolet transilluminator and photographed using the BIO IMAGINE Gel Documentation system (Bio-Rad Laboratories, Inc., Hercules, California, USA). 18S rRNA was used as the normalization gene. The PCR conditions were: sample pre-heated at 94 °C for 5 min, followed by 28 cycles of denaturing temperature at 94 °C for 30 sec, an annealing temperature of 55 °C for 30 sec and an extension temperature at 72 °C for 30 sec.

Statistical analysis

A completely randomized design was used in this study. Five replicates per treatment, one inflorescence per replicate (bottle), were used to monitor ethylene production. Ten inflorescences per treatment were used to determine the progression of senescence. Five replicates per treatment and one inflorescence per replicate (bottle) were used to monitor ethylene production. All experiments were repeated at least once on a separate occasion, with very similar results. Data were investigated statistically based on analysis of variance and the statistical significance of the differences between means was estimated based on the least significant difference at $p < 0.05$, using the SPSS Statistics 23.0 software package (IBM; Armonk, NY, USA).

Results

Senescence

Inflorescences of *Dendrobium* cv. Sonia Bom #17 were pretreated with and without 1-MCP prior to pollination. Pollinated florets showed earlier senescence than pollinated florets that had been treated with 1-MCP prior to pollination

(Fig. 1). Epinasty, petal yellowing, perianth venation and water soaking of the tissues of non-pollinated florets all occurred more rapidly in pollinated than in non-pollinated florets (Table 1). The initial symptoms of epinasty and perianth venation occurred within 2 d of pollination. The times for the development of each of these symptoms were statistically the same in the control as in those florets treated with 1-MCP. In contrast, pollination negated the effect of 1-MCP (Table 1). The ovary diameter of the pollinated flowers was significantly larger than that of non-pollinated florets 7 d after pollination. The ovary growth amounts of non-pollinated and pollinated florets with 1-MCP treatment were not significantly different from that of non-pollinated florets (Table 2).

Ethylene production

Differences in the ethylene production of florets among treatments were not significant within 3 h of treatment. However, subsequent changes were rapid and significant. The ethylene production rate of the pollinated florets increased 14-fold above that of the non-pollinated controls and reached a maximum 15 h after pollination. Similarly, the ethylene production of the pollinated florets that had been pre-treated with 1-MCP increased to a maximum 15 h after pollination but the peak rate was lower than that of pollinated florets without 1-MCP treatment. The ethylene production of the non-pollinated control florets with and without 1-MCP treatment remained unchanged up to 12 h after pollination and then increased slightly to a maximum 15 h after treatment and then declined (Fig. 2).

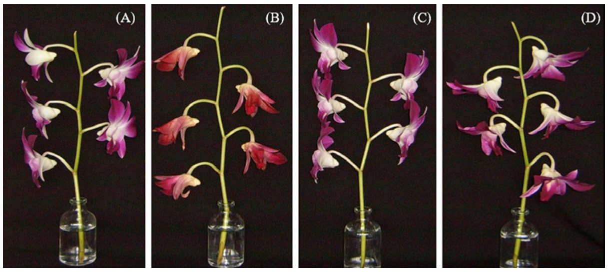

Fig. 1 Senescence of *Dendrobium* cv. Sonia #17 open florets using pollinia of cv. White 5n on day 7: (A) control; (B) pollinated; (C) 1-methylcyclopropene (1-MCP); (D) 1-MCP plus pollination

Table 2 Ovary diameter determined 7 d after pollination in *Dendrobium* cv. Sonia Bom#17 flowers using pollinia of cv. White 5n, with 1-methylcyclopropene (1-MCP) applied for 4 h before pollination.

Treatment	Ovary diameter (mm)	
	Day 0	Day 7
Control	3.49±0.06	3.41±0.29^{b}
Pollination	3.60±0.21	4.05±0.30^{a}
1-MCP	3.62±0.07	3.69±0.03^{b}
1-MCP + Pollination	3.55±0.08	3.71±0.16^{b}
Mean ± SD	3.56±0.28	3.71±0.07

Results are mean ± SD from five replicates and each replicate had two inflorescences.

[a] Values (mean ± SD) within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

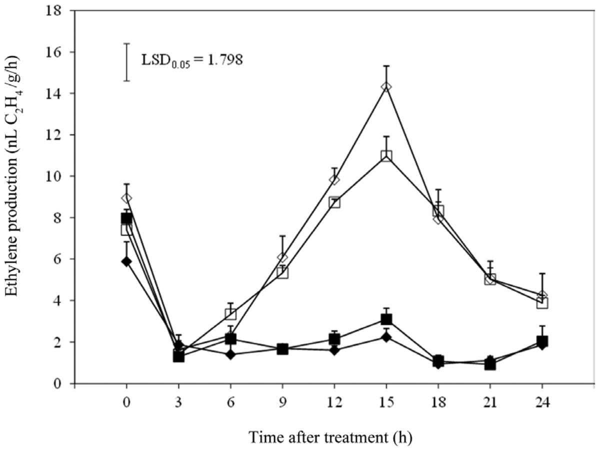

Fig. 2 Ethylene production of *Dendrobium* cv. Sonia Bom #17 open florets for control (♦), pollination using pollinia of cv. White 5n, (◊), 1-methylcyclopropene (1-MCP; ■) and 1-MCP + pollination using pollinia of cv. White 5n (□), where results are means of five replications (five florets per inflorescence, five inflorescences) ± SD and LSD = least significant difference

Table 1 Time to visible post-pollination symptoms in *Dendrobium* cv. Sonia Bom #17 flowers using pollinia of cv. White 5n, with 1-methylcyclopropene (1-MCP) applied for 4 h, prior to pollination

Treatment	Epinasty	Perianth venation	Yellow labellum	Perianth water soaking
	Time (days)			
Control	10.9±0.16^{a}	7.5±0.08^{a}	15.8±0.06^{a}	31.3±0.39^{a}
Pollination	1.5±0.09^{b}	1.6±0.06^{c}	5.2±0.06^{c}	21.4±0.10c
1-MCP	11.6±0.06^{a}	8.0±0.38^{a}	11.1±0.18^{ab}	28.9±0.07^{ab}
1-MCP + Pollination	2.5±0.08^{b}	4.8±0.07^{b}	7.9±0.02^{bc}	23.1±0.07^{bc}

Results are shown as mean ± SD from five replicates and each replicate had two inflorescences.

Values (mean ± SD) within each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

1-Aminocyclopropane-1 carboxylic acid content

Differences in the ACC content in the perianth (petals plus sepals) of pollinated and non-pollinated florets, with or without 1-MCP treatment, were not apparent for the 12 d after pollination. From day 12 onwards, the ACC content tended to be highest in the pollinated florets and lowest in the non-pollinated control florets. However, the differences among treatments were not consistent over time (Fig. 3A).

The ACC content in the column plus ovary tissue at zero time was comparable to that in the perianth. However, the ACC content of both pollinated florets and pollinated florets with 1-MCP treatment rapidly increased at 6 h and reached a maximum at 12 h. Differences in the ACC content between these two treatments were minor. In contrast, the ACC content of the non-pollinated florets with and without 1-MCP remained unchanged at a very low value and differences between the two were not significant at any time (Fig. 3B). Overall, the ACC content of the column plus ovary of pollinated florets increased considerably more (by three-fold) than that of the perianth.

1-Aminocyclopropane-1 carboxylic acid synthase activity

The ACS activity in all treatments was largely unchanged in the perianth (petals and sepals) during the first 12 h following pollination. This activity in the petals and sepals of pollinated florets increased markedly after 12 h, reached a maximum rate at 15 h and then declined to a minimal value at 18 h (about 0.5 nmol ACC /mg protein/h). A similar pattern occurred in the pollinated florets that had been pre-treated with 1-MCP but the maximum rate was less than that of the pollinated florets that had not been pre-treated with 1-MCP. In contrast, the ACS activities of non-pollinated florets without and with 1-MCP treatment remained largely unchanged throughout the 24 h of the study (Fig. 4A).

The ACS activity in the column plus ovary of all treatments remained low throughout (0.5–1.1 nmol ACC/mg protein/h), with no consistent differences occurring among treatments (Fig. 4B).

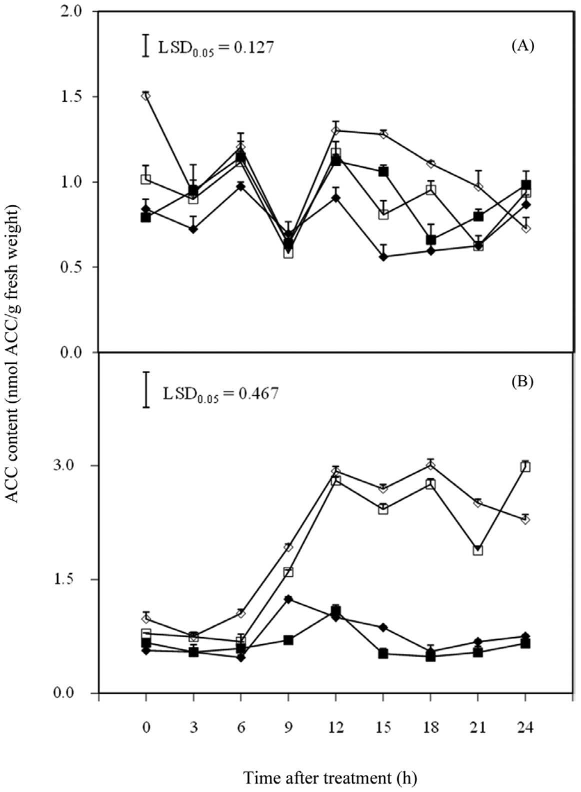

Fig. 3 ACC content in perianth (petals and sepals) (A) and column plus ovary (B) of *Dendrobium* cv. Sonia Bom #17 open florets for control (♦), pollination using pollinia of cv. White 5n (◊), 1-methylcyclopropene (1-MCP; ■) and 1-MCP + pollination using pollinia of cv. White 5n (□), where results are means of five replications (five florets per inflorescence, five inflorescences) ± SD and LSD = least significant difference

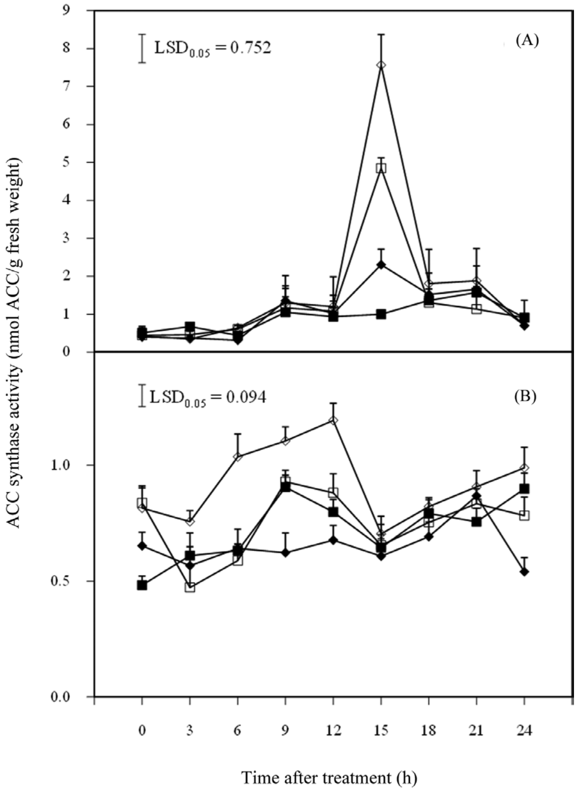

Fig. 4 1-Aminocyclopropane-1 carboxylic acid synthase (ACS) activity in perianth (petals and sepals) (A) and column plus ovary (B) of *Dendrobium* cv. Sonia Bom #17 open florets for control (♦), pollination using pollinia of cv. White 5n (◊), 1-methylcyclopropene (1-MCP; ■) and 1-MCP + pollination using pollinia of cv. White 5n (□), where results are means of five replication (five florets per inflorescence, five inflorescences) ± SD and LSD = least significant difference

1-Aminocyclopropane-1 carboxylic acid oxidase activity

The ACO activity in the perianth of the control and pollinated florets without and with 1-MCP treatment increased to a maximum at 3 h and thereafter decreased to minimum values (Fig. 5A).

The ACO activity in the column plus ovary of the pollinated florets, with or without 1-MCP treatment, reached a maximum at 15 h after pollination and then declined. The maximum ACO rate of the pollinated florets that had been pre-treated with 1-MCP was 40% lower than that of pollinated florets. The ACO rates of the non-pollinated control, with and without 1-MCP treatment remained low throughout (Fig. 5B).

Before pollination, the *DenACO* gene expression was just detectable in the control perianth (Fig. 6A). *DenACO* gene expression in the perianth of pollinated florets without the 1-MCP treatment was low at 6 h after pollination, while *DenACO* gene expression in the perianth of the non-pollinated florets with the 1-MCP treatment increased significantly at 6 h of pollination (Fig. 6A). *DenACO* gene expression in the pollinated florets with the 1-MCP treatment increased significantly at 6 h, 12 h and 15 h after pollination but expression at 12 h was higher than the expression at 6 h and 15 h after pollination (Fig. 6A).

The *DenACO* gene expression in the column plus ovary of the non-pollinated florets without the 1-MCP treatment was low at 0 h and only barely detectable thereafter. The *DenACO* gene expression in the column plus ovary of the pollinated florets without the 1-MCP treatment was detected at 6 h and reached a maximum at 15 h after pollination. In contrast, the *DenACO* gene expression levels in the column plus ovary of the non-pollinated florets with the the 1-MCP treatment and the pollinated florets with the 1-MCP treatment were barely detectable at 6 h, 12 h and 15 h after pollination (Fig. 6B).

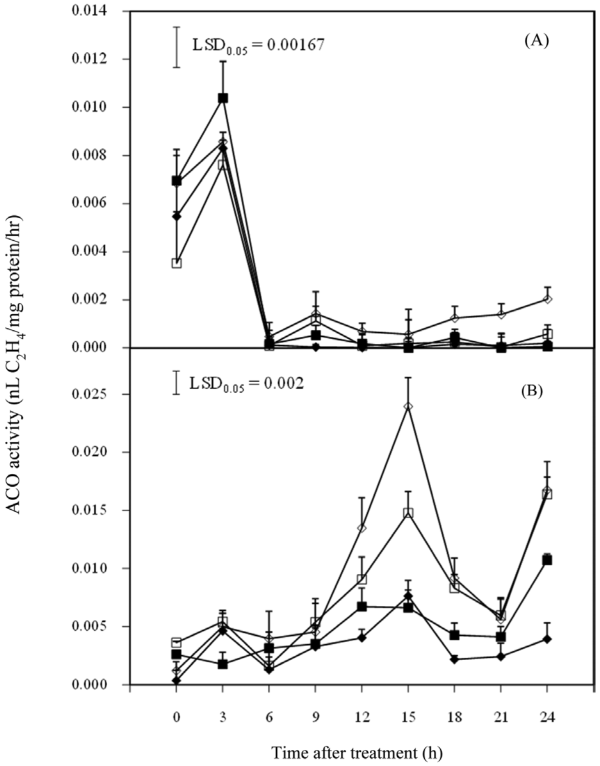

Fig. 5 1-Aminocyclopropane-1 carboxylic acid oxidase (ACO) activity in perianth (petals and sepals) (A) and column plus ovary (B) of *Dendrobium* cv. Sonia Bom #17 open florets for control (♦), pollination using pollinia of cv. White 5n (◊), 1-methylcyclopropene (1-MCP; ■) and 1-MCP + pollination using pollinia of cv. White 5n (□), where results are means of five replication (five florets per inflorescence, five inflorescences) ± SD and LSD = least significant difference

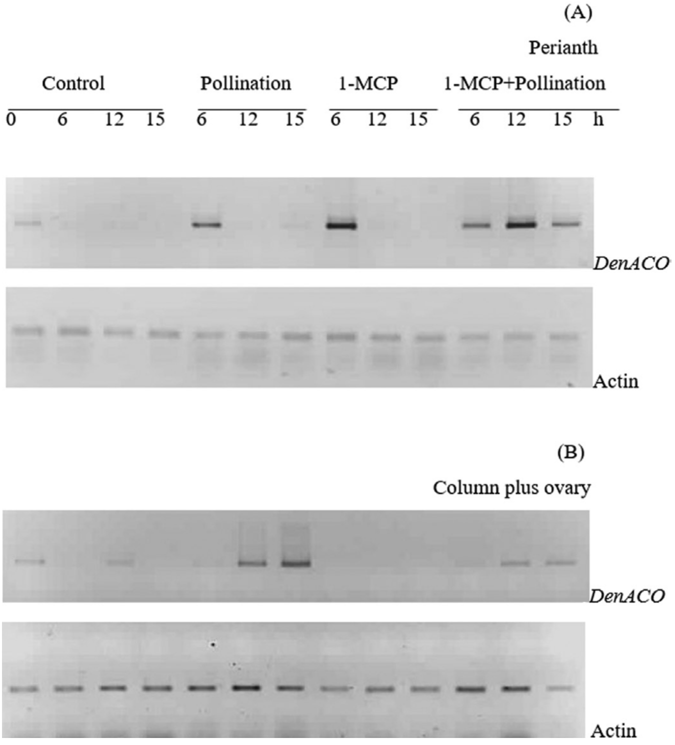

Fig. 6 Expression of *DenACO* (30 cycles) of *Dendrobium* cv. Sonia Bom #17 flowers based on semi-quantitative reverse transcriptase polymerase chain reaction analysis performed on extracts from: (A) perianth; (B) column plus ovary, where lanes 1–4 = total RNA extracted from perianth and column plus ovaries of control at 0 h, 6 h, 12 h, 15 h; lanes 5–7 = pollination using pollinia of cv. White 5n at 6 h, 12 h and 15 h; lanes 8–10 = 1-methylcyclopropene (1-MCP) at 6 h, 12 h and 15 h; lanes 11–13 = 1-MCP + pollination using pollinia of cv. White 5n at 6 h, 12 h and 15 h; and 1-MCP = 1-methylcyclopropene

Discussion

The senescence of the pollinated *Dendrobium* cv. Sonia Bom #17 florets using pollinia from cv. White 5n exhibited visible symptoms, including yellowing of the labellum, perianth venation and water soaking of the perianth considerably earlier than that which occurred in the non-pollinated florets (Fig. 1, Table 1). These results were similar to those obtained in other studies on *Dendrobium* (Ketsa and Rugkong, 1999; Luangsuwalai et al., 2008), *Phalaenopsis* (Porat et al., 1994), and petunia (Ichimura and Suto, 1998). 1-MCP, which is a powerful inhibitor of ethylene action (Blankenship and Dole, 2003), resulted in a delay of this premature senescence of pollinated florets when applied prior to pollination. These results indicated that post-pollination developmental processes that are associated with perianth senescence are regulated by ethylene (O'Neill et al., 1993; O'Neill and Nadeau, 1997; van Doorn and Ketsa, 2021). 1-MCP also inhibited the ovary growth of the pollinated florets (Table 2) indicating that ethylene induced by pollination was one of the plant hormones playing an important role at least in the initial ovary growth of *Dendrobium* florets following pollination (Ketsa et al., 2006).

Isolated perianth parts had previously been observed to show earlier senescence following treatment with ethylene; however, this effect was blocked by treatment with 1-MCP (unpublished data). This indicated that the isolated perianth parts could be induced to senesce using ethylene and that this process required ethylene perception. A plausible explanation of the present data was that ethylene was transported into the perianth parts, as was shown to be the case in *Cymbidium* and other flowering plants (Woltering 1990b; O'Neill et al., 1993, Woltering et al., 1997a, b). However, ACC was not found to move from the column into the perianth after emasculation in *Cymbidium* (Woltering, 1990b). The data presented here on the ACC content in the perianth tended to confirm this conclusion for the effect of pollination in *Dendrobium*, as very little change in the ACC content occurred over the 24 h following pollination. Indeed, there was little evidence to support the idea of transfer of ACC to the perianth. When ACC was applied to distal parts of the column of *Cymbidium* orchids, it did not move to basal parts of the column. In that research, radio-labeled ACC was applied to the stigma of non-pollinated flowers which had previously been treated with aminoethoxyvinylglycine and cobalt chloride to block both ACS and ACO activities. No ethylene was produced in the following 20 h in that study. The radioactivity (99%) remained at the site of application, with only 0.15% being found in the perianth. The conclusion was that ACC did not move to the perianth when applied to the top of the column of *Cymbidium* (Woltering et al., 1997b). In *Petunia* flowers, when radio-labeled ACC was applied to the stigma, the data indicated that the ACC did move down the style (Reid et al., 1984); however, the data did not allow for the label being moved as ethylene and not as ACC.

In *Dendrobium* in the current study, a considerable increase in ACO activity was observed in the columns after pollination. A similar effect was found in *Cymbidium* after emasculation (Woltering, 1990a; Woltering et al., 1997a) and in *Phaleanopsis* after pollination (O' Neill et al., 1993). Pollination stimulated ethylene production (Fig. 2), increased ACC content (Fig. 3) and the activities of both ACS (Fig. 4) and ACO (Fig. 5). These results supported the conclusion that pollination induced ethylene production in pollinated florets via an increase in the ACS and ACO activities as previously shown in the *Phalaenopsis* orchid by O'Neill et al. (1993). 1-MCP, an inhibitor of ethylene action (Blankenship and Dole, 2003), reduced ethylene production, the ACC content and the activities of ACS and ACO in the pollinated florets, suggesting that ethylene production in the pollinated florets was an autocatalytic system (Satoh et al., 2005). Pollination increased the ACC content and ACO activity in the columns more rapidly and to considerably higher amounts than those in the perianth, while pollination increased the ACS activity in the perianth more rapidly and at a higher rate than that in the column. The ACC content in the perianth was considerably lower (by approximately three-fold) than that in the column, while the ACS activity in the perianth was higher than that in the column plus ovary over the 12–18 h period. These differences in the ACC content in the perianth may have been due to a more rapid turnover of ACC in the perianth than in the column plus ovary or because the ACC in the perianth may have been transported into the column plus ovary and converted to ethylene which, in turn, regulated perianth senescence. The higher ACO activity in the column plus ovary supported the latter conclusion.

Expression of the *DenACO* gene was highly upregulated in the perianth and in the column plus ovary, within 6–12 h of pollination (Fig. 6). This increase might have been partially responsible for the increase in perianth and in column ACO activity. The expression of the *DenACO* gene, after pollination, was similar to that of an *ACO* gene in *Phalaenopsis*, where a rapid and large up-regulation was observed in the column of pollinated flowers (Nadeau et al., 1993) and similar to that reported for *Doritaenopsis* (Nadeau and O'Neill, 1995), *Phalaenopsis* (Bui and O'Neill, 1998), *Vanda* (Lokkamlue and Huehne, 2013, 2020), plum (Fernandez-Otero et al., 2006), and tomato (Llop-tous et al., 2000). The *DenACO* gene at 6 h in the column plus ovary of the pollinated florets with the 1-MCP treatment was highly expressed throughout the study period. This suggested that ethylene production in the column plus ovary of pollinated florets had negative feedback, since 1-MCP up-regulated the expression of the *DenACO* gene in the column plus ovary of pollinated florets. A similar response was reported in carnation flowers (Jones, 2003). Ethylene production in the column plus ovary of the pollinated florets of *Dendrobium* cv. Sonia Bom #17 inhibited its own production (negative feedback) similar to the response of wounding ethylene in melon, persimmon and plum (Bouquin et al., 1997; Nakano et al., 2002). The current finding of a 1- MCP effect on both the ACO activity and on the *DenACO* gene was similar to that found in apple (Yang et al., 2013), banana (Pathak et al., 2003), durian (Amornputti et al., 2016), nectarine (Bregoli et al., 2005), pear (Zhao et al., 2020), and plum (Khan and Singh, 2007). In each case, there was a resulting reduction in ethylene production. Whether ACS gene expression plays a role in this ethylene production is still in question as attempts reported to date to isolate ACS partial sequences from *Dendrobium* florets have not been successful (Luangsuwalai, 2007).

It was concluded that the pollination of *Dendrobium* cv. Sonia Bom #17 open florets with pollinia of cv. White 5n induced rapid and early perianth senescence. Pollination increased ethylene production in the whole open florets and increased the ACC content and the activity of ACO in the column plus ovary, and the activity of ACS in the perianth. The 1-MCP treatment prior to pollination resulted in delayed perianth senescence and a decrease in the peak ethylene production, ACC content and the activities of ACS and ACO. The column was the likely source of ethylene production in the pollinated flowers of *Dendrobium* cv. Sonia Bom #17. The increased ethylene production of pollinated florets might have been partially due to an increase in the expression of the *DenACO* gene.

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