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Ethylene and 1-MCP treatments involved in differential expression of signal transduction and ethylene biosynthesis genes in *Dendrobium* cut flowers during senescence

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ABSTRACT: *Dendrobium* orchids represent a significant portion of Thailand's cut flower exports, with their longevity being influenced by ethylene sensitivity. This research aimed to elucidate the regulatory mechanisms of ethylene and its inhibitor, 1-methylcyclopropene (1-MCP), on the premature senescence of *Dendrobium* 'Khao Chaimongkol' cut flowers at different developmental stages. Using a combination of treatments, the inflorescences were treated with 500 $nL.L^{-1}$ 1-MCP, 0.4 $\mu L.L^{-1}$ ethylene, and a combination of 500 $nL.L^{-1}$ 1-MCP followed by 0.4 $\mu L.L^{-1}$ ethylene. Senescence symptoms and gene expression profiles related to ethylene signaling pathways and ethylene biosynthesis were assessed in both floral buds and open florets, for five days. The results indicated that ethylene-treated inflorescences rapidly displayed senescence symptoms, with the first visible signs being venation followed by drooping within a day. Exogenous ethylene triggered the upregulation of ethylene receptors *ERS1*, signal transduction genes *CTR1*, *EIL1*, and *ERF1*, and stimulated the expression of *ACS1* and *ACO1* genes responsible for ethylene production in floral buds. In contrast, in open florets, exogenous ethylene upregulated *ERS1*, *CTR1*, and *ACO1* expression but did not induce *EIL1*, *ERF1*, and *ACS1*. Consequently, floral buds exhibited more pronounced premature senescence compared to open florets. This differential response indicated distinct effects of exogenous ethylene on ethylene signaling transduction between floral buds and open florets. Conversely, the application of 1-MCP led to competitive binding of ethylene receptors, resulting in the suppression of *ERS1*, *CTR1*, *EIL1*, *ERF1*, and *ACO1* expression in floral buds. In open florets, however, 1-MCP did not suppress the expression of *EIL1*, *ERF1*, and *ACS1*. Consequently, premature senescence was inhibited in both floral buds and open florets. This differential response indicated distinct mechanism effects of ethylene and 1-MCP on ethylene signaling pathway across the differential sensitivity of floral tissues and developmental stages to ethylene.
Keywords: *Dendrobium*; ethylene; 1-MCP; gene expression; senescence

Introduction

Dendrobium orchids have the highest export volume among Thailand's cut flowers. The longevity of cut *Dendrobium* flowers is influenced by a combination of internal and external factors, with *Dendrobium* cut flowers commonly categorized as ethylene-sensitive (Woltering and van Doorn, 1988; Ketsa and Rugkong, 2000; Ketsa and van Doorn, 2009). The phytohormone ethylene, a key regulator of various physiological and developmental

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processes in plants (Binder, 2020), and significantly influences symptoms related to flower senescence (Woltering and van Doorn, 1988). In ethylene-sensitive orchids, post-pollination-induced endogenous ethylene elevates ethylene production and respiratory rates, resulting in visible senescence symptoms (Burg and Dijkman, 1967; Arditti et al., 1971; Arditti, 1979; van Doorn, 1997; Ketsa and Rugkong, 2000; Attri et al., 2008; van Doorn and Ketsa, 2021; Thanomchit et al., 2022). Additionally, exogenous ethylene accumulated during transportation and storage can also induce flower senescence, manifesting as epinasty, drooping, color changes, venation of petals and sepals, water soaking, wilting, and eventual flower abscission. (Woltering and van Doorn, 1988; Sukhotu, 2006; Uthaichay et al., 2007; Ketsa and van Doorn, 2009; Kirasak et al., 2023). However, sensitivity to ethylene in *Dendrobium* flowers varied depending on the cultivars and the stage of flower blooming (Bunya-atichart et al., 2006; Rungruchkanont et al., 2007). Regarding the orchid flower structure, the column is identified as the primary source of ethylene involved in premature senescence (Arditti et al., 1973). In *Dendrobium* 'Khao Sanan', exogenous ethylene treatment resulted in the columns producing significantly more ethylene than the perianth, leading to increased autocatalytic ethylene production and premature senescence within five days of treatment. (Lerslerwong and Ketsa, 2008).

For several decades, molecular aspects and genetic approaches of flower senescence have been employed across various flowers exhibiting ethylene-sensitive, ethylene-insensitive, and intermediate pattern of senescence (van Doorn et al., 2003). The induction of flower senescence by ethylene is associated with transcriptional regulation of the 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (*ACS*) and ACC oxidase (*ACO*) genes, as well as ethylene receptor genes (Jones et al., 2003; Kuroda et al., 2004). In *Dendrobium* 'Khao Chaimongkol', a commercial cultivar sensitive to ethylene, pollination-induced endogenous ethylene has been shown to trigger autocatalytic ethylene biosynthesis and activate the signaling pathway, resulting in visible senescence symptoms and differential expression of ethylene signaling genes. Compatible pollination in this cultivar led to rapid ethylene production and the fastest onset of senescence symptoms, characterized by drooping, while concurrently stimulating *DenACS1* and *DenACO1* in the ethylene biosynthesis pathway (Thanomchit et al., 2022). Also, in *Dendrobium* 'Khao Sanan', *Den-ACS* and *Den-ACO* in the column and perianth were positively regulated by both endogenous and exogenous ethylene, contributing to the autocatalytic system of *Dendrobium* flower senescence. Additionally, the expression levels of *ACS* genes reflect the rate-limiting step in ethylene biosynthesis (Lerslerwong and Ketsa, 2008).

Genes related to the ethylene receptors and ethylene signaling pathway have been isolated and characterized in various plant species, including *Arabidopsis*, tomato, and geranium (Chang et al., 1993; Ecker, 1995; Alonso et al., 1999; Dervinis et al., 2000). These investigations contribute valuable insights into the intricate processes associated with ethylene signaling across different plant species. The perception of ethylene involves a family of receptor genes, with the primary receptor gene in *Dendrobium* being the ethylene response sensor (*ERS*). Cloned from *Dendrobium* 'Pompadour', *Den-ERS1* encoded a protein featuring three ethylene binding domains, a GAF domain, and a histidine kinase domain. In *Dendrobium*, high expression of *Den-ERS1* occurred in the young flower bud stage (B3) and declined with flower development (Thongkum et al., 2009). Additionally, studies have consistently shown that both endogenous and exogenous ethylene up-regulated the expression of *Den-ERS1* during *Dendrobium* flower senescence (Thongkum et al., 2009; 2015). A similar pattern was observed for the

ethylene receptor gene *OgERS1* in *Oncidium* 'Gower Ramsey', where mRNA levels increased in fully open flowers during senescence, reaching their maximum on the fifth day (Huang et al., 2007).

The early stages of the ethylene signaling pathway involve constitutive triple response (CTR1) and ethylene insensitive 2 (EIN2). CTR1, localized at the endoplasmic reticulum (ER), possesses an amino-terminal domain with a high potential for interaction with the histidine kinase domains of both ETR1 and ERS1 ethylene receptors (Clark et al., 1998; Guo and Ecker, 2004). Downstream in the signaling pathway, EIN3 and EIN3-Like1 (EIL1) are recognized as crucial transcription factors for the immediate early gene expression of ethylene. EIN3 acts as a primary transcriptional regulator that activates *ERF* genes, which modulate the expression of a wide range of ethylene-responsive genes in the ethylene signaling pathway (Guo and Ecker, 2003). Further downstream in the transcriptional regulation, the ethylene response factor *ERF* is an immediate target for *EIN3* (Solano et al., 1998). *ERF* genes are critical components of the ethylene signaling pathway. ERF proteins act downstream of EIL/EIN3 transcription factors. They regulate the expression of a wide array of ethylene-responsive genes by binding to specific DNA sequences GCC-box or DRE/CRT motifs in their promoters of target genes (Lorenzo et al., 2003). This binding can lead to either activation or repression of gene transcription, depending on the specific ERF involved and the context of the response. Both *EIL* and *ERF* genes are integral to the ethylene signaling pathway, playing distinct but interconnected roles in mediating plant responses to ethylene. *EIL* genes act as primary transcriptional regulators that activate *ERF* genes, which in turn modulate the expression of a wider range of ethylene-responsive genes, ensuring appropriate physiological and developmental responses. However, ethylene signal transduction gene expressions in various plants revealed significant variability depending on the species and the environmental conditions. Especially between ethylene-sensitive and ethylene-insensitive cultivars. Ethylene-sensitive flowers tended to upregulate ethylene biosynthesis and signaling genes, such as *ACO1* and ethylene receptor 2 (*ETR2*), more intensely compared to ethylene-insensitive cultivars. Additionally, the ethylene-insensitive flowers exhibited a lower or delayed response in activating ethylene signal transduction genes like EIN2, affecting their sensitivity to ethylene and related physiological processes (Ha et al., 2019).

1-Methylcyclopropene (1-MCP) is widely utilized as an inhibitor of ethylene perception. It binds to ethylene receptors in plants, thereby extending the longevity and vase life of cut flowers (Serek et al., 1994; Serek and Sisler, 2004; Naing et al., 2022). The binding affinity of 1-MCP for these receptors is approximately ten times greater than that of ethylene (Blankenship and Dole, 2003; Almasi and Mohamed, 2020). Notably, 1-MCP has proven effective in preventing bud and petal abscission as well as flower senescence in various species, including begonia, miniature roses, kalanchoe (Serek et al., 1994) and orchids (Bunya-atichart et al., 2006; Uthaichay et al., 2007; Phetsirikoon et al., 2016). In *Dendrobium* 'Karen', 1-MCP reduced ethylene production by decreasing both ACS activity in open flowers and ACO activity in floral buds, thereby prolonging flower abscission. For the commercial export of orchid flowers, 1-MCP is used as a pretreatment to reduce the ethylene levels inside cardboard boxes, thereby prolonging vase life during shipment (Ketsa and Uthaichay, 2012). A comprehensive understanding of the regulatory mechanisms governing the effects of exogenous ethylene and 1-MCP on *Dendrobium* flowers, both within floral buds and open florets, has the potential to reveal intricate insights into plant responses within ethylene signaling pathways and the regulatory complexities associated with ethylene biosynthesis. This research is designed to elucidate the regulatory mechanisms of exogenous ethylene during

flower export, its interplay with ethylene inhibitor 1-MCP in gene signaling transduction, and the modulation of ethylene biosynthesis. The objective is to understand how these regulatory networks contribute to the premature senescence observed at two developmental stages, floral bud and open floret of ethylene-sensitive *Dendrobium* 'Khao Chaimongkol' flowers. The findings are expected to benefit the export market by decreasing losses.

Materials and methods

Plant Materials

Dendrobium 'Khao Chaimongkol' cut flowers were sourced from a commercial orchid farm in Ratchaburi province. The inflorescences of *Dendrobium* with 4-6 open florets and 6-8 floral buds were used in this study. Four treatments were applied: control, treated with 500 $nL.L^{-1}$ of 1-MCP for 3 hours at 25°C, exposure to 0.4 $\mu L.L^{-1}$ of ethylene for 24 hours at 25°C, and treatment with 500 $nL.L^{-1}$ of 1-MCP for 3 hours at 25°C, followed by exposure to 0.4 $\mu L.L^{-1}$ of ethylene for 24 hours at 25°C. Peduncles were recut and placed in 15-mL plastic tubes before treatment. Ethylene and 1-MCP treatments were followed by Bunya-atichart et al. (2006) with some modifications. Ethylene was applied by introducing an appropriate volume through an injecting port and brought to a final concentration of 0.4 μLL^{-1}, 1-MCP (EthylBlocTM, Floralife, SC, USA) was added to the chamber to a final concentration of 500 nLL^{-1}. Control inflorescences were placed in plastic chambers without added ethylene and 1-MCP. The experiments were conducted into four treatments with ten replicates (one inflorescent/replicate) in a completely randomized design (CRD). Senescence symptoms, including drooping, venation, epinasty, yellowing, wilting, and dropping of open florets, as well as yellowing, wilting, and dropping of floral buds, were recorded as a percentage of each criterion over five days. Perianths of floral buds and open florets from ten inflorescences were pooled separately and frozen for RNA extraction.

Total RNA extraction and cDNA synthesis

Total RNA was extracted from frozen perianths of floral buds and open florets using a method previously described by Thanomchit et al. (2022). Total RNA was treated with deoxyribonuclease I (Turbo DNAfree™ Kit, Ambion, TX, USA) to remove contaminating genomic DNA. The first strand cDNA was synthesized from total RNA following the protocol of the SuperScript (SuperScriptTM III First-Strand Synthesis System for RT-PCR, Invitrogen, USA) as a template to amplify the target genes by PCR and a template for quantitative real-time PCR. The first strand cDNA was stored at -20°C until use.

qPCR Analysis of ethylene biosynthesis, ethylene receptor, and signaling genes

For the qPCR analysis, primer sets were designed based on the 3'-untranslated regions (UTR) of individual genes using the vector NTI **(Table 1)**. The length of all PCR products ranged from 95 to 120 base pairs. The gene-specificity of these primer sets was confirmed through the following steps: (1) individual PCR products were separated on 1% agarose gels and stained with SYBR-safe to examine their size; (2) the PCR products were cloned into the pGEM®-T Easy Vector System (Promega, USA), and sequence analysis was performed.

qPCR was conducted using the Light Cycler® 480 system (384-well plates) and the Light Cycler® 480 SYBR Green I Master kit (Roche Diagnostics, Germany), following the manufacturer's instructions. All reactions were separately performed in three replications using 3 µL of the diluted cDNA template (50x), 2 µL of each primer (2.5 µM), and 5 µL of 2 × Master mix to a final volume of 10 µL. The PCR program began with an initial step of 5

minutes at 95°C, followed by 50 cycles of 95°C for 5 seconds, 60°C for 5 seconds, and 72°C for 10 seconds. The program concluded with a melt curve analysis.

The *Dendrobium* actin gene served as an internal control to normalize slight differences in template amounts, with the forward primer 5'–GCC CAA AAG ACC ATC AGA CAA GC-3' and the reverse primer 5'–CGG AAG GAC CAA AAG TGA CAA CC-3'. Relative gene expression data were calculated using PCR efficiency calculations. An average of three replicates of each sample value was represented with an error bar.

Statistical analyses

The data were analyzed using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$. Statistical analysis was performed using R version 4.2.0 (2022-04-22 ucrt) Copyright © 2022. (Thermo Fisher Scientific Inc.)

Table 1 Primers for qPCR analysis

Genes	Specific primer (qPCR)	Product size (bp)	Accession number
DenACS1	F-TGGGTGAATATGGAGACGTTGATG	120	MK312251
	R-AGAACAGCAACAAGAAGAACCAGGA		
DenACO1	F-GCGGAGGAGAAGAAGAAGGAAGTT	113	MK312252
	R-CCATAGTCTTCATAGCCTCAAACCTT		
DenERS1	F-TTAAGTTTACAAAGGAGGG	100	MK312250
	R-CTTGGGATAGGGTGGAAC		
DenCTR1	F-GCAAAGGGAATGAACTATCT	95	MK312253
	R-ACCGTGTACTTCTTGTCAAC		
DenEIL1	F-GGTGCCCAACAAATCAGTCG	110	MK312254
	R-TGGCTTTGGCTGGTTGAGAA		
DenERF1	F-CCAGCTAGTTCATCACCTGCTT	117	MK312255
	R-ATCATGTTCCCAGCCAAAGTCT		

Results and discussion

Senescence symptoms

In the experiments examining the impact of ethylene, 1-MCP, and the sequential application of 1-MCP followed by ethylene on *Dendrobium* florets, distinct effects were observed compared to untreated florets (control). These treatments elicited diverse senescence symptoms in floral buds and fully opened florets.

Exposure to exogenous ethylene triggered premature senescence, the onset of senescence of floral buds began as early as one day after exposure to ethylene treatment, manifested by the development of yellowing symptoms. By day 3, approximately 60% of the floral buds displayed discernible changes **(Figure 1 and Figure 2A)**. Subsequently, wilting symptoms began to appear, with noticeable alterations observed from day 2 to day 5 **(Figure 2B)**. Notably, the appearance of dropping symptoms marked the final visible stage of senescence on day 5 **(Figure 2C)**.

In open florets exposed to ethylene, venation, and drooping symptoms increased rapidly from day 1, reaching 100% on days 2 and 3, respectively **(Figure 3)**. Subsequently, displayed rapid escalation of epinasty, reaching 50% severity by day 2 **(Figure 4A)**. This rapid increase in symptoms contrasted with non-treated florets and those treated with 1-MCP and 1-MCP followed by ethylene. The appearance of venation and drooping symptoms resembled the response observed in exogenous ethylene-treated *Dendrobium* 'Khao Sanan' (Lerslerwong et al., 2009), *Dendrobium* 'Lucky Duan' (Kirasak et al., 2023), and pollinated *Dendrobium* 'Pompadour' (Thongkum et al., 2009), and *Dendrobium* 'Khao Chaimongkol' (Thanomchit et al., 2022), where endogenous ethylene was regulated by evident pollination. Consequently, it can be deduced that these cultivars display high sensitivity to ethylene. However, the wilting and yellowing symptoms in open florets developed slowly across ethylene treatment, persisting until day 5 **(Figure 4B and 4C)**.

This finding revealed that the flowers exhibited asynchronously different senescence symptoms when exposed to exogenous ethylene due to the differential sensitivity of various flower parts, such as sepal, petal, columns, and perianth (Reid and Wu, 1992). Moreover, the developmental stage of the flower also affected its sensitivity to ethylene (van Doorn, 2001). These differential responses can be attributed to factors such as variations in ethylene receptor expression, the presence of ethylene signaling components, and the differential activation of ethylene-responsive genes in different floral tissues (O'Neill, 1997), which cause them to respond to ethylene at different rates.

In contrast, floral buds and open florets treated with 1-MCP, and 1-MCP followed by ethylene exhibited a slower onset of these senescence symptoms than those treated with ethylene alone **(Figure 1-4)**. This suggests that 1-MCP can delay senescence in this sensitive-ethylene *Dendrobium* 'Khao Chaimongkol' orchid. 1-MCP is an ethylene action inhibitor that binds to ethylene receptors in the plant tissues, preventing ethylene from attaching to the receptors and thereby blocking the ethylene signaling pathway. Since ethylene is a key hormone regulating senescence, this inhibition can significantly delay the process. The application of 1-MCP has consistently demonstrated its capacity to effectively delay senescence symptoms and extend the vase life of open florets, a phenomenon consistently observed in various flower species such as *Dendrobium* 'Jacky', and 'Lucky Duan' (Kirasak et al., 2023), *Oncidium* 'Gower Ramsey' (Huang et al., 2007), and *Mokara* hybrids (Wongjunta et al., 2021). Studies conducted on *Phalaenopsis* 'Allen' and 'Venice' have revealed that 1-MCP, when applied either before or concurrently with ethylene exposure, effectively delayed the onset of flower senescence. However, it is noteworthy that the timing of the 1-MCP application is crucial. When applied after the flowers have been exposed to ethylene for 42 hours, there is a notably higher percentage of senescence, particularly evident in the 'Allen' cultivar, as opposed to the response observed in the 'Venice' cultivar (Favero et al., 2016). Additionally, pre-treatment with 1-MCP can prevent the autocatalytic production of ethylene and protect against damage to cellular structures, including malfunctions in mesophyll parenchyma cells near vascular bundles, plasma membranes, mitochondria, and nuclei (Kirasak et al., 2023).

The effectiveness of 1-MCP in delaying senescence is influenced by several factors, including its concentration, the duration of exposure to the flower, and its ability to interfere with signaling pathways in various tissues (O'Neill, 1997). The developmental stage of the flower also plays a critical role; younger tissues, such as floral buds, may be less sensitive to ethylene than fully developed open florets (Bunya-atichart et al., 2006), affecting the

efficacy of 1-MCP depending on the maturity of the floral tissue (van Doorn, 2001). Despite the application of 1-MCP, senescence can still occur as ethylene receptors are continuously produced.

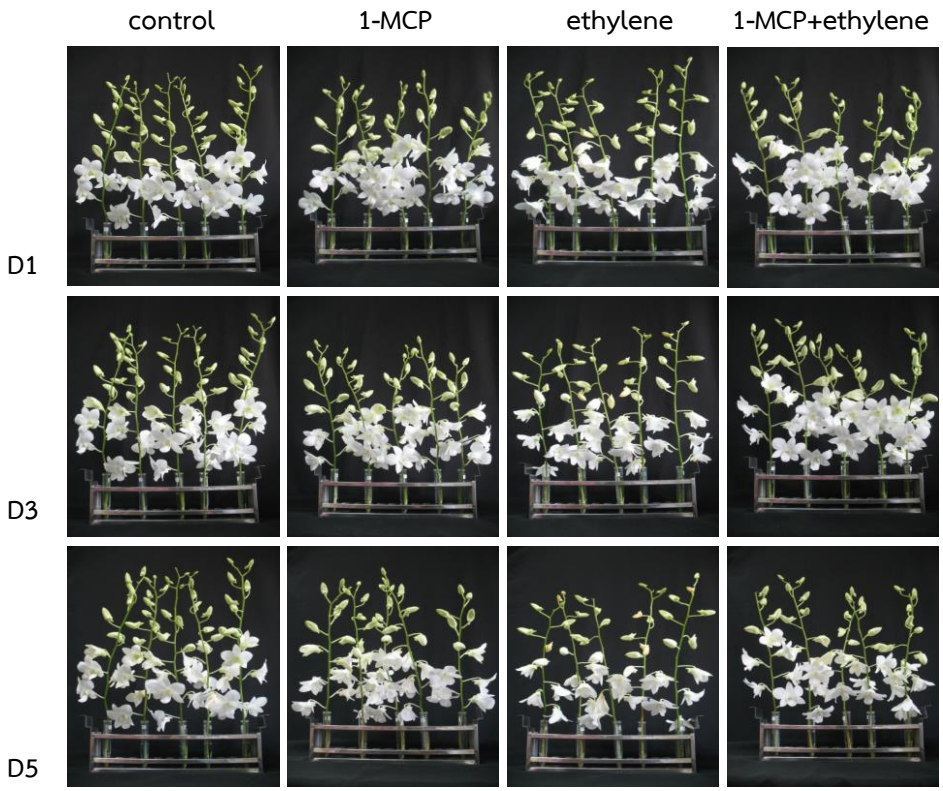

Figure 1 Senescent appearance of *Dendrobium* 'Khao Chaimongkol' inflorescences which were untreated or treated with 500 nL.L^{-1} 1-MCP for 3 h, 0.4 µL.L^{-1} C_2H_4 for 24 h or 500 nL.L^{-1}1-MCP for 3 h followed by 0.4 µL.L^{-1} C_2H_4 at 25°C for 24 h. at 1, 3 and 5 days.

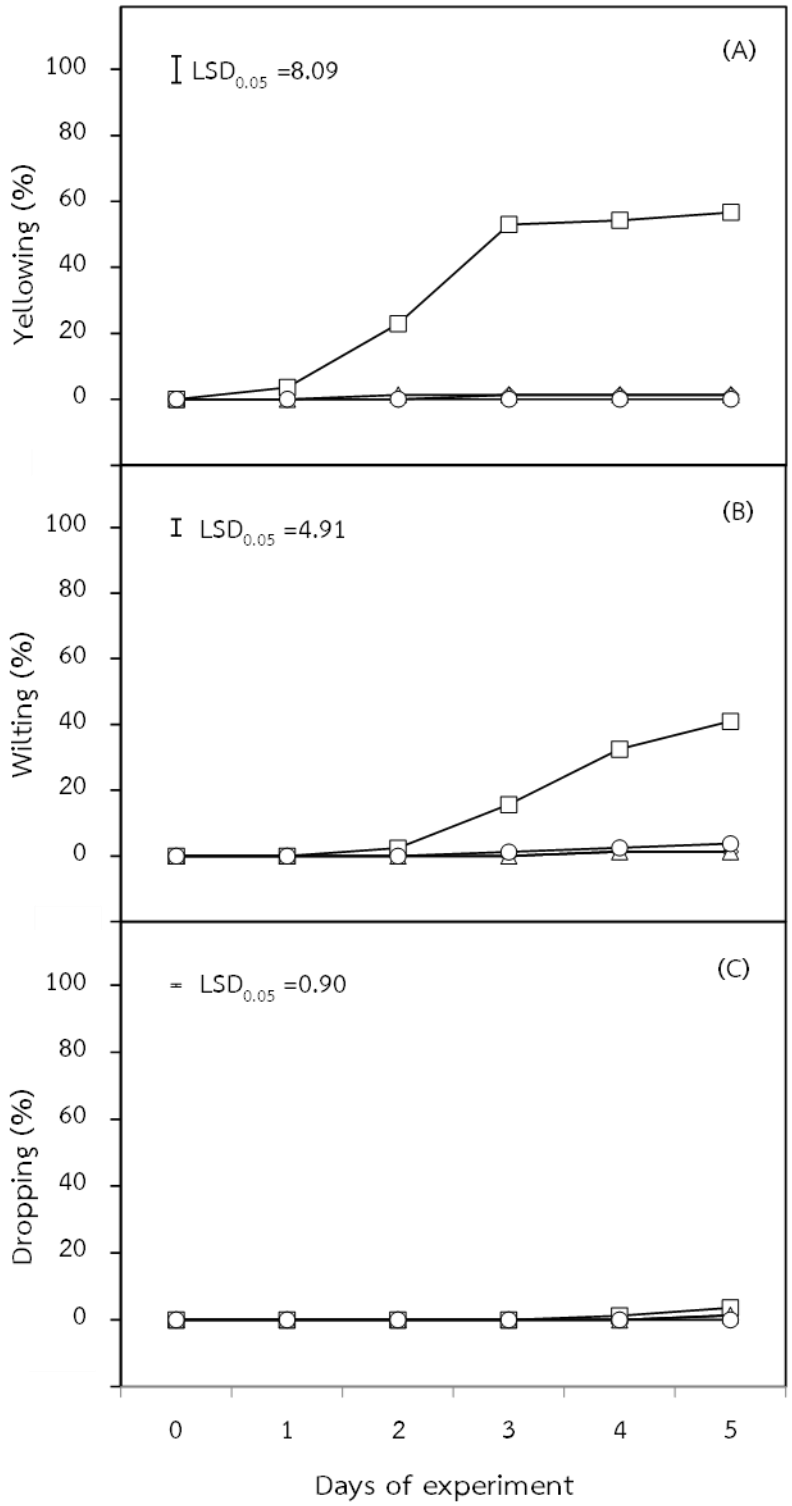

Figure 2 Yellowing (A), wilting (B) and dropping (C) symptoms of *Dendrobium* 'Khao Chaimongkol' floral buds; untreated (control) (◇) or treated with 500 nL.L^{-1} 1-MCP for 3 h (Δ), or 0.4 μ L.L^{-1} C_2H_4 for 24 h (□), or 500 nL.L^{-1} 1-MCP for 3 h followed by 0. 4 μL.L^{-1} C_2H_4 for 24 h. (○). LSD is indicated by a bar (n=10).

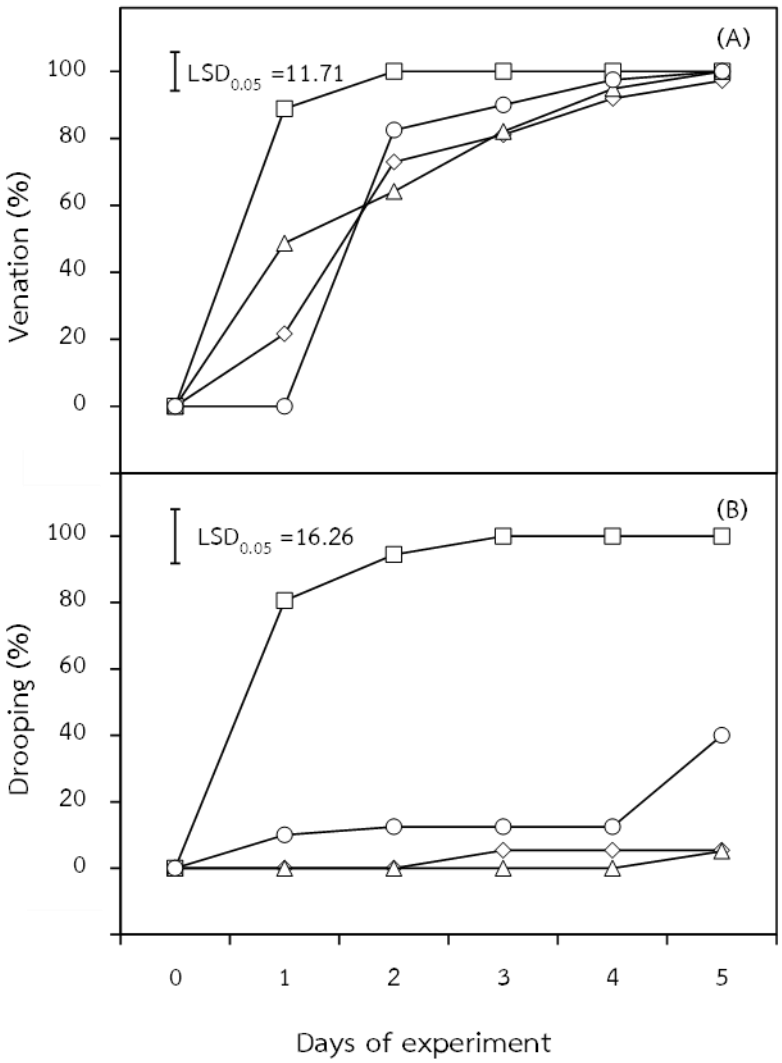

Figure 3 Venation (A), and drooping (B) symptoms of *Dendrobium* 'Khao Chaimongkol' open florets; untreated (control) (◇) or treated with 500 nL.L^{-1} 1-MCP for 3 h (△), or 0.4 µL.L^{-1} C_2H_4 for 24 h (□), or 500 nL.L^{-1} 1-MCP for 3 h followed by 0. 4 µL.L^{-1} C_2H_4 for 24 h. (○). LSD is indicated by a bar (n=10).

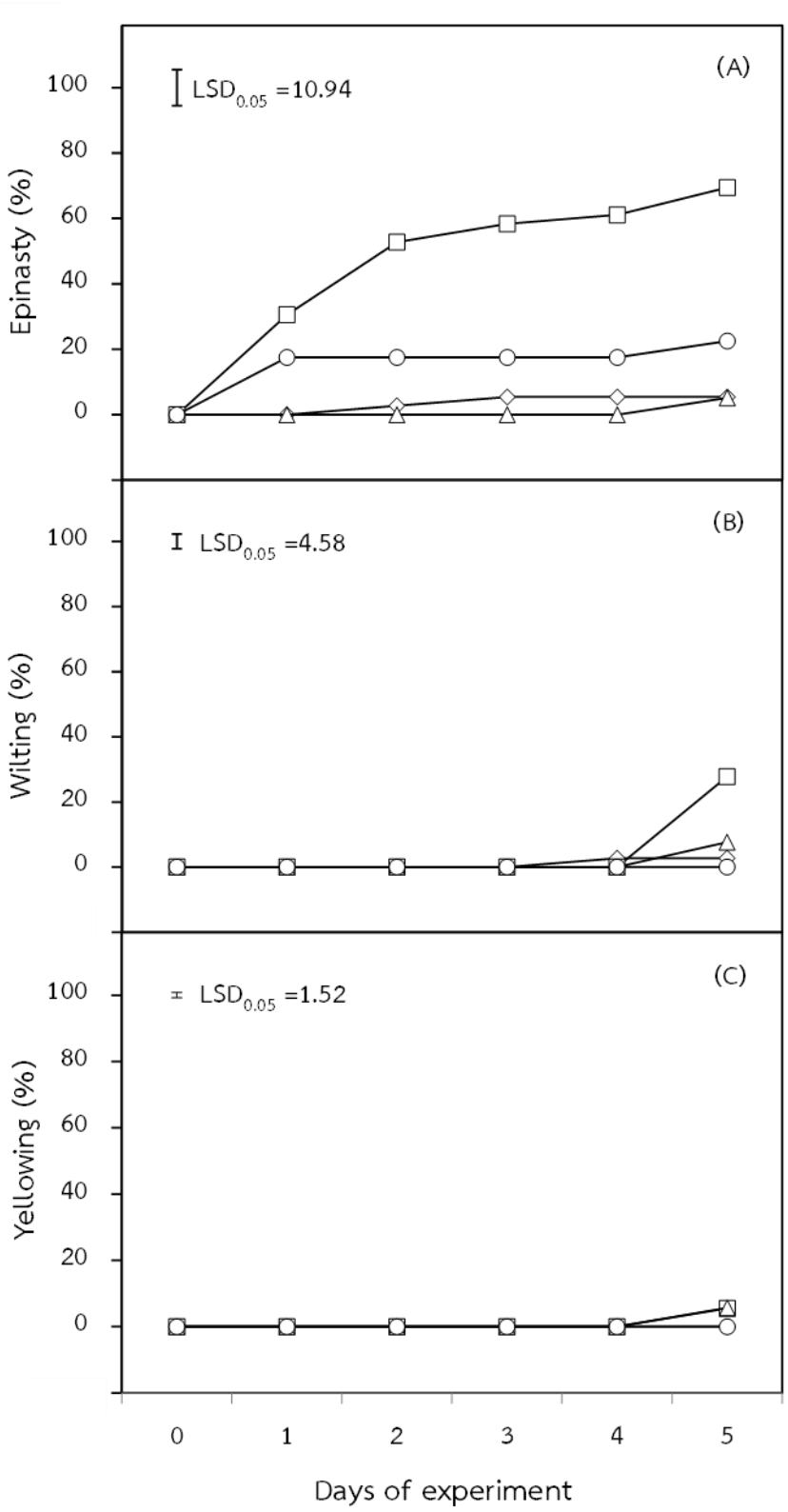

Figure 4 Epinasty (A) wilting (B) and yellowing (C) symptoms of *Dendrobium* 'Khao Chaimongkol' open florets; untreated (control) (◇) or treated with 500 nL.L^{-1} 1-MCP for 3 h (△), or 0.4 µ L.L^{-1} C_2H_4 for 24 h (□), or 500 nL.L^{-1} 1-MCP for 3 h followed by 0. 4 µL.L^{-1} C_2H_4 for 24 h. (○). LSD is indicated by a bar (n=10).

Gene expression

The mRNA expression profiles of the ethylene receptor, signal transduction genes, and ethylene biosynthesis genes were comprehensively investigated utilizing quantitative PCR analysis. The expression of *DenERS1* was higher in open florets compared to floral buds. Conversely, in *Dendrobium* 'Pompadour,' high expression of *DenERS1* was observed during the early flower bud stage (Thongkum et al., 2009). However, across the 5-day 1-MCP treatment period, the expression of *DenERS1* was significantly reduced in both floral buds and open florets (Figure 5B), whereas an increase in *DenERS1* expression was observed in florets treated with ethylene **(Figure 5C)**. Pre-treatment with 1-MCP before ethylene exposure markedly diminished the expression of *DenERS1*, particularly within the initial 1-2 days post-ethylene exposure, with expression levels rising again on the last day of senescence (Figure 5D). Notably, the transcript abundance of *DenERS1* in the florets, especially in open florets exhibited a burst of expression on days 1-2, reminiscent of the induction triggered by exogenous ethylene. These findings supported that exogenous ethylene triggered the expression of *DenERS1* ethylene receptor, which subsequently bound to ethylene, the signal is transduced through a series of proteins CTR, EIN, EIL, and ERF that modulate the expression of ethylene-responsive genes including ethylene biosynthesis *ACS* and *ACO*, leading to senescence, similar to its behavior in *Dendrobium* 'Khao Sanan' was induced by exogenous ethylene (Lerslerwong and Ketsa, 2008; 2009) and pollinated *Dendrobium* 'Khao Chaimongkol', which senescence was induced by endogenous ethylene (Thanomchit et al., 2022). In this study, 1-MCP treatment effectively suppressed the expression of the *DenERS1* gene by promptly competitively binding to this ethylene receptor, thereby surpassing the influence of ethylene and preventing the accumulation of these receptor genes **(Figure 5B)**. Notably, this suppressive effect of 1-MCP persisted even after subsequent exposure to exogenous ethylene for 5 days in floral buds and 3 days in open florets **(Figure 5D)**, leading to reduced senescence symptoms in both floral buds and open florets **(Figure 1-4)**.

The expression profiles of ethylene signaling transduction genes in both floral buds and open florets showed that the expression of *DenCTR1* induced by exogenous ethylene was significantly higher than that observed under 1-MCP treatment, and slightly higher than that under 1-MCP followed by ethylene treatment **(Figure 5E-H)**. Additionally, in floral buds, downstream genes in the ethylene signaling transduction pathway, such as *DenEIL1* **(Figure 5I-L)** and *DenERF1* **(Figure 5M-P)**, exhibited notable peaks specifically on the first day following exposure to ethylene **(Figure 5K, O)**. Conversely, the expression levels of *DenEIL1* and *DenERF1* showed a slight reduction after performing the 1-MCP application **(Figure 5J, N)**. Notably, in response to exogenous ethylene, the expressions of *DenEIL1* and *DenERF1* were significantly upregulated in floral buds, whereas these genes remained unaffected in open florets following the treatments. This observation supports the understanding that *EIL1* genes govern the transcriptional control of ethylene responses within the floral bud is different from that of open florets.

Subsequently, the expression levels of ethylene-responsive genes, including those involved in ethylene biosynthesis were investigated. In the floral bud, the expression of the *DenACS1* gene exhibited a remarkable difference among various treatments. Expression levels of the *DenACS1* gene in the control, 1-MCP, and 1-MCP followed by ethylene-treated floral buds were notably lower than those treated with ethylene **(Figure 6A-D)**. Ethylene-treated floral buds displayed a continuous and remarkable increase in *DenACS1* gene expression from day 1 to day 3, and this upregulation was observed again on day 5 **(Figure 6C)**. This initial upregulation of *DenACS1* during the first 1-3 days might be a response to exogenous ethylene exposure, consistent with the upregulation of

ethylene signal transduction genes, particularly *ERS1* and *ERF1* **(Figure 5C, O)**. However, the increased *DenACS1* expression observed at the end of the senescence phase (day 5) might be due to a response to the autocatalysis of endogenous ethylene production. In open florets, *DenACS1* expression was not elevated in response to ethylene treatment compared to the control, the expression of *DenACS1* was just elevated only on day 1 and 5 **(Figure 6A-D)**. This observation highlights the prominent role of ethylene in modulating *DenACS1* gene expression specifically in the floral buds. Conversely, open florets and floral buds treated with 1-MCP exhibited high levels of *DenACS1* expression compared to the control **(Figure 6B)**, reminiscent of the increased expression of *Rh-ACS3* in cut rose cv. Samantha indicated that 1-MCP did not inhibit its up-regulation (Ma et al., 2006). However, the expression of *DenACS1* in the 1-MCP followed by ethylene-treated floral buds and open florets was lowest compared to the others (Figure 6D). This finding underscores the complex interplay between ethylene and 1-MCP in the regulation of *DenACS1* and highlights the potential interactions between ethylene signaling pathways at different floral developmental stages.

The expressions of *DenACO1* genes in floral buds and open florets treated with 1-MCP and open florets subjected to a sequential 1-MCP followed by ethylene treatment consistently exhibited lower transcript levels **(Figure 6E-H)**. This consistent pattern suggests that applying 1-MCP exerts a suppressive influence on *DenACO1* gene expression. Similar effects were also observed in various flowers, such case of *Rh-ACO1* expression in roses (Ma et al., 2006) and *Nn-ACO* in 1-MCP-treated lotus flowers (Imsabai et al., 2010). In contrast to 1-MCP treatment, the expressions of *DenACO1* increased when treated with ethylene, particularly in open florets **(Figure 6G)**. Hence, the expression levels of *ACO* genes might reflect the rate-limiting step in ethylene biosynthesis in this *Dendrobium* cultivar. Thus, the increase in *ACS1* expression when treating *Dendrobium* florets solely with 1-MCP implies that it might be a response to the disruption caused by 1-MCP in the ethylene production and signaling pathway through suppression of *ACO1* abundance. Understanding these distinctions could provide insights into how plants respond to disruptions in ethylene signaling pathways and the regulatory mechanisms involved in ethylene biosynthesis.

In this study, the findings illuminated the differential mechanisms underlying exogenous ethylene-induced premature senescence in floral buds and open florets of *Dendrobium* 'Khao Chaimongkol'. In floral buds, ethylene treatment upregulated the expression of the ethylene receptor *ERS1*, signal transduction genes *CTR1*, *EIL1*, and *ERF1*, as well as the ethylene biosynthesis genes *ACS1* and *ACO1*, leading to senescence. However, in open florets, ethylene significantly increased the expression of *ERS1*, and mildly upregulated *CTR1* and *ACO1*, without affecting the expression of *EIL1*, *ERF1,* and *ACS1*. Consequently, floral buds exhibited more pronounced premature senescence compared to open florets. This differential response indicated distinct effects of exogenous ethylene on ethylene signaling transduction between floral buds and open florets. Conversely, the application of 1-MCP led to competitive binding of ethylene receptors, resulting in the suppression of *ERS1*, *CTR1*, *EIL1*, *ERF1*, and *ACO1* expression in floral buds. In open florets, however, 1-MCP did not suppress the expression of *EIL1*, *ERF1*, and *ACS1*. Consequently, premature senescence was inhibited in both floral buds and open florets. These findings demonstrated that floral buds exhibited a heightened sensitivity to ethylene relative to open florets. The expression levels of ethylene receptor genes, signaling genes, and *ACO1* play a significant role in modulating ethylene sensitivity.

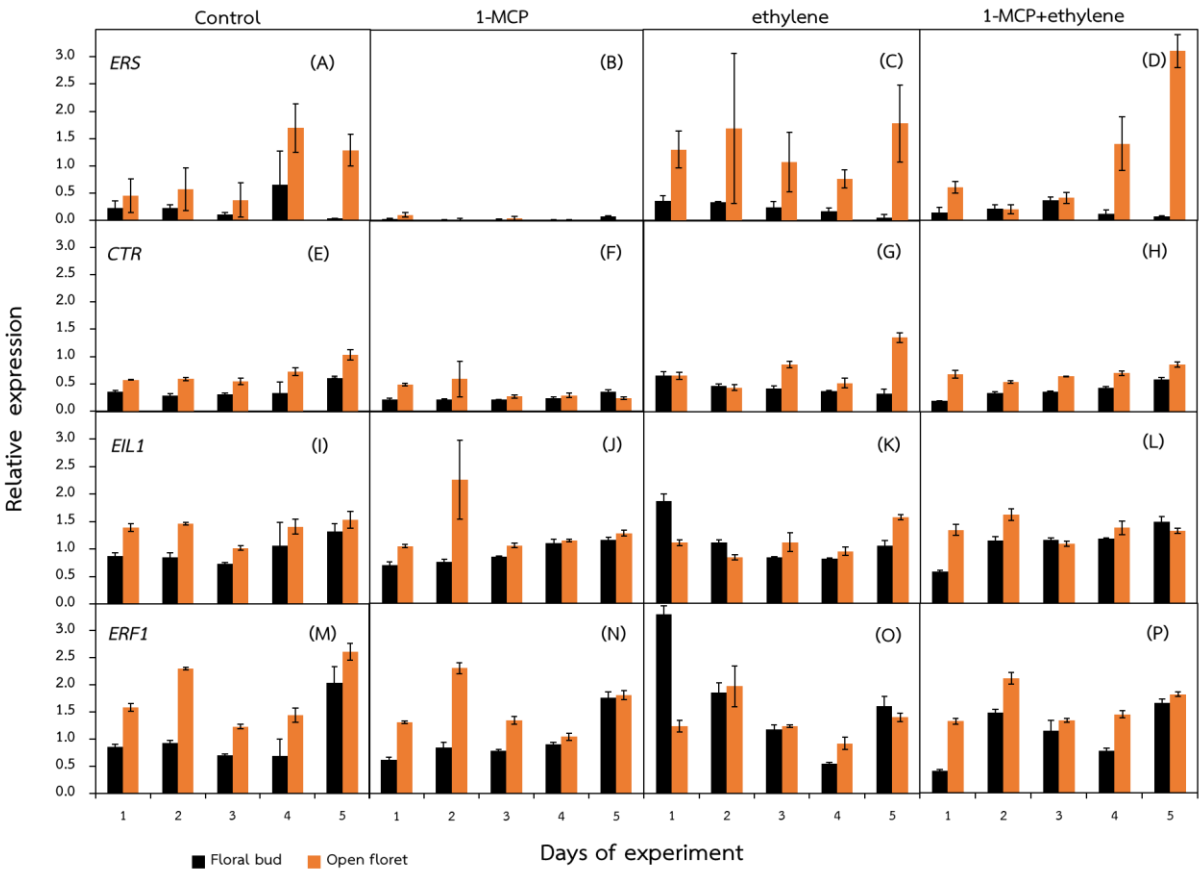

Figure 5 Expression of *DenERS1* (A-D), *DenCTR1* (E-H), *DenEIL1* (I-L) and *DenERF* (M-P) genes in in floral buds and open florets of *Dendrobium* ‘ Khao Chaimongkol’ under various treatments: untreated, 1-MCP treated (500 $nL.L^{-1}$ for 3 hours), ethylene treated (0.4 $\mu L.L^{-1}$ C_2H_4 at 25°C for 24 hours), and a combination of 1-MCP treatment (500 $nL.L^{-1}$ for 3 hours) followed by ethylene treatment (0.4 $\mu L.L^{-1}$ C_2H_4 at 25°C for 24 hours). Expression levels were determined, and data represent the mean from three technical replicates $\pm$ SD.

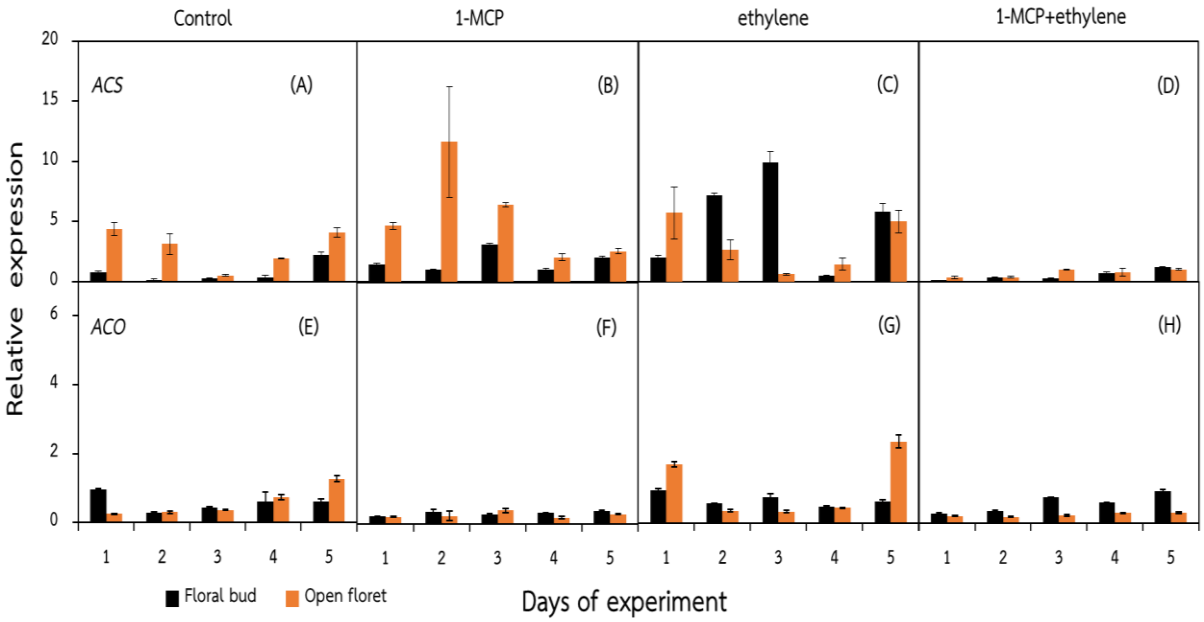

Figure 6 Expression of *DenACS1* (A-D) and *DenACO1* (E-H) genes in floral buds and open florets of *Dendrobium* ' Khao Chaimongkol' under various treatments: untreated, 1-MCP treated (500 nL.L^{-1} for 3 hours), ethylene treated (0.4 µL.L^{-1} C_2H_4 at 25°C for 24 hours), and a combination of 1-MCP treatment (500 nL.L^{-1} for 3 hours) followed by ethylene treatment (0.4 µL.L^{-1} C_2H_4 at 25°C for 24 hours). Expression levels were determined, and data represent the mean from three technical replicates ± SD.

Conclusions

This research illuminated the distinct mechanisms by which ethylene and 1-MCP regulate gene expression during premature senescence in *Dendrobium* 'Khao Chaimongkol'. Significant variations were observed in the expression of ethylene receptor, signaling, and ethylene biosynthesis genes across the differential sensitivity of floral tissues and developmental stages to ethylene. These findings contributed to a deeper understanding of the molecular responses to ethylene and its inhibition, providing insights to improve the post-harvest longevity of *Dendrobium* cut flowers.

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