

Effects of Putrescine on Vase Life of Cut Dendrobium Inflorescences

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ABSTRACT

The effects of three concentration levels of putrescine 10, 20 and 40 ppm in combination with 200 ppm (8-HQS), with or without 1% (w/v) sucrose on the quality and vase life of cut Dendrobium cv. Khao Sanan inflorescences were tested in this experiment. Thereafter, best performed treatment was tested with three commercial cut Dendrobium cultivars such as Khao Sanan, Sonia Bom and Queen Pink. The treated inflorescences were placed under 4 µmol m⁻² s⁻¹ fluorescent light intensity at 25°C temperature and 80% relative humidity. Physiological changes were examined in every two days interval. The highest bud opening was exhibited from vase solution of 20 ppm putrescine with 200 ppm 8-HQS and 1% sucrose solution (63.25%). The minimum bud yellowing (9.71%) and maximum vase life (25.6 days) were reported from 10 ppm putrescine with 200 ppm 8-HQS and 1% sucrose solution. it was subjected to efficiency test with other two cultivars. The selected vase solution; a mixture of 10 ppm putrescine, 200 ppm 8-HQS and 1% sucrose was significantly prolonged the vase life of cut Dendrobium cvs. Khao Sanan, Sonia Bom and Queen Pink inflorescence. Meanwhile, it increased the quality of flowers, reduced the bud yellowing and ethylene production as well as promoted the bud opening. Therefore, putrescine could be used as the vase treatment for prolonging vase life of cut Dendrobium inflorescences.

Keywords: Dendrobium, putrescine, ethylene, vase solution

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INTRODUCTION

The total orchid cut flower trade of the world mostly consists of 85% Dendrobium species and 15% Phalaenopsis and Cymbidium species. Asia is the main source of orchid to enter the world market (Sheehan and Sheehan, 1994; De and Medhi, 2015). Dendrobium inflorescences showed early abscission of open flowers and flower buds, after shipment in cardboard boxes and placement of the inflorescence stems in vase water. These effects were thought to be due to ethylene accumulation in

the boxes, during shipment. The levels of ethylene in the boxes during shipment may promote abscission (Uthaichay et al., 2007). Petal wilting appears to be regulated by endogenous ethylene (Woltering and Van Doorn, 1988) and wilting or dropping of Dendrobium might be induced by ethylene produced by flower buds (Ketsa and Thampitakorn, 1995). Pollination and physical damage also helps to induce the endogenous ethylene production (Woltering and Van Doorn, 1988) and the senescence or abscission of non-pollinated orchid flowers or buds that response to exogenous ethylene (Uthaichay et al., 2007; De et al., 2014). Hence, ethylene produced by inappropriate postharvest handling, exhausted gas from vehicles, long storage and shipping operations can induce the premature senescence of orchid flowers, resulting in short vase life and quality concerns (De et al., 2014).

Biosynthesis of ethylene and some polyamines traverse the common pathways and use a common precursor, s-adenosylmethionine, for their biosynthesis. This is interesting since ethylene usually is associated with senescence, while polyamines have opposite effects (Leshem et al., 1986; Pandey et al., 2000). Putrescine is one of the most important polyamines, influences many biochemical and physiological processes such as cell division, cell elongation, flowering, fruit set and fruit development, ripening and senescence (Pandey et al., 2000; Unsanan et al., 2016). Also, it has the potential to prolong the vase life of cut flowers (Unsanan et al., 2016). The effects of putrescine at different concentrations in combination with 8-hydroxyquinoline sulfate (8-HQS) sucrose on the vase-life of cut inflorescences of Dendrobium were examined and described in this study.

MATERIALS AND METHODS

Plant Material

Inflorescences of Dendrobium were purchased from a commercial grower in Nakhon Pathom province, Thailand. Harvesting high quality inflorescences was done in the morning with 4-5 open florets and 3-5 flower buds. Peduncles of each individual inflorescences were cut at the 12 cm from the basal end of the first floret and kept in transparent tubes containing 50 ml of different vase solutions (Ketsa et al., 1995). The treated inflorescences were placed under 4 µmol m⁻² s⁻¹ fluorescent light intensity at 25°C and 80% relative humidity.

Chemical Treatments

Three concentration levels of putrescine 10, 20 and 40 ppm in combination with 200 ppm

8-hydroxyquinoline sulfate (8-HQS), with or without 1% (w/v) sucrose were used for the vase solutions. To examine the effects of putrescine on vase life of cut Dendrobium cv. Khao Sanan inflorescences. distilled water and 8-HQS were used as the control treatment. Best performed treatment was selected and it was tested again with three commercial cut Dendrobium cultivars such as 'Khao Sana', 'Sonia Bom' and 'Queen Pink'. Ten replicates were used and each replicate was consisted with a single inflorescence.

Flower Senescence

Color strip was used to demarcate the position between last opened flower and first unopened flower bud. Bud opening, bud yellowing, bud dropping and flower dropping were recorded at each data collection and percentage of each parameter was calculated.

Vase Life

The average of vase life was determined by flower senescence which was marked by the cumulative drooping of flower. Cumulative flower drooping was calculated by the sum of flower drooping, flower venation and flower dropping. The wilting or cumulative drooping of flower was considered as senescence of individual flower. Day of more than 50% flowers drooping was considered as the vase life of each individual inflorescences.

Fresh Weight Changes

The difference between consecutive fresh weight (FW) of each inflorescence was used to calculate the fresh weight changes. It was presented as percentage of weight changes per day.

Water Uptake

The difference between consecutive volumes of the vase solution (without an inflorescence) was used to calculate daily water uptake (ml/day) and cumulative water uptake (ml/100 g FW). Evaporative water losses from the surface of the solution was negligible.



Ethylene and CO, Production

Twelve inflorescences of each treatment were placed in three empty air tight containers (11.65 Litres) for one hour and 5 ml gas sample withdrawn from the headspace for ethylene and CO₂ determination by gas chromatograph (GC-2014, SHIMADZU). Reading data from gas chromatograph were used to calculate the ethylene production (nl C₂H₄/g FW.hr) rate and CO₂ production (mg CO₃/g FW.hr) rate.

Statistical Analysis

Collected data were subjected to analysis of variance (ANOVA) and two sample t-test using SAS® studio (University edition) and Statistix 8.0 at the p value of 0.05 and least significant difference (LSD) was used to compare the means of each treatment.

Place and Duration

The experiment was conducted at Postharvest Physiology and Technology Laboratory, Department of Horticulture, Faculty of Agriculture, Kasetsart University at the period of June 2017 to May 2018.

RESULTS AND DISCUSSION

Effects of Putrescine at Different Concentrations. 8-HQS and Sugar on Quality and Vase Life of Dendrobium cv. Khao Sanan Flowers

Bud opening, bud yellowing, bud dropping, bloomed flower dropping and vase life of cut Dendrobium cv. Khao Sanan flowers treated with different concentrations of putrescine in combination with 8-HQS with and without sucrose were illustrated in Table 1. The vase solution containing 20 ppm putrescine, 200 ppm 8-HQS and 1% sucrose exhibited the highest bud opening (63.2%), that was highly significant with all other treatments except the solutions which contained 10 or 40 ppm putrescine with 8-HQS and sucrose those were 60.84% and 57.7%, respectively. Meanwhile, 10 ppm putrescine with 8-HQS and sucrose exhibited the lowest percentage of bud yellowing (9.7%), bud dropping (3.5%) and no dropping of early bloomed flowers. The maximum vase life (25.6 days) also was recorded from the same treatment. Means of bud opening, yellowing and vase life of the above treatments were significantly different from the control treatments (Table 1). Therefore, vase solution of 10 ppm putrescine with ppm 8-HQS and 1% sucrose was selected for efficiency testing with the other commercial cultivars of cut Dendrobium flowers such as cv. Sonia Bom and cv. Queen Pink.

Table 1 Effects of putrescine, 8-HQS and sugar on the bud opening, bud yellowing, bud dropping, flower dropping and vase life of cut *Dendrobium* cv. Khao Sanan inflorescences

	Physiological changes during 18 days of treatment				
Vase treatments	Bud opening (%)	Bud yellowing (%)	Bud dropping (%)	Flower dropping (%)	(Days)
Distilled water	35.3 ^{cd}	24.3 ^{abc}	9.4	11.3	18.0 ^{bc}
8-HQS	34.8 ^{cd}	26.2 ^{ab}	7.3	11.3	18.4 ^{bc}
8-HQS + 1% Suc	47.1 ^{bc}	14.8 ^{abc}	4.2	10.7	18.8 ^{bc}
10 ppm PUT + 8-HQS	30.4 ^d	28.8ª	18.8	4.0	17.6 ^{bc}
20 ppm PUT + 8-HQS	33.8 ^d	30.0ª	15.1	6.7	19.6 ^{bc}
40 ppm PUT + 8-HQS	30.4 ^d	26.8 ^{ab}	17.6	10.0	16.4°
10 ppm PUT + 8-HQS + 1% Suc	60.8ª	9.7°	3.5	0.0	25.6ª
20 ppm PUT + 8-HQS + 1% Suc	63.2ª	12.0 ^{bc}	6.0	0.0	22.0ab
40 ppm PUT + 8-HQS + 1% Suc	57.8 ^{ab}	11.7 ^{bc}	3.9	3.3	22.8 ^{ab}
LSD	**	*	NS	NS	*
CV %	23.2	58.8	111.8	161.7	21.7

Note: Means within a column not sharing the same letters were significantly different at P < 0.05 by LSD * = significant, ** = highly significant, NS = non significant 8-HQS = 200 ppm 8-hydroxyquinoline sulfate, PUT = putrescine and Suc = sucrose

Effects of Putrescine, 8-HQS and Sugar on Quality and Vase life of The Commercial Cultivars of **Dendrobium Flowers**

Physiological changes of three different cut Dendrobium cultivars which were held in the vase solutions containing 10 ppm putrescine with 8-HQS and sucrose were investigated and compared with the one those were held in distilled water (Figure 1).

Highest bud opening was observed by the cut Dendrobium cv. Khao Sanan, cv. Sonia Bom and cv. Queen Pink flowers which were held in selected vase solution. Cut Dendrobium cv. Khao Sanan and cv. Queen Pink were shown highly significant different at day 18. The maximum value of bud opening (80.4%) was recorded by cv. Queen Pink. Similarly, percentages of bud yellowing (9.2%) and bud dropping (9.2%) were comparatively low in the Dendrobium cv. Queen pink inflorescences which were treated with vase solution. Highest percentages of flower dropping (71.8%) and lowest vase life (9.4 days) were recorded in the Dendrobium cv. Queen Pink flowers held in distilled water. The highest vase life (23.2 days) was observed in the Dendrobium cv. Khao Sanan flowers held in selected solution. Hence, vase life of Dendrobium cvs. Sonia Bom, Khao Sanan and Queen Pink flowers in the vase solution were highly significant different from control treatment (Table 2).



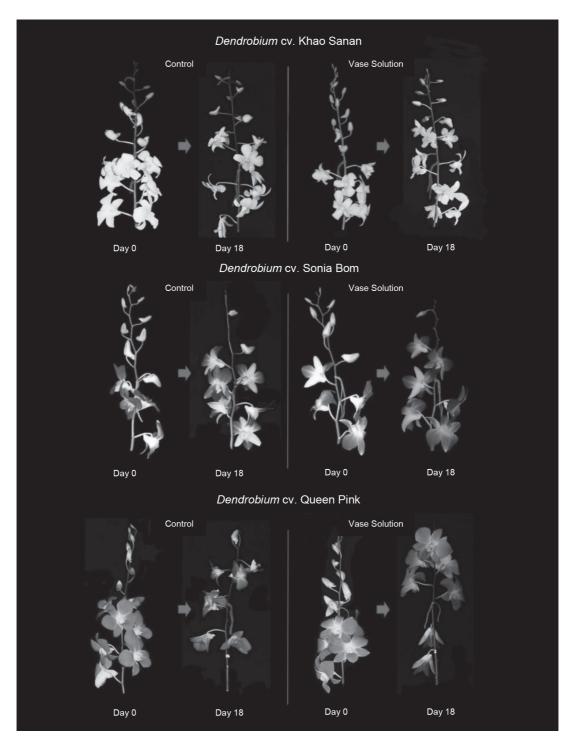


Figure 1 Effects of vase solution with 10 ppm putrescine, 200 ppm 8-HQS and 1% sugar on display quality of three different cut Dendrobium cultivars

Table 2 Effects of 10 ppm putrescine with 200 ppm 8-HQS and 1% sugar on bud opening, bud yellowing, bud dropping, flower dropping and vase life of three different cut *Dendrobium* cvs. Khao Sanan, Sonia Bom and Queen Pink

Cultivar	Treatment	Physiological changes during 18 days of treatment				
		Bud opening (%)	Bud yellowing (%)	Bud dropping (%)	Flower dropping (%)	Vase life (Days)
Khao Sanan	Control	42.4b	17.9	15.9	24.6	16.0 ^b
	Vase solution	58.6ª	11.9	10.9	12.8	23.2ª
t-test		**	NS	NS	NS	**
Sonia Bom	Control	34.4	62.7	56.0	24.0	18.6⁵
	Vase solution	40.5	51.5	48.2	24.3	21.8ª
t-test		NS	NS	NS	NS	*
Queen Pink	Control	62.0 ^b	22.3	21.2	71.8	9.4 ^b
	Vase solution	80.4ª	9.2	9.2	66.4	16.6ª
t-test		**	NS	NS	NS	**

Note: Means within a column not sharing the same letters were significantly different at P < 0.05 by t-test; *= significant, ** = highly significant and NS= non-significant

Percentage of average weight changes of inflorescences were increased by both treatments up to 4 days. The control treatment maintained the fresh weight over than the initial weight up to 7 days and selected vase solution could maintain the fresh weight over than initial weight up to 10 days. This result indicated that 10 ppm putrescine in combination with 200 ppm 8-HQS and 1% sucrose could maintain the freshness of inflorescences without losing the initial weight up to 10 days. Dendrobium cv. Queen Pink showed comparatively high weight

changes (Figure 2C). Meanwhile, Dendrobium cv. Khao Sanan exhibited the less percentage of weight changes. Dendrobium cv. Khao Sanan inflorescences which treated with selected vase solution did not show any weight reduction compared to the initial fresh weight. However, selected vase solution treated inflorescences of cut Dendrobium cv. Khao Sanan and cv. Queen pink were significantly different from the control treatment during the period of observation (Figure 2A and 2C).

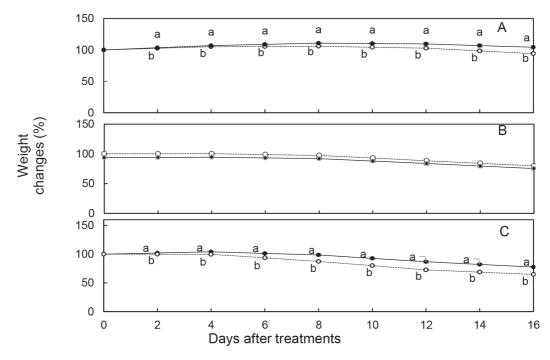


Figure 2 Effects of vase solution with 10 ppm putrescine, 200 ppm 8-HQS and 1% sugar on percentage of weight changes of three different cut Dendrobium cultivars. (A: cv. Khao Sanan, B: cv. Sonia Bom, C: cv. Queen Pink, — distilled water (control) and → vase solution)

Daily water uptake on selected vase solution and distilled water were shown in Figure 3. The highest daily water uptake was observed from both treatments at first two days. Thereafter, daily water uptake gradually declined. Similar pattern of water uptake was observed in all three Dendrobium cultivars (Figure 3A-C). Comparatively highest cumulative water uptake was observed from the flowers of all three cultivars in the selected vase solution compared to distilled water. However, Dendrobium cv. Khao Sanan and cv. Queen Pink were highly significantly different at day 10 onwards (data not presented).

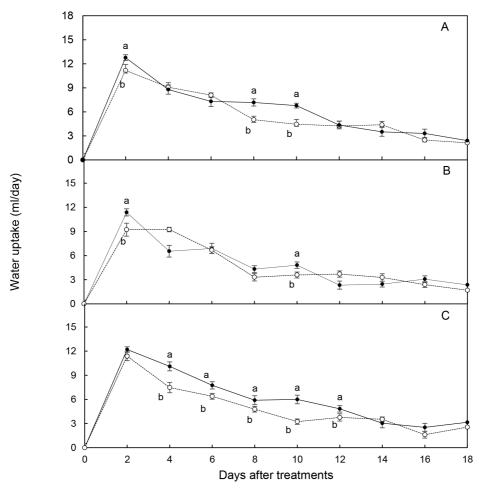


Figure 3 Effects of vase solution with 10 ppm putrescine, 200 ppm 8-HQS and 1% sugar on daily water uptake of three different cut Dendrobium cultivars (A: cv. Khao Sanan, B: cv. Sonia Bom, C: cv. Queen Pink, — distilled water (control) and → vase solution)

Both treatments of distilled water and selected vase solution were monitored the highest ethylene production at day 2 of experiment. Thereafter, ethylene production gradually declined up to day 6. After that, continually increased ethylene production was observed by control treatment onwards day 6 of experiment. Meanwhile, vase solution of 10 ppm putrescine in combination with 200 ppm 8-HQS and 1% sucrose exhibited comparatively less ethylene production. However, the means were not significantly different from each cultivar. Hence, Dendrobium cv. Khao Sanan

(Figure 4A) and cv. Sonia Bom (Figure 4B) clearly showed the similar observation. However, Dendrobium cv. Queen Pink exhibited the comparatively high ethylene production from the vase treatment at day 8, 10 and 14 (Figure 4C). Thus figure 1 illustrated the quality changes of each cultivar. Hence, Dendrobium cv. Queen Pink flowers with vase treatment clearly showed the comparatively high senescence rate of flowers which were opened before the treatment. Moreover, the highest percentage of flower dropping was also observed in this cultivar (Table 2).

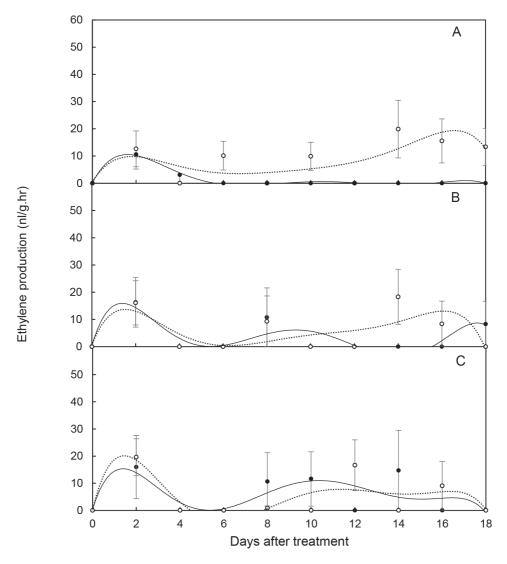


Figure 4 Effects of vase solution with 10 ppm putrescine, 200 ppm 8-HQS and 1% sugar on ethylene production of three different cut Dendrobium cultivars. (A: cv. Khao Sanan, B: cv. Sonia Bom, C: cv. Queen Pink, — distilled water (control) and → vase solution)

The CO₂ production of the inflorescences placed in selected vase solution was comparatively higher than that of in the control. Similar pattern was observed in all three cultivars (Figure 5A-C). However, Dendrobium cv. Queen Pink showed the highest CO2 production followed by cvs. Sonia Bom and Khao Sanan. The result showed

that CO₂ production in *Dendrobium* cv. Khao Sanan was significantly different at day 2, 4 and 18. Meanwhile, Dendrobium cv. Sonia Bom was significantly different at day 0, 12, and 18 and Dendrobium cv. Queen Pink was recorded the significant difference at day 0 and 4.

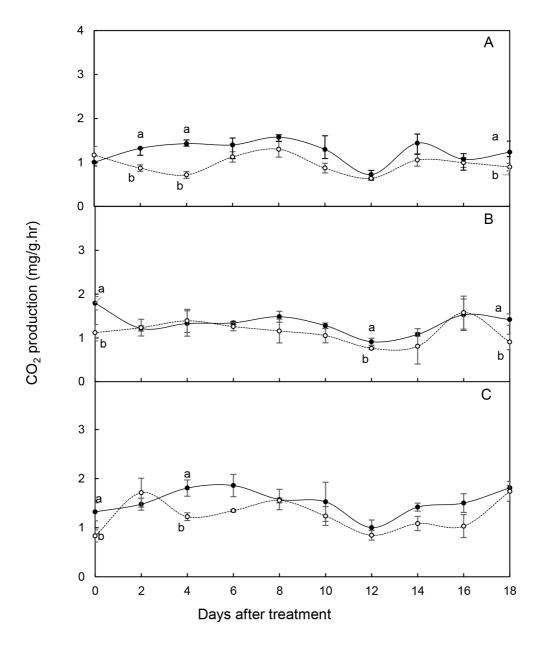


Figure 5 Effects of vase solution with 10 ppm putrescine, 200 ppm 8-HQS and 1% sugar on CO, production of three different cut Dendrobium cultivars. (A: cv. Khao Sanan, B: cv. Sonia Bom, C: cv. Queen Pink, —distilled water (control) and → vase solution)



The vase solution consisted of putrescine, 8-HQS and sugar, exhibited the best performance on physiological changes and vase life of Dendrobium cv. Khao Sanan flowers, compared to the vase solution which consisted of only putrescine or 8-HQS. Specially, high bud yellowing, less bud opening and less vase life were observed in the flowers which were held in the absence of sugar in vase solutions compared to the control treatment.

pH of putrescine and sucrose solutions were closed to neutral (6.2 and 6.6, respectively), on the other hand 8-HQS was acidic (pH 4.2). Hence, combinations of putrescine with 8-HQS or putrescine with 8-HQS and sugar showed the pH scale ranged from 4.1 to 4.2 (data not presented). Thus, the combination with 8-HQS resulted in the reduction of pH in each vase solution. However, in this study the pH of vase solution had no effect on the vase life of cut Dendrobium cv. Khao Sanan, since the vase life of inflorescences treated with 8-HQS and control treatment were not significantly different.

Utilization of putrescine and decarboxy S-adenosylmethionine (DC-SAM) are converted to spermidine by spermidine synthase. Spermidine and DC-SAM converted to spermine by spermine synthase. Methionine converted to S-adenosylmethionine (SAM) by SAM-synthase and SAM converted to DC-SAM by SAM-decarboxylase (Pandey et al., 2000; Liu et al., 2006). Tiburcio et al. (1990) reported that the biosynthesis of spermidine requires ATP in addition to putrescine and methionine. Adams and Yang (1979) also suggested that methionine is converted to SAM by SAM synthetase at the expense of ATP utilization, this biosynthesis pathway is called as the Yang cycle. Based on above explanations, we can conclude that, providing the exogenous polyamine increased the utilization demand of endogenous SAM. Hence, increase the methionine to SAM conversion and increase the demand of ATP. Therefore, the cut inflorescence exposed to exogenous putrescine potentially increased the respiration rate and additional source of energy must be supplied. On the other hand, sugars provided during bud opening, supplied energy and the carbon skeleton required for the development of floral structure (Pun and Ichimura, 2003). Meantime, sucrose antagonized the effects of abscisic acid (ABA), which promoted senescence (Nair et al., 2003).

Results of high bud yellowing and bud dropping could be suggested that, increased of exogenous putrescine might result in reduced sugar levels inside the plant cells. In case of lack of energy, level of ABA might be increased. Especially, ABA can promote the bud senescence, which was symbolized by high bud yellowing and bud dropping. In fact, that formulating the vase solutions with polyamine, the exogenous source of energy must be included. That can be help to fulfill the requirement of energy. This is the reason why vase solutions contained putrescine, 8-HQS and sugar exhibited high performance, especially low bud yellowing compared to combinations of putrescine and 8-HQS without sugar.

However, treatment with only sugars could promote microbial growth. Hence, the combination of sugars and biocides might extend the vase life of cut flowers. The 8-HQS is a very important germicide in preservatives used in floral industry and acts as an antimicrobial agent (Ketsa, 1989; Ketsa et al., 1995). Accordance with the report of Asrar (2012) that 200 ppm 8-HQS combined with 2% sucrose solution had the potential to be used as a commercial cut flower preservative solution to delay flower senescence, enhance post-harvest quality and prolong the vase life of cut flowers. Ichimura et al. (1999) also reported that soluble carbohydrate was an important factor in determining the vase life of cut flowers.

In this experiment, the maximum daily water uptake and elevated ethylene production were observed at day 2 from both control and vase treatment in all cultivars (Figure 3 and 4). High water uptake at day 2 might be caused by high evaporation or dehydration during the transportation of inflorescence before the experiment. Hence, the inflorescences were placed in vase solutions, water uptake was increased due to the rehydration. Elevated ethylene production might be also caused

by this stress condition. Wang et al. (2002) reported that ethylene biosynthesis was induced by the varieties of stress signals such as mechanical wounding, chemicals and metals, drought or water stress, extreme temperatures and pathogen infection.

Biosynthesis of ethylene and some polyamines (spermidine and spermine) traverse the common pathways and use a common precursor: SAM for their biosynthesis. During the ethylene biosynthesis, SAM is converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) and ACC to ethylene conversion by ACC oxidase (ACO). ACC synthase is known as the rate limiting enzyme in this pathway. Putrescine is precursor for spermidine and spermine (Leshem et al., 1986; Pandey et al., 2000). Biosynthesis of spermidine and spermine by exogenous putrescine can be promoted the utilization of SAM by conversion of SAM to DC-SAM, which affected in the biosynthesis of ethylene. Also, polyamines inhibited the conversion of ACC to ethylene and reduced the endogenous level of ACC, indicated that these polyamines inhibited at least two steps in the biosynthetic pathway of ethylene formation (Apelbaum et al., 1981; Suttle, 1981). Especially, putrescine that could inhibit auxin-induced ethylene production, the conversion of methionine and conversion of ACC to ethylene in fruit protoplasts (Apelbaum et al., 1981) and 'Angelino' plum (Adams and Yang, 1979). According to Liu et al. (2006), the inverse relationship between ethylene and polyamine was primarily illustrated by their opposite synthesis patterns during flower and fruit development. Thus, polyamine could delay or inhibit ethylene production. In addition, Unsanan et al. (2016) found that the transcript levels of the ACS and ACO genes in the *Dendrobium* cv. 5N flowers treated with putrescine were lower than the control flowers, suggesting that the expression of these two genes was suppressed by putrescine. Therefore, the ethylene production was reduced.

Dendrobium cv. Khao Sanan and cv. Sonia Bom which treated with selected vase solution exhibited the higher respiration rate with lower ethylene production compared to the control. However, higher respiration rate was expected

to elevate ethylene production during the flower senescence. Some experiments which based on cut carnation flowers (Bufler et al., 1980), Dendrobium cv. Sonia Red (Sapbua et al., 2013), and Dendrobium cv. Khao Sanan (Tonboot et al., 2015) had been conformed this phenomenon. This suggested that ACC is oxidized by ACC oxidase to ethylene, CO₂, and HCN (Wang et al., 2002) which is oxygen dependent, and under anaerobic condition ethylene formation is completely suppressed (Bulens et al., 2012). Thus, O₂ and SAM become limiting factors for ethylene biosynthesis. Exogenous putrescine might use the SAM directly to biosynthesize spermidine and spermine. Also, indirectly used O₂ in plant cell by increasing the respiration rate. Gorny and Kader (1997) suggested that elevated CO₂ and reduced O₂ environment inhibited the ethylene biosynthesis. Chavez-Franco and Kader (1993) also reported that high concentration level of CO₂ inhibited the ACC synthase activity while low concentrate stimulated.

The observation of low ethylene production with high CO₂ production rate from the selected vase solution treated flowers could be interpreted as overall effects of exogenous putrescine on reparation rate, spermidine and spermine biosynthesis process and inhibition and/or delay the conversion of SAM to ethylene.

Thickness of sepals and petals or/and cuticle layer play the critical role regarding the transpiration rate (Yeats and Rose, 2013). Specially, less thickness of cuticle layer might be triggered the transpiration rate and weight losses as well as flower senescence. From the experiment, Dendrobium cv. Queen Pink has less thickness of sepals and petals when compared with the others, therefore Dendrobium cv. Queen Pink exhibited high susceptibility of senescence and comparatively high weight losses, ethylene production, respiration and less vase life. Those results might be expected by the thickness of petals, sepals, cuticle layer or/and other genotypic characters. However, all three cultivars performed well and enhanced the vase life under the vase solution of 10 ppm putrescine with 200 ppm HQS and 1% sucrose.



CONCLUSION

A mixture of 10 ppm putrescine, 200 ppm 8-HQS and 1% (w/v) sucrose could prolong the vase life of Dendrobium cvs. Khao Sanan, Sonia Bom and Queen Pink inflorescences. Since it increased the quality of flowers, promoted the bud development and opening, reduced the bud yellowing and ethylene production. Thus, a mixture of 10 ppm putrescine, 200 ppm 8-hydroxyquinoline sulfate and 1% (w/v) sucrose could be suggested as a potential vase treatment for cut *Dendrobium* inflorescences.

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