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Involvement of Ethylene in Physiological Processes Determining the Vase Life of Various Hybrids of *Mokara* Orchid Cut Flowers

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Abstract: There is limited information about the postharvest performance and physiology of *Mokara* orchid cut flowers, which are a special group of artificially created trigenetic hybrids of *Vanda* × *Arachnis* × *Ascocentrum*. Therefore, we first characterized the patterns of various physiological parameters during vase life of five *Mokara* hybrids, which differ in their longevity. Then, we examined the effects of ethephon and ethylene inhibitors on these physiological parameters, and on parameters of the ethylene biosynthesis pathway, during vase life of two selected *Mokara* hybrids, "Moo-deang" and "Dao-lai", which showed significant differences in their vase life duration and senescence symptoms. The results demonstrate that the differences in vase life longevity among the five *Mokara* hybrids are due to differences in their ethylene production rates, which regulate flower development processes expressed in bud opening and floret senescence. The results clearly show that ethylene is involved in the regulation of the *Mokara* flower senescence, and pretreatment with ethylene inhibitors significantly improved their vase life longevity. Thus, ethylene seems to be the main factor that determines the longevity differences of the *Mokara* hybrids, rather than their water relations parameters. This study can serve as a research tool for developing effective postharvest treatments for *Mokara* hybrids.

Keywords: cut flowers; ethephon; ethylene inhibitors; ethylene pathway; floret senescence; flower development; x *Mokara* orchid hybrids; respiration; vase life; water relations

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1. Introduction

The *Orchidaceae* family includes groups of ethylene-sensitive genera, which show a great variable sensitivity to ethylene among the species and cultivars [1–4]. The previous study of Woltering and Van Doorn (1988) [4] reported that the orchid flowers of *Cattleya*, *Paphiopedilum*, *Dendrobium* and *Phalaenopsis* are highly sensitive to ethylene. *Cymbidium* cut flowers were sensitive to exogenous ethylene, manifested in color fading and wilting of sepal tips, induction of anthocyanin formation in female reproductive parts, and floret abscission [5,6]. Cut flowers of *Dendrobium* orchid cv. "Karen" responded to endogenous ethylene by abscission of young floral buds and fastening the opening of mature buds [7].

Recently we reported that exposure of cut flowers of *Vanda* orchids cv. "Patchara Delight", "Pure Wax" and "Sansai Blue" to exogenous ethylene (10 $\mu L \cdot L^{-1}$) for 24 h significantly reduced their vase life by 50% [8]). A further study by our group suggested that the ethylene-induced rapid bleaching in petals of *Vanda* "Sansai Blue" cut flowers is independent of the flower senescence process, and is an outcome of anthocyanin degradation, partially mediated by increase in peroxidase activity [9].

During development and senescence, ethylene biosynthesis in plants is under metabolic regulation. S-adenosyl methionine (SAM) is converted to 1-aminocyclopropene-1-carboxylic acid (ACC) by ACC synthase (ACS) enzyme, which is the first rate-limiting step in the ethylene biosynthesis pathway. Subsequently, ACC is converted to ethylene by ACC oxidase (ACO) enzyme [10–12]. Cut flowers can be categorized as either climacteric or nonclimacteric according to whether or not, respectively, ethylene plays a pivotal role in coordinating their senescence [13–16]. Climacteric flowers may also have an increase in respiration during senescence. The ethylene climacteric peak in cut flowers appears to be under autocatalytic regulation, since exposure to ethylene induces endogenous ethylene biosynthesis by turning on genes and enzymes responsible for this process, which coincides with the ethylene climacteric rise [13–16].

Various ethylene inhibitors were developed and commercially used to overcome the deleterious effects of ethylene and prolong the vase life of many ornamentals including orchids [17]. Thus, an inhibitor of ethylene biosynthesis, aminooxyacetic acid (AOA) [18], and two inhibitors of ethylene perception, silver thiosulfate (STS) [19], and 1-methylcyclopropene (1-MCP) [20,21] are commonly used with ornamentals. Therefore, it is possible that these inhibitors could be effective in *Mokara* hybrid orchids.

Mokara orchids are a special group of artificially created tri-genetic hybrids of *Vanda* × *Arachnis* × *Ascocentrum* [22]. The *Mokara* orchid flowers are tropical and exotic flowers, with several freckled and broad starfish-shaped florets on the stem, and therefore the inflorescences are commonly used as cut flowers. The information about the postharvest physiology of *Mokara* orchid cut flowers is extremely limited [23]. The vase life is often shortened due to floret senescence and problems in water uptake due to xylem plugging by the presence of bacteria and fungi [24,25]. Addition of leaf extracts with antifungal activity from *Jatropha curcas*, *Psidium guajava*, and *Andrographis paniculata* to the vase solution, reduced microbial populations, improved water uptake, and extended the inflorescence longevity comparing to 8-hydroxyquinoline citrate (8-HQC) control [24,25].

To the best of our knowledge, there is no report regarding ethylene involvement in the senescence of *Mokara* orchid cut flowers. Here, we report for the first time on a negative correlation between ethylene production rates and vase life of five *Mokara* hybrids, which widely vary in their vase life longevity. To investigate further the effects of ethylene on the flowers' quality, we challenged two *Mokara* hybrids with exogenous ethylene, and analyzed their responses. In addition, we examined the effects of inhibitors of ethylene biosynthesis or perception on inflorescences quality and vase life, as well as on activities of ethylene biosynthesis enzymes, which represent autocatalytic effects. The results suggest that ethylene is involved in the regulation of the *Mokara* orchid flower senescence, and pretreatment with ethylene inhibitors can improve their vase life longevity. Accordingly, the rate of endogenous ethylene biosynthesis can be used as a criterion for selection of *Mokara* hybrids for cut flowers with long vase life.

2. Materials and Methods

2.1. Plant Material and Treatments

Inflorescences of five hybrids of *Mokara* (*Arantha* × *Ascocentrum* × *Vanda*) orchids were obtained from Thai Orchid Co. Ltd., Bangkok, Thailand. The appearance of the florets of the five hybrids, "Moo-deang", "Jao-pra-ya", "Duang-porn", "Nora-pink", and "Dao-lai", is presented in Figure 1. The *Mokara* inflorescences were harvested at a commercial maturity stage (7–9 open florets and 4–5 buds), were selected for uniformity and lack of defects, packed dry, and transported to King Mongkut's University of Technology Thonburi (KMUTT), Bangkhuntien Campus. Within 1 h after arrival to the laboratory, inflorescence stems were recut (in water) to a 30-cm length. The inflorescences were held in vases with distilled water in the observation room maintained at 21 + 2 °C, 70–80% RH, and cool-white fluorescent light for a 12-h photoperiod.

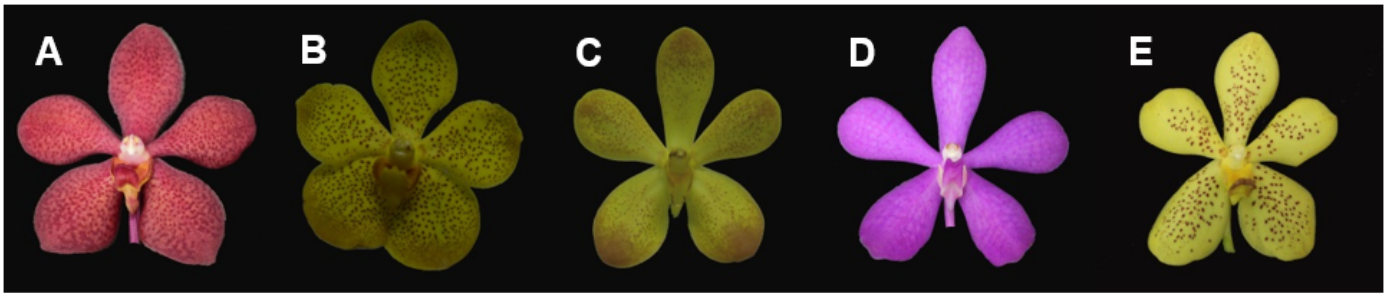

Figure 1. Florets appearance of five *Mokara* orchid flower hybrids: "Moo-deang" (**A**), "Jao-pra-ya" (**B**), "Duang-porn" (**C**), "Nora-pink" (**D**), and "Dao-lai" (**E**).

Application of (2-chloroethyl)-phosphonic acid (ethephon): Inflorescences of "Moo-deang" and "Dao-lai" hybrids were pulsed with either distilled water (control) or with 10 $\mu L \cdot L^{-1}$ ethephon for 24 h in the observation room.

Application of inhibitors of ethylene biosynthesis or perception: Inflorescences of "Moo-deang" and "Dao-lai" hybrids were pulsed with either 0.5 mM AOA or 0.05 mM STS for 24 h in the observation room. For application of 1-MCP, the inflorescences were exposed to 200 $nL \cdot L^{-1}$ 1-MCP (0.14% *w*:*w* 1-MCP; Floralife, Inc., Walterboro, SC, USA) in 43-L glass chambers for 6 h in the observation room. Control flowers were exposed to air under the same conditions.

Following treatments, all treated inflorescences were transferred to vases with distilled water and were held in the observation room throughout the experimental period. Samples of 45 inflorescences per treatment were used for all the experiments.

2.2. Measurement of Fresh Weight and Water Uptake

Fresh weight (FW) changes of *Mokara* orchid inflorescences were calculated daily and expressed as % FW relative to the initial FW on day 0 (g g^{-1} initial FW day^{-1}). Changes of water uptake (mL day^{-1}) were recorded daily from the volume losses of the vase solutions during the vase life evaluation period.

2.3. Measurement of Bud Opening, Floret Senescence, and Vase Life Duration

Open buds and senescent florets were monitored every two days, and their numbers were presented as percent of open buds and senescent florets in each inflorescence. Vase life was defined according to the number of days until 30% senescent florets were observed. The percentage of senescent florets in each inflorescence was calculated according to the sum of florets with petal enrolling and abscised florets.

2.4. Measurement of Ethylene Production and Respiration Rates

Ethylene production rate of whole inflorescences was monitored by enclosing three inflorescences in an airtight polyethylene container and allowing ethylene to accumulate for 1 h at 21 + 2 °C. Three replicates were analyzed per each time point. Gas samples were collected with an 1-mL syringe and analyzed for ethylene in a gas chromatograph (GC8A, Shimadzu, Kyoto, Japan), equipped with a 2-m stainless steel column packed with Unibeads C 80/100 mesh, and a flame ionized detector. Nitrogen was used as the carrier gas, and the temperatures of the column, injector and detector were 220, 100 and 100 °C, respectively. Ethylene production rate was expressed as $\mu L\ C_2H_4\ kg^{-1}FW\ h^{-1}$.

Respiration rate of the inflorescences was monitored by recording the carbon dioxide concentration, which was analyzed in a gas chromatograph (GC2014, Shimadzu, Kyoto, Japan), equipped with a 1.8-m stainless steel column packed with WG100 KA1144 and a flame ionized detector. Helium was used as the carrier gas, and the temperature of the column, injector and detector was 50 °C. Respiration rate was expressed as $mg\ CO_2\ kg^{-1}FW\ h^{-1}$.

2.5. Evaluation of ACC Content and Activities of ACS and ACO Enzymes

ACO was extracted and assayed according to the method described by Kato and Hyodo [26]. The tissues of two lower florets per inflorescence were pooled, immediately frozen in liquid nitrogen, and stored at −70 °C until used. Samples of 3-g florets were homogenized in 2 mL of extraction buffer consisting of 0.1 M Tris-HCl, pH 7.2, 5 mM dithiothreitol (DTT), 30 mM Na-ascorbate, and 10% glycerol (v/w) at 2 °C. The homogenate was centrifuged at 14,000× g for 20 min at 4 °C, and the supernatant was used for assays of the ACC content and ACO enzyme activity.

The assay tubes were held in an ice bath and after 3 min a gas sample was withdrawn for measurement of ethylene concentration in a gas chromatograph (GC8A, Shimadzu, Kyoto, Japan). The efficiency of ACC conversion to ethylene in each sample was determined by adding a known amount of ACC (at least three times the anticipated ACC content of the tissue sample), as an internal standard to each replicate assay tube. The identity of ACC in the Mokara orchid tissue was verified by cochromatography with authentic ACC on a Whatman No. 3 paper developed with butanol-acetic acid-water (4:1:5, v/v), as described by Lizada and Yang [27].

ACO activity in the supernatant was assayed in a reaction medium consisting of 0.1 M Tris-HCl buffer, pH 7.2, 30% glycerol, 1 mM ACC, 10 mM sodium ascorbate, 50 mM $FeSO_4$ and 10 mM $NaHCO_3$ in a total volume of 1 mL. The test solution was placed in 6-mL sealed test tubes, which were incubated at 37 °C and gently shacked for 30 min. A 1-mL sample of the headspace gas was then withdrawn for ethylene determination in a gas chromatograph (GC-8A, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector. ACO activity was determined as the amount of ethylene produced from ACC during the reaction period, and expressed as nL C_2H_4 mg protein^{-1} h^{-1}.

For the analysis of ACS activity, a 5-g sample of floret tissue was rinsed twice with distilled water, blotted with a paper towel, and ground in 100 mM *N*-2-hydroxyethylpiperazine propane sulfonic acid (EPPS) buffer (pH 8.5) containing 4 mM DTT and 0.5 mM pyridoxal phosphate (1:2, w/v), using mortar and pestle. The homogenate was centrifuged at 12,000× g for 20 min. The supernatant extract was placed in a dialysis bag, soaked in dialysis buffer containing 2 mM EPPS buffer, 0.1 mM DTT and 0.2 µM pyridoxal phosphate (1:10, v/v), and dialyzed for 24 h. All steps were carried out on ice or at 4 °C. The ACS activity was assayed as described by Hoffman and Yang [28], by incubating 0.4-mL extract in a 6-mL tube with 50 µL of 0.5 mM SAM and 90 µL of distilled water at 30 °C for 2 h. The ACC produced was determined as described above. ACS activity was expressed as nmol ACC mg protein^{-1} h^{-1}.

2.6. Experimental Design and Statistics Analysis

The experiments were conducted in a completely randomized design (CRD). The quality parameters (FW, water uptake, percent of open buds and senescent florets) were analyzed using 8–10 replicate inflorescences per treatment, and the physiological and enzymatic parameters (rates of respiration and ethylene production, ACC content, and activities of ACS and ACO enzymes) were analyzed using three replicate samples. The data were analyzed using ANOVA, and differences among means were compared by Duncan's New Multiple Rang Test (DMRT), using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). The coefficient of determination between vase life and ethylene production rates was analyzed by linear regression model in Excel.

3. Results

3.1. Characterization of the Different Vase Life Performance among the Five Mokara Hybrids

After an initial slight increase from day 0 to day 2, the relative FW of four *Mokara* orchid hybrids inflorescences continuously decreased throughout the vase life period (Figure 2A). Unlike this pattern, the relative FW of the fifth hybrid, "Dao-lai", started to decrease only after the fourth day, in a slower rate than that of the other hybrids. The "Jao-pra-ya" hybrid showed the fastest rate in FW decrease, reaching about 85% relative

FW on day 8 (Figure 2A), when its vase life was terminated (Table 1). Thus, the five hybrids can be arranged in the following order of decrease in their relative FW: "Jao-pra-ya" > "Duang-porn" > "Moo-deang" = "Nora-pink" > "Dao-lai" (Figure 2A).

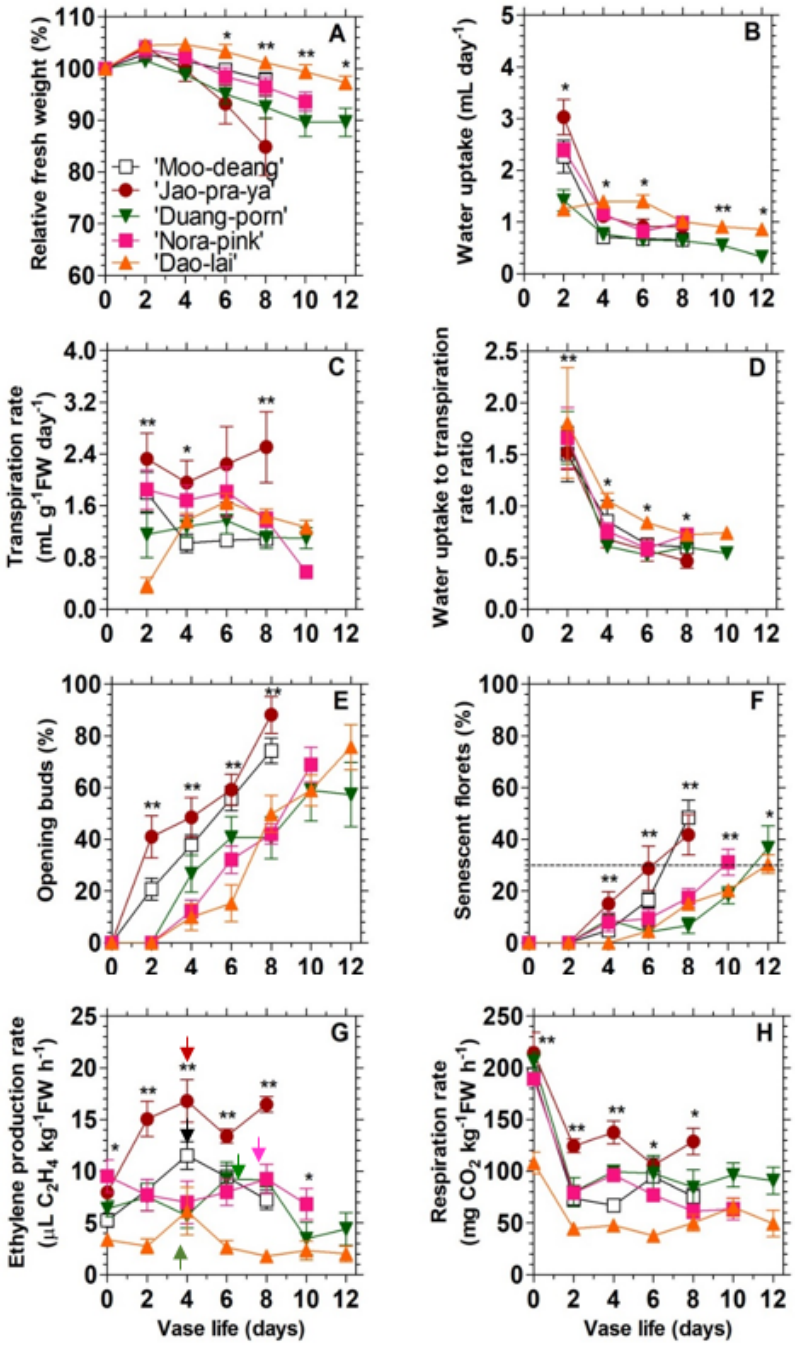

Figure 2. Changes in different flower quality parameters of cut inflorescences of five *Mokara* orchid hybrids during 12 days of vase life. The examined parameters included: relative fresh weight (**A**), water uptake rate (**B**), transpiration rate (**C**), water uptake to transpiration ratio (**D**), percent of open buds (**E**), percent of senescent florets (**F**), ethylene production rate (**G**), and respiration rate (**H**). Inflorescences were held in distilled water in the observation room throughout the experimental period. The dashed line in graph **F** indicates the termination of vase life, when 30% senescent florets were observed. The colored arrows in graph **G** indicate the climacteric peaks of each hybrid. The results represent means of 8–10 replicates ± SE. *, ** represent significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively, compared for the five hybrids according to DMRT test.

The differences in the rates of water uptake among the five hybrids were relatively large on day 2 of vase life (Figure 2B). The results showed a sharp decline in the rate of water uptake by the inflorescences in all hybrids between days 2 and 4, which remained almost steady thereafter, showing a slight and sustained decline throughout the vase life period. The rates of water uptake of the *Mokara* hybrids "Moo-deang" and "Duang-porn" inflorescences were lower than the rates of the other hybrids. The "Jao-pra-ya" inflorescences had the highest water uptake rate in the initial day 2, which decreased sharply thereafter. However, "Dao-lai" inflorescences had the highest relative FW (Figure 2A) and rate of water uptake (Figure 2B) after day 4 in vase life.

The changes in FW during vase life depend on the ratio between the water uptake and the transpiration rates. The "Jao-pra-ya" hybrid showed the highest transpiration rate (Figure 2C), and a lower ratio of water uptake/transpiration (Figure 2D) than the other hybrids during vase life. On the other hand, the 'Dao-lai' hybrid showed the highest ratio of water uptake/transpiration compared to the other hybrids during vase life (Figure 2D). It is important to emphasize that despite the differences in relative FW among the hybrids during vase life, the inflorescences of all hybrids were turgid, and this allowed the buds to open at constant rates during 12 days of vase life (Figure 2E). Quite surprisingly, it was found that in the "Jao-pra-ya" hybrid, which showed the fastest FW decrease, the rates of bud opening and of floret senescence were the fastest (Figure 2E,F). A similar but opposite pattern was observed in the 'Dao-lai' hybrid, in which the decrease in the relative FW was the slowest, and the rates of bud opening and florets senescence were the slowest (Figure 2E,F). These results suggest that the development stages of bud opening and floret senescence are positively correlated and similarly regulated. The vase life of the five hybrids of *Mokara* orchids held in distilled water varied from 7.6 to 11.5 days after harvest (Table 1). Thus, "Nora Pink", "Duang-porn", and "Dao-lai" inflorescences had long lasting quality with 10.3, 11.5 and 11.5 days of vase life, respectively, while "Jao-pra-ya" and "Moo-deang" inflorescences showed a short vase life of 7.6 and 8.0 days, respectively (Table 1), reflecting their faster floret senescence rates (Figure 2F).

The rates of ethylene production (Figure 2G) and respiration (Figure 2H) varied significantly among the five *Mokara* hybrids. *Mokara* orchids produced a detectable high ethylene production rate on day 0 after harvest, which peaked on day 4 in three of them. The other two hybrids, "Nora-pink" and "Duang-porn" showed a small ethylene production peak on day 8 (Figure 2G). "Jao-pra-ya" and "Moo-deang" inflorescences showed on day 4 high ethylene production rates of 16.80 and 11.53 μL C_2H_4 kg^{-1}FW h^{-1}, respectively, which were more than twice higher than the rates produced by "Nora-pink", "Dao-lai" and "Duang-porn" inflorescences, which reached levels of 7.00, 6.16 and 5.71 μL C_2H_4 kg^{-1}FW h^{-1}, respectively, on this day. Similar to the ethylene patterns, *Mokara* orchids had high respiration rates on the initial day 0, which decreased sharply on day 2, and remained quiet constant later on throughout the vase life period (Figure 2H). Thus, on day 4 of vase life, "Jao-pra-ya" inflorescences had the highest respiration rate of 137.66 mg CO_2 kg^{-1}FW h^{-1}, while "Dao-lai" inflorescences showed the lowest rate of 44.50 mg CO_2 kg^{-1}FW h^{-1}.

The results showed a correlation between the rates of ethylene production and respiration (Figure 2G,H), and the percentage of bud opening and senescent florets (Figure 2E,F). 'Jao-pra-ya' inflorescences showed the highest rates and 'Dao-lai' the lowest rates of these parameters, while the other hybrids showed intermediate values. Ethylene production rates were negatively correlated with vase life longevity of the five hybrids, but the correlation was statistically significant only on day 4 (Table 1). The results suggest that the differences in vase life longevity among the five *Mokara* hybrids are due to the differences in their ethylene production rates, especially at the peak of production, which regulate the flower development processes expressed in bud opening and floret senescence. We therefore examined the effect of ethylene on these processes, which are usually accompanied by high respiration rates.

Table 1. Correlation of vase life and ethylene production rates during the initial six incubation days of cut inflorescences of five *Mokara* orchid hybrids. The inflorescences were incubated in distilled water in the observation room throughout the experimental period. The results represent means of 8–10 replicates ± SE. Different letters represent significant differences at $p \leq 0.01$ according to DMRT test. *, ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively according to linear regression; the higher R-Squared value the better (>0.70). NS, not significant.

Hybrids	Vase Life	Ethylene Production Rates (μL C_2H_4 kg^{-1} FW h^{-1})		
		Day 2	Day 4	Day 6
'Moo-deang'	8.0 ^{c}	8.22 ^{b}	11.54 ^{ab}	9.46 ^{b}
'Jao-pra-ya'	7.6 ^{c}	15.06 ^{a}	16.81 ^{a}	13.42 ^{a}
'Duang-porn'	11.5 ^{a}	7.52 ^{b}	5.71 ^{b}	9.28 ^{b}
'Nora-pink'	10.3 ^{b}	7.70 ^{b}	7.00 ^{b}	7.98 ^{b}
'Dao-lai'	11.5 ^{a}	2.77 ^{c}	6.16 ^{b}	2.66 ^{c}
F-test	**	**	**	**
Coefficient of determination of vase life and ethylene production		y = −0.3191x + 12.354 R^2 = 0.5698 NS	y = −0.366x + 13.176 R^2 = 0.8656 *	y = −0.3383x + 12.616 R^2 = 0.4976 NS

3.2. Ethylene Regulates Floret Development and Inflorescence Longevity in Two Mokara Hybrids

3.2.1. Effects of Exogenous Ethylene Treatment

We pre-treated two *Mokara* hybrids with ethephon during 24 h of incubation, which generates endogenous ethylene production, for examining the changes of the various parameters in response to high ethylene levels. The control inflorescences of 'Moo-deang' hybrid showed a fast floret abscission after petal enrolling started (data not shown), and had a shorter vase life of 8 days (Table 1), while 'Dao-lai' florets showed delayed abscission after petals enrolling (data not shown), and had a longer vase life of 11.5 days (Table 1). Both hybrids similarly responded to ethephon treatment (Table 2, Figure 3). Ethephon accelerated bud opening (Figure 3A,B,E) and floret senescence (Figure 3C,D,E) of both 'Moo-deang' and 'Dao-lai' *Mokara* hybrids. As a result, ethephon treatment reduced the vase life longevity of 'Moo-deang' and 'Dao-lai' by 47% and 42%, respectably (Table 2). The differences in total bud opening of control inflorescences in this experiment (40–50%) (Figure 3A,B), compared to about 80% of bud opening on days 8 or 12 in these two hybrids in a former experiment (Figure 2E), could be probably a result of different environmental growth conditions, expressed in warmer temperatures in the second growing season.

Table 2. Effect of ethephon on vase life duration of cut inflorescences of 'Moo-deang' and 'Dao-lai' *Mokara* orchid hybrids. Cut inflorescences were pulsed with either distilled water (control) or 10 μL·L^{-1} ethephon for 24 h, and then transferred to distilled water throughout the experimental period in the observation room. The results represent means of 8–10 replicates ± SE. Different letters represent significant differences at $p \leq 0.01$, compared to distilled water according to DMRT test.

Hybrids	Treatments	Vase Life (Days)
'Moo-deang'	Control	8.1 ^{b}
	Ethephon	4.3 ^{d}
'Dao-lai'	Control	11.3 ^{a}
	Ethephon	6.6 ^{c}
F-test		**

Ethylene production rates did not change significantly in the 'Moo-deang' hybrid after ethephon treatment, and a small endogenous climacteric ethylene production peak of about 10 μL C_2H_4 kg^{-1} FW h^{-1} was already obtained in control untreated flowers

(Figure 4A). On the other hand, 'Dao-lai' inflorescences exhibited a typical classic ethylene autocatalytic production rate in response to ethephon treatment, reaching a value of 15.83 μL C_2H_4 kg^{-1}FW h^{-1}, which was 3-times higher than that of the control untreated inflorescences (Figure 4B). ACO activity significantly increased in response to ethephon treatment, which could be observed from day 4 in the 'Moo-deang' hybrid (Figure 4C), and already on day 2 in the 'Dao-lai' hybrid (Figure 4D). Increase in ACS activity as part of the autocatalytic ethylene production, could be observed in the ethephon-treated inflorescences of the 'Moo-deang' hybrid during days 2–6 (Figure 4E), and on day 8 in the 'Dao-lai' hybrid (Figure 4F). A small peak of increased ACC content in the ethephon-treated inflorescences was obtained on day 4 in the 'Moo-deang' hybrid (Figure 4G), and a similar but sharper peak increase was observed in the 'Dao-lai' hybrid (Figure 4H). This sharp ACC increase induced by ethephon, coincided with the high ethephon-induced ethylene production rates observed in this hybrid during days 4–8 (Figure 4B).

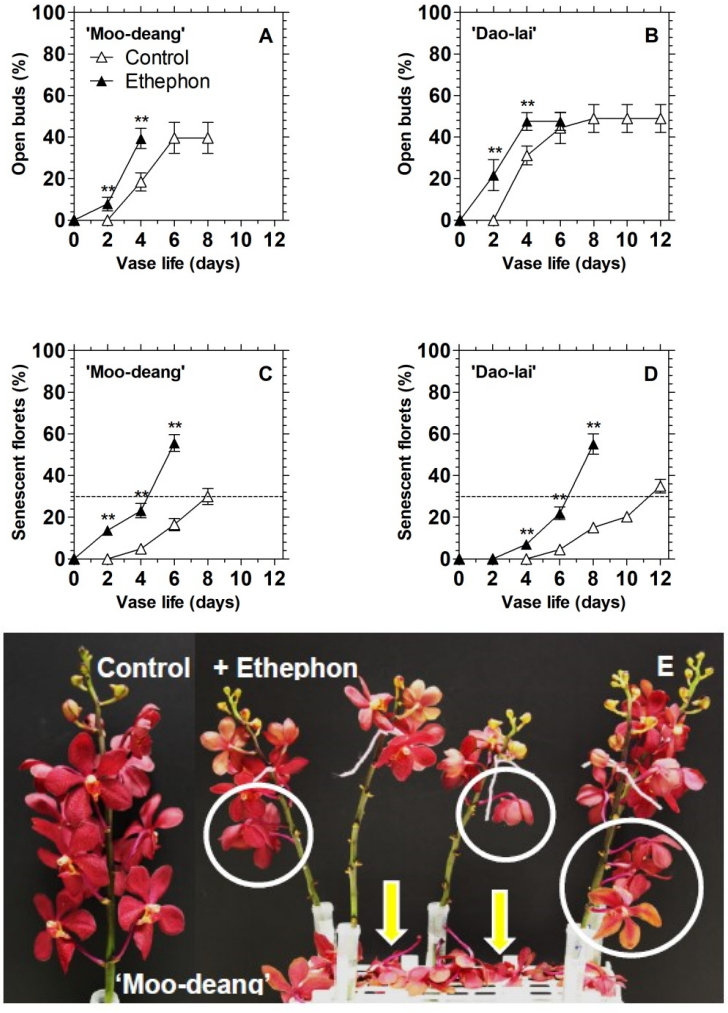

Figure 3. Ethylene enhances bud opening and floret senescence. Effect of ethephon treatment on percent of open buds (**A**,**B**) and senescent florets (**C**,**D**) during vase life of cut inflorescences of two *Mokara* orchid flower hybrids, 'Moo-deang' (**A**,**C**) and 'Dao-lai' (**B**,**D**), and on appearance of petal enrolling (circles) and abscission symptoms (arrows) in 'Moo-deang' inflorescences on day 4 of vase life (**E**). Inflorescences were pulsed with either distilled water (control) or 10 μL·L^{-1} ethephon for 24 h, and then transferred to distilled water throughout the experimental period in the observation room. The results represent means of 8–10 replicates ± SE; ** represent significant differences at $p \leq 0.01$. The dashed lines in graphs C and D indicate the termination of vase life when 30% senescent florets were observed.

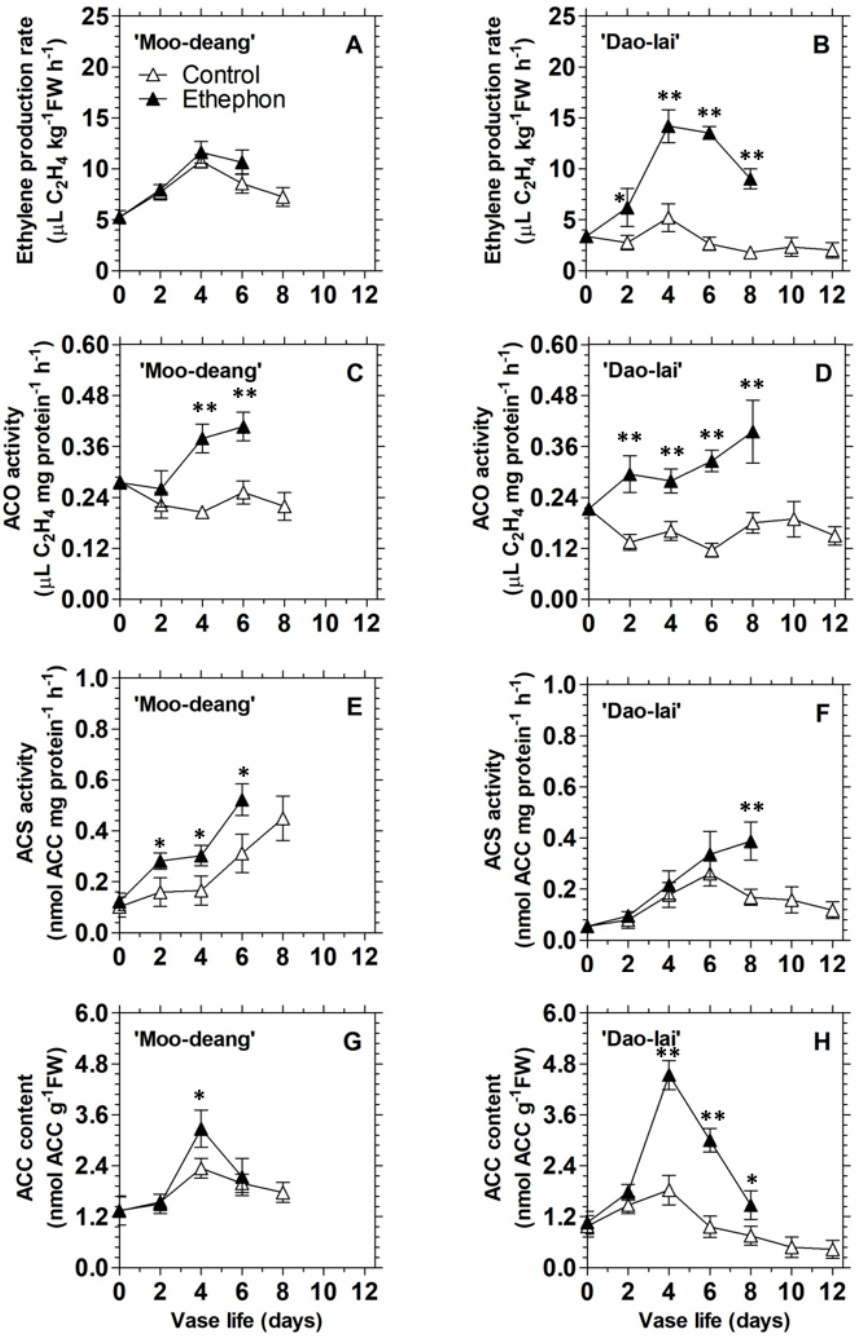

Figure 4. Ethylene enhances the parameters of ethylene biosynthesis pathway. Effect of ethephon treatment on ethylene production rates (**A**,**B**), ACO activity (**C**,**D**), ACS activity (**E**,**F**), and ACC content (**G**,**H**), during vase life of cut inflorescences of two *Mokara* orchid hybrids, 'Moo-deang' and 'Dao-lai'. Inflorescences were pulsed with either distilled water (control) or 10 $\mu L \cdot L^{-1}$ ethephon for 24 h, and then transferred to distilled water throughout the experimental period in the observation room. Each parameter was examined with samples of two florets. The results represent means of three replicates ± SE; *, ** represent significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively.

3.2.2. Effects of Treatments with Ethylene Inhibitors

Ethylene inhibitors, AOA, STS, and 1-MCP significantly decreased *Mokara* floret development rates: They delayed the increase in the percentage of open buds and senescent florets in both 'Moo-deang' and 'Dao-lai' hybrids, as compared to control inflorescences pulsed with distilled water or pretreated with air (Figure 5). Control 'Moo-deang' inflorescences had 40% open buds on day 6 and 30% senescent florets on day 8, while

inflorescences pretreated with AOA, STS or 1-MCP had 40% open buds on days 9, 11 and 10, and 30% senescent florets only on days 12 and 14, respectively. Similarly, control 'Dao-lai' inflorescences had 40% open buds on day 8, and 30% senescent florets on day 11 of the vase life period. 'Moo-deang' inflorescences pretreated with either one of the three ethylene inhibitors, showed 40% open buds and 30% senescent florets for a longer time of 14 days (Figure 5A,C). 'Dao-lai' inflorescences pretreated with AOA, STS, or 1-MCP, had 40% open buds on days 10, 11 and 12, and 30% senescent florets on days 13, and 14 of the vase life period, respectively (Figure 5B,D). Thus, by using ethylene inhibitors, we were able to show that ethylene regulates *Mokara* orchid floret development, expressed by the processes of bud opening and floret senescence. Consequently, ethylene inhibitors significantly increased by averages of 61% and 26.5%, respectively, the vase life longevity of 'Moo-deang' and 'Dao-lai' hybrids (Table 3).

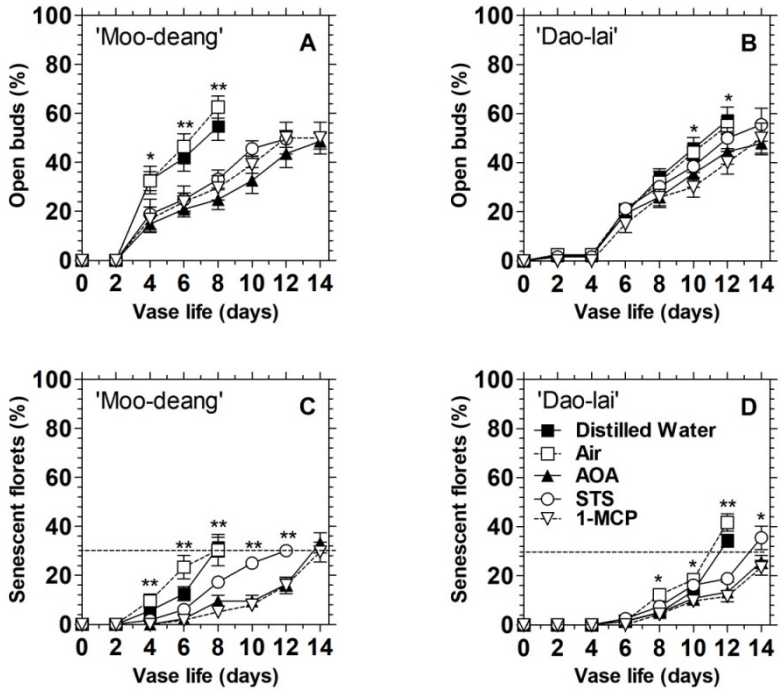

Figure 5. Ethylene inhibitors delay bud opening and floret senescence. Effect of inhibitors of ethylene biosynthesis or perception on percent of open buds (**A**,**B**), and percent of senescent florets (**C**,**D**) during vase life of cut inflorescences of two *Mokara* orchid hybrids, 'Moo-deang' and 'Dao-lai'. The following treatments were applied to *Mokara* inflorescences: Pulsing with distilled water (control), with 0.5 mM AOA or with 0.05 mM STS for 24 h. For 1-MCP treatment, inflorescences in distilled water were either pretreated with air (control) or with 200 $nL \cdot L^{-1}$ 1-MCP for 6 h in closed chambers. Following treatments, the inflorescences were transferred to distilled water, and incubated in the observation room. The results represent means of 8–10 replicates ± SE. *, ** represent significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively, compared for the five treatments according to DMRT test. The dashed lines in graphs **C** and **D** indicate the termination of vase life when 30% senescent florets were observed.

Table 3. Effect of inhibitors of ethylene biosynthesis or perception on vase life duration of cut inflorescences of 'Moo-deang' and 'Dao-lai' *Mokara* orchid hybrids. Application of treatments was as detailed in Figure 5. Following treatments, the inflorescences were transferred to distilled water, and incubated in the observation room. The results represent means of 8–10 replicates ± SE. Different letters and ** represent significant differences at $p \leq 0.01$ compared to distilled water or air controls according to DMRT test.

Treatments	Vase Life (Days)	
	'Moo-Deang'	'Dao-Lai'
Distilled water	8.1 ^{b}	11.3 ^{b}
0.5 mM AOA	13.4 ^{a}	14.3 ^{a}
0.05 mM STS	12.7 ^{a}	13.8 ^{a}
Air	8.4 ^{b}	11.3 ^{b}
200 nL·L^{-1} 1-MCP	13.7 ^{a}	14.8 ^{a}
F-test	**	**

The ethylene biosynthesis inhibitor AOA effectively inhibited ethylene production rates in both *Mokara* hybrids (Figure 6A,B), as expected. All three ethylene inhibitors reduced ethylene autocatalysis, which was manifested in a decrease in ACO activity (Figure 6C,D), and in delaying and reducing the peaks of endogenous ethylene production (Figure 6A,B). The three ethylene inhibitors similarly inhibited ACS activity, as part of the ethylene autocatalytic activity, in the 'Moo-deang' hybrid (Figure 6E), which resulted in reduction of the ACC content in this hybrid (Figure 6G). However, the three ethylene inhibitors had only minor effects on the ACS activity (Figure 6F) and on the ACC content of the 'Dao-lai' hybrid (Figure 6H).

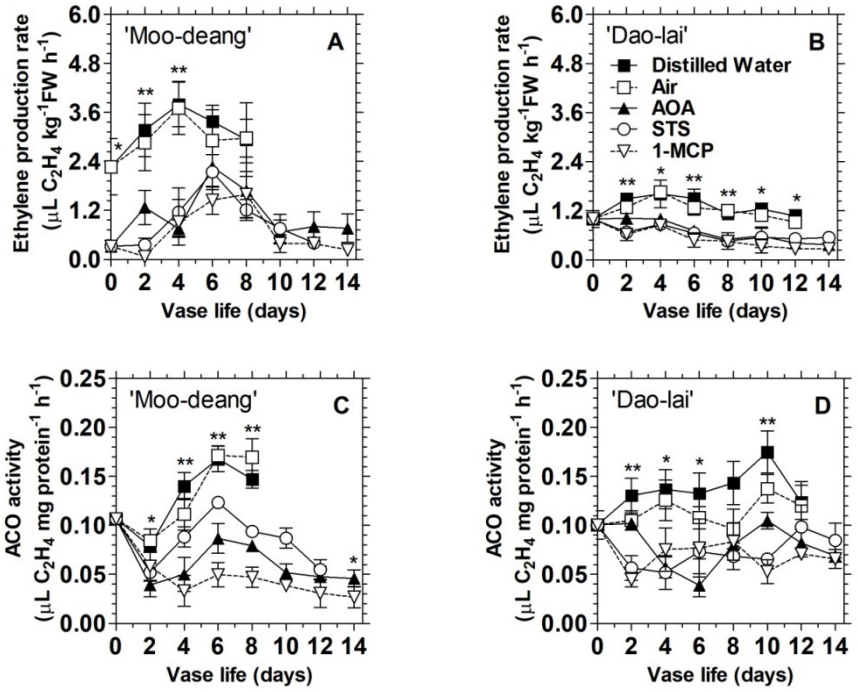

Figure 6. *Cont.*

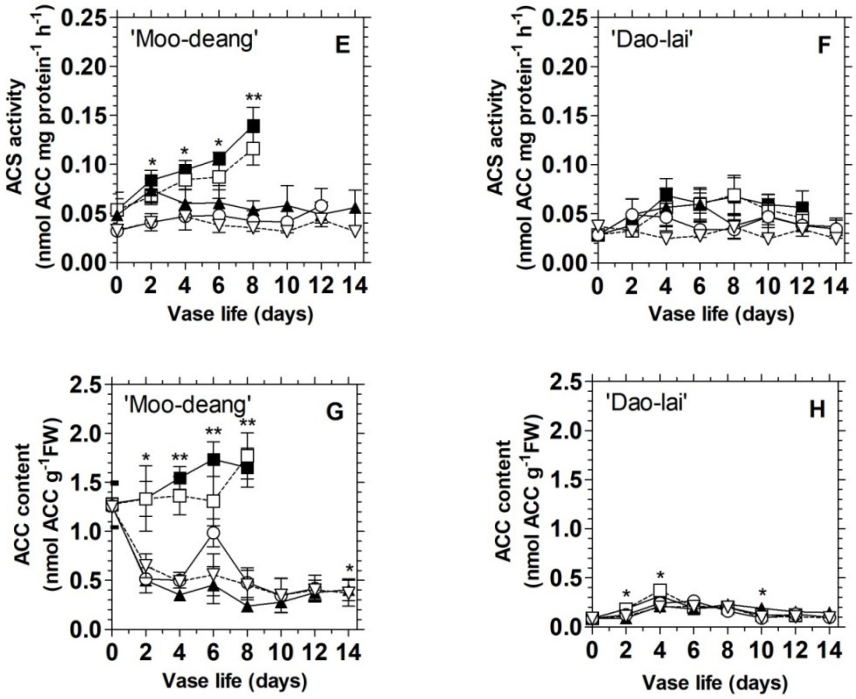

Figure 6. Effect of inhibitors of ethylene biosynthesis or perception on ethylene production rates (**A**,**B**), ACO activity (**C**,**D**), ACS activity (**E**,**F**), and ACC content (**G**,**H**) during vase life of cut inflorescences of two *Mokara* orchid hybrids, 'Moo-deang' and 'Dao-lai'. Application of treatments was as detailed in Figure 5. Each parameter was examined with samples of two florets. The results represent means of three replicates ± SE. *, ** represent significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively, compared for the five hybrids according to DMRT test.

4. Discussion

The vase life of cut orchid flowers is often terminated by floret wilting and withering, accompanied with improper bud opening, which wither and abscise due to a failure in water relations. The floret senescence symptoms are usually expressed in petal enrolling, wilting and abscission [29,30]. The information about the postharvest performance and physiology of *Mokara* orchid cut flowers is extremely limited [23–25]. Therefore, studying various *Mokara* hybrids (Figure 1), which differ in their vase life longevity, can serve as a research tool for developing effective postharvest treatments. The results of the present research show that the vase life of five hybrids of *Mokara* orchids held in distilled water varied from 7.6 to 11.5 days after harvest (Table 1). The termination of the inflorescences' vase life was defined when 30% of their florets were senesced. The main senescence symptoms, manifested in petal enrolling, wilting and floret abscission (Figure 3E), are known to be regulated by ethylene, and are followed by water deficit symptoms [3,4,29–33]. Thus, the ethylene-induced petal enrolling followed by wilting in florets of *Phalaenopsis*, *Doritaenopsis*, *Dendrobium* and *Cymbidium* orchid cut flowers, were accompanied by water loss from cells of the upper layer of the petals, leading to their upward folding [31]. Therefore, the question was whether the variation in vase life duration of the five *Mokara* hybrids stems from the differences in their water relations or in ethylene production parameters.

The results of the present study showed significant differences among the five *Mokara* hybrids, in their water relations parameters (Figure 2A–D). Lower rates of water uptake and higher rates of transpiration resulted in the lowest ratio of water uptake/transpiration, and in the fastest decrease in the relative FW during vase life of the 'Jao-pra-ya' hybrid, as compared to the 'Dao-lai' hybrid. These patterns of water relations may apparently lead to the significant differences in the vase life longevity of these two hybrids, varying between 7.6 and 11.5 days, respectively (Table 1). However, the 'Duang-porn' hybrid, which also showed a fast decrease in its relative FW, had a long vase life of 11.5 days. In

addition, all the hybrids, including 'Jao-pra-ya', did not show water deficit symptoms, such as inflorescences wilting and apex bending, or reduced bud opening. Actually, the two hybrids with the shortest vase life longevity, 'Jao-pra-ya' and 'Moo-deang' (Table 1), showed the highest percent of bud opening during vase life, and their buds opened in the fastest rate compared to the other hybrids (Figure 2E). The process of bud opening usually requires active water uptake and high turgor, as it results in increased floret FW [34]. Therefore, to our opinion, the differences in the vase life longevity observed among the five *Mokara* hybrids are not exclusively due to the differences in their water relations parameters. Additionally, the slight and sustained decline of the water uptake rates, observed from day 4 to the end of vase life in all hybrids (Figure 2B), further suggests that there was no significant blockage in the vascular system of the inflorescences, although the vase solution contained only distilled water without addition of antimicrobial compounds. This conclusion is in contrast to a previous study, performed with '*Mokara Red*' orchid flowers, which suggested that blockage in the vascular system, resulting from a rapid growth of bacteria in the vase solution, is the cause of its short vase life [24,25]. However, to avoid possible contamination during vase life, it is suggested to use preservatives also for *Mokara* hybrids.

It was previously reported that ethylene can affect the water status in some cut orchid flowers. For example, ethephon treatment decreased water uptake and enhanced the decrease in flower FW of *Vanda* orchid flowers cv. 'Sansai Blue' [35], while the ethylene action inhibitors STS or 1-MCP delayed their FW loss or negated the ethephon effects, respectively [35,36]. Similarly, 1-MCP treatment delayed the decrease in FW of two *Dendrobium* cultivars 'Red Sonia' and 'Burana Jade' [37,38]. Accordingly, the possibility that the differences in the water relations parameters observed among the *Mokara* hybrids during vase life, such as decreased FW and reduced ratios of water uptake to transpiration, can be ascribed in part to the differences in their endogenous ethylene production rates.

Orchid flowers are classified as climacteric flowers in which senescence is accompanied by a climactic increase in rates of respiration and ethylene production [29,30,39]. Ethylene climacteric peak in cut flowers appears to be under autocatalytic regulation, since exposure to exogenous ethylene induces endogenous ethylene biosynthesis and higher respiration rates [13–16]. The results of the present study show that ethylene production rates in *Mokara* orchid hybrids had different patterns during vase life. While the patterns of ethylene production rates showed climacteric peaks that varied in timing and magnitude among the hybrids (Figure 2G), their respiration rates did not change much during vase life after the sharp decrease from day 0 to day 2 (Figure 2H). These results suggest that the *Mokara* orchid hybrids could be categorized by the combined differences in their respiration and ethylene production rates, according to the following three patterns: (**1**) low respiration and ethylene production rates ('Doa-lai'); (**2**) moderate respiration and ethylene production rates ('Duang-porn' and 'Nora-pink'); (**3**) high respiration and ethylene production rates ('Moo-deang' and 'Jao-pra-ya'). Thus, the differences in respiration rates, as well as ethylene production patterns and rates, which are probably genetically derived, can serve as markers for developing other *Mokara* cultivars.

The increase in ethylene production rates in the *Mokara* hybrids closely coincided with their senescence symptoms that terminated their vase life longevity. Indeed, ethylene production rates were negatively correlated with the vase life longevity of the five hybrids, which were statistically significant on day 4 (Table 1). These results suggest that the differences in vase life longevity among the five *Mokara* hybrids are due to the differences in their ethylene production rates, especially at the day of their peak production, which regulate their flower development manifested in bud opening and floret senescence.

It was previously suggested that the variation in the postharvest life among flower species and cultivars can be partly ascribed to differences in their endogenous ethylene biosynthesis, as well as to differences in their sensitivity to endogenous and exogenous ethylene [3,4,40]. The responses to ethylene vary widely according to the species [18], although they are often consistent within either families or subfamilies [29]. This sensitivity

to exogenous ethylene plays a vital role in their quality and lasting characteristics [41]. Variation in ethylene sensitivity may be related to differences in the concentration and affinity of the ethylene receptors and/or the activity of downstream components in the signal transduction pathway, which activates gene transcription and translation [42]. It was previously reported that *Orchidaceae* flowers are highly sensitive to ethylene, even after exposure to very low concentrations of ethylene [4], and this sensitivity is generally manifested in flower bud drops or abscission [43]. Ethylene also hastened senescence of petals in flowers of the *Orchidaceae* family, e.g., *Cymbidium* and *Dendrobium*, that initially stay attached to the flower [44].

In the present study, we exposed two *Mokara* hybrids, 'Moo-deang' and 'Dao-lai', to exogenous ethylene using ethephon treatment. These hybrids were chosen based on the differences in their vase life duration, and the symptoms causing their vase life termination. The 'Moo-deang' hybrid showed a fast floret abscission after petal enrolling started, which ended in vase life of 8 days, and 'Dao-lai' florets showed petals enrolling and delayed abscission after 11.5 days of vase life (Figure 2H; Table 1). Both hybrids showed high sensitivity to the same ethephon treatment, which significantly enhanced the typical senescence symptoms of each hybrid (Figure 3C,D), and reduced their vase life longevity very significantly (Table 2). In addition, the sensitivity to ethephon was manifested in enhanced bud opening (Figure 3A,B), and acceleration of various parameters of ethylene autocatalysis (Figure 4). Ethylene was reported to promote or inhibit flower opening, depending on the species and the cultivar [34,45]. In the five *Mokara* hybrids examined in the present study, ethylene promoted bud opening, as buds opened faster (Figure 2E) in the hybrids that produced higher ethylene (Figure 2G). Additionally, in the two selected Mokara hybrids, bud opening and florets senescence were enhanced by ethephon treatment (Figure 3), and were slowed down by ethylene inhibitors (Figure 5). It seems therefore, that in *Mokara* orchids, ethylene regulates the flower developmental processes, manifested in bud opening, floret senescence and abscission. The ethylene autocatalysis in response to ethephon treatment was more pronounced in the 'Dao-lai' hybrid than in the 'Moo-deang' hybrid, as all its ethylene biosynthesis parameters were significantly increased by ethephon during vase life (Figure 4). Unlike this, in the 'Moo-deang' hybrid, the increases in ethylene production rates and in ACC content in response to ethephon were minor and were observed only on day 4. A similar pattern of response to exogenous ethylene as that obtained in the *Mokara* 'Moo-deang' hybrid was reported in *Cattleya* Aliliances orchids, showing reduced vase life, increase in ACO activity and no effect on ACS activity and ACC content [46]. In general, it seems that the increases in ACS and mainly in ACO activities are very sensitive to ethylene in these two *Mokara* hybrids.

To avoid detrimental effects of endogenous ethylene on cut flower quality we treated the two hybrids with inhibitors of ethylene biosynthesis and perception. It was our interest to find whether inhibitors of ethylene can inhibit senescence of *Mokara* orchid cut flowers and increase their vase life longevity. The effects of three ethylene inhibitors, AOA, STS, and 1-MCP, were examined on various quality parameters of the two *Mokara* hybrids, 'Moo-deang' with a short vase life, and 'Dao-lai' with long lasting vase life. Our results revealed that all the ethylene inhibitors effectively and significantly extended the vase life duration of both hybrids (Table 3). These effects could be ascribed to the delaying effects of the inhibitors on floret senescence (Figure 5C,D), as well as to their effects in reducing the endogenous ethylene production rates and ACO activities in both hybrids (Figure 6A–D). However, there were several differences in the responses of the two hybrids to the ethylene inhibitors, which probably resulted from the differences in their original vase life longevity. Thus, the ethylene inhibitors significantly decreased the percentage of bud opening but kept them open for a longer time in the 'Moo-deang' hybrid (Figure 5A), while they had almost no effect on the bud opening in the 'Dao-lai' hybrid (Figure 5B). Similarly, the ethylene inhibitors significantly decreased ACS activity and ACC content only in the 'Moo-deang' hybrid (Figure 6E,G), probably because these parameters were already very low in the control inflorescences of the 'Dao-lai' hybrid (Figure 6F,H). These

differences indicate that the longer vase life of the 'Dao-lai' hybrid as compared to that of the 'Moo-deang' hybrid, could be due to its lower sensitivity to ethylene and its lower ethylene production rates. Taken together, these ethylene inhibitors can be further used as effective postharvest treatments for preserving the quality of *Mokara* hybrid cut flowers.

5. Conclusions

In summary, this study demonstrates that ethylene is involved in the physiological processes that determine inflorescence quality and the vase life of various hybrids of *Mokara* orchid cut flowers. Thus, ethylene was found to regulate *Mokara* orchid flower development, manifested in bud opening and floret senescence during vase life, and to also affect their water relations. Hence, ethylene seems to be the main factor that affects the differences in the vase life longevity of the five *Mokara* hybrids, rather than their water relations parameters. Based on these findings, it is suggested that ethylene production and respiration rates and patterns can be used as criteria for further breeding of new *Mokara* orchid cultivars for cut flowers. Additionally, treatments with ethylene inhibitors can serve as effective postharvest tools for preserving the quality of the *Mokara* cut flowers and extending their vase life.

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