Effect of Putrescine and Sucrose on Vase Life of *Dendrobium* cv. 5N

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Abstract

The effect of different concentrations of putrescine and sucrose on the vase life of *Dendrobium* cultivar 5N was investigated. *Dendrobium* inflorescences were immersed in putrescine (0.25, 0.50, 1, and 2 mM) and sucrose solutions (1, 2, 4, and 6%) for 4 h at 25±2ºC, then transferred into deionized water and kept in the culture room at 25±2ºC. Putrescine at 2 mM significantly increased the vase life of *Dendrobium* cultivar 5N flowers (24.37 days) compared to the control (20.87 days). A holding solution containing putrescine at 2 mM and sucrose at 1% also prolonged the vase life of the orchid flowers (20.28 days). Vase life of *Dendrobium* flowers treated with 2 mM putrescine alone, or a mixture of 2 mM putrescine and 1% sucrose was comparable to flowers treated with silver thiosulfate (STS) and a commercial holding solution. The effect of putrescine on the expression of the *ACS* and *ACO* genes was determined by reverse transcription polymerase chain reaction (RT-PCR) using primers synthesized on the *ACS* and *ACO* genes from the orchid flowers, and RNA isolated from putrescine treated flowers as the template. Results revealed that putrescine suppressed expression of the *ACS* and *ACO* genes similar to STS treatment, and suggested that putrescine and sucrose have high potential as a holding solution for prolonging the vase life of *Dendrobium* cultivar 5N.

Keywords: *Dendrobium*, putrescine, vase life, holding solution

Introduction

Orchids belong to the Orchidaceae which is one of the largest families composed of about 800 genera with approximately 25,000 species. Orchids are a major commercially cut flower with the longest vase life in the Thai cut flower industry. *Dendrobium* is the second largest genus in the family after Bulbophyllum [1]. It has unique characteristics among the cut orchids and is one of the most important industrially with more than 50% of the flowers exported from Thailand [2]. Because of varieties of color, a larger number of florets in the inflorescence display recurrent flowering.

Vase life is an important factor for the longevity of cut flowers and an important target for improving flower characteristics, whether by chemical treatment or plant breeding [3]. The vase life of cut flowers is affected by two main factors; ethylene which accelerates senescence, and microorganisms which cause vascular blockage [4]. A shorter vase life and loss of aesthetic value due to the senescence of the flower caused by exogenous or endogenous ethylene are major problems in the cut flower industry [5]. Increasing ethylene gas inside the flower packages during shipment can cause premature senescence [1]. Generally, ethylene is produced through the conversion of *S*-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS), and ACC to ethylene by ACC oxidase (ACO) [1]. Thus, one approach to prevent the synthesis of ethylene in plants is to inhibit the synthesis or activity of both enzymes.

Putrescine, one of the polyamines, is widely found in higher plants and has the potential to prolong the vase life of cut flowers. Putrescine influences many biochemical and physiological processes such as cell division, cell elongation, flowering, fruit set and fruit development, ripening, senescence, and storage life [6]. It has been widely used to prolong the vase life as well as the quality of many plants such as *Rosa hybrida* cv. Dolcvita [6], *Narcissus* [4] and *Lisianthus* [7]. In addition, it has also been used to prevent softening by fruit flies during the storage of strawberry (*Fragaria ananassa* Duch.) [8], banana fruit (cultivar 'Hom Thong') [9] and Mango (*Mangifera indica*) [3]. The effects of putrescine at different concentrations on vase life and quality of the *Dendrobium* cultivar 5N flowers were examined in this study. The effects of sucrose in combination with putrescine on vase life of flowers was also described.

Materials and methods

Plant Material

The inflorescences of *Dendrobium* hybrid cultivar 5N were provided by Bangkok Green Co., Ltd., Thailand. High quality inflorescences composed of 5 to 12 flower buds and 6 to 10 open florets were used. The cut flowers were held in centrifuge tubes containing 10 ml of distilled water (control) and holding solutions containing putrescine or sucrose at various concentrations for 4 h at 25±2ºC. They were then transferred into distilled water and kept in the culture room under natural light conditions at ambient temperature and humidity of 25±2ºC and 70-80%, respectively.

Chemical treatments

The effects of putrescine or sucrose on vase life and quality of the flowers were examined by immersing the inflorescences in a holding solution containing putrescine (0.25, 0.50, 1, and 2 mM) or sucrose (1, 2, 4, and 6%), in the culture room at 25±2ºC for 4 h. A combination of putrescine and sucrose on vase life and flower quality were also tested by immersing the inflorescences in a holding solution composed of putrescine at 2 mM and sucrose at 1, 2, 4 and 6% w/v in the culture room at 25±2ºC for 4 h. Silver thiosulfate (STS) was used as a control treatment.

Vase life

The average vase life of cut flowers was determined from the day of transfer of the inflorescences to the holding solution, and was assessed to be terminated when 50% of the flowers had senesced, characterized by petal wilting. Petal senescence was marked by the loss of turgor in the petal tissue, followed by complete wilting.

Water uptake

The difference between consecutive weighings of the centrifuge tube plus holding solution (without the flower) was used to calculate water uptake.

RNA extraction

Total RNA was extracted from petals of *Dendrobium* flowers after putrescine or sucrose treatments using RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). The extraction of RNA

was carried out essentially as recommended by the manufacturer's instruction. The first strand cDNA was used as a template in the PCR reaction or stored at -20ºC until required.

Gene expression analysis by RT-PCR

The expression of the orchid *ACS* and *ACO* genes was detected by RT-PCR. Total RNA isolated from the petal of orchid flowers was used as the template in the RT-PCR reaction. RT-PCR was carried out using OneStep RT-PCR Kit (QIAGEN, Hilden, Germany) with specific primers designed based on the *ACS* and *ACO* sequences in the GenBank database [8]. The forward ACSFor4 (5'- ATC TTA GCC GAT CCC GGC GA -3') and reverse ACSRor2 (5'- ACA C(G/T)C AAT CCC TGC TTC CT -3') were used to amplify the *ACS* gene. The forward ACOFor1 (5'- ATGGAGCTTCT(C/T)(G/C)AGGGTTC-3') and reverse ACORor2 (5'- TCAAGCAGTAGGAATC (A/G)GCT -3') were used to amplify the *ACO* gene. The reaction mixture (50 µl) consisted of 10 µl of 5xQIAGEN OneStep RT-PCR buffer, 0.4 µM dNTP, 0.6 µM of each primer (forward and reverse), 2 µl of QIAGEN OneStep RT-PCR enzyme mix, 1 µg of RNA template, and 33 µl RNase-free water. RT-PCR conditions were set as follows: 1 cycle of reverse transcription at 50ºC for 45 min, 1 cycles of initial PCR activation step at 95ºC for 15 min, then 40 cycles of PCR as following: denaturation at 95ºC for 1 min, annealing at 48ºC (for *ACS* gene) or 55ºC (for *ACO* gene) for 1 min and extension at 72ºC for 2 min. The PCR products (8 µL) were collected at cycle 25, 28, 31, 34, 37, and 40 and analyzed by agarose gel electrophoresis on a 1% agarose gel. After staining with 0.5 µg/ml ethidium bromide, the relative amounts of the products were compared using the gel ImageMaster [3].

Statistical analysis

All experiments were carried out using completely randomized design (CRD) with seven replications. Data collected was subjected to analysis of variance (ANOVA) using SPSS at the *p* value of 0.05. Duncan's New Multiple Range Test (DMRT) was used to compare the means of each treatment.

Results and discussion

Effects of putrescine and sucrose on vase life and quality of orchid flowers

As shown in Table 1, the vase lives of *Dendrobium* inflorescences treated with 2 mM putrescine and control treatment were significantly different, whereas no significant different

was found among the other tested treatments. Putrescine at 2 mM significantly prolonged the vase life of *Dendrobium* cultivar 5N (24.37 days) as compared to the control treatment (20.87 days). In addition, it also promoted the blooming of flower buds, and delay the abscission of opened flowers (data not shown), suggesting a high potential of putrescine to be used as a holding solution for cut orchid flowers. With respect to sucrose treatment, there were no significant different in the vase life of orchid flowers as compared to the STS treatment (23 days). However, sucrose at 4% (w/v) tend to provide longer vase life than other concentration tested.

Table 1. Effect of putrescine and sucrose on vase life of *Dendrobium* cv. 5N

Treatment	**Vase life (day)[a]**
Distilled water (control)	20.87[a]
0.25 mM Putrescine	22.75[abc]
0.50 mM Putrescine	22.12[ab]
1 mM Putrescine	23.12[abc]
2 mM Putrescine	24.37[bc]
1% Sucrose	21.62[ab]
2% Sucrose	23.00[abc]
4% Sucrose	23.25[abc]
6% Sucrose	21.87[ab]
STS	23.00[abc]
Coefficient of variation (CV%)	9.71

[a] Means within the column not sharing the same letter were significantly different at $p = 0.05$ by Duncan's Multiple Range Test.

The water uptake rates of *Dendrobium* flowers held in different concentrations of putrescine or sucrose were not significantly different. However, flowers held in 2 mM putrescine and 4% (w/v) sucrose had higher water uptake rates than the other treatments (Figure 1).

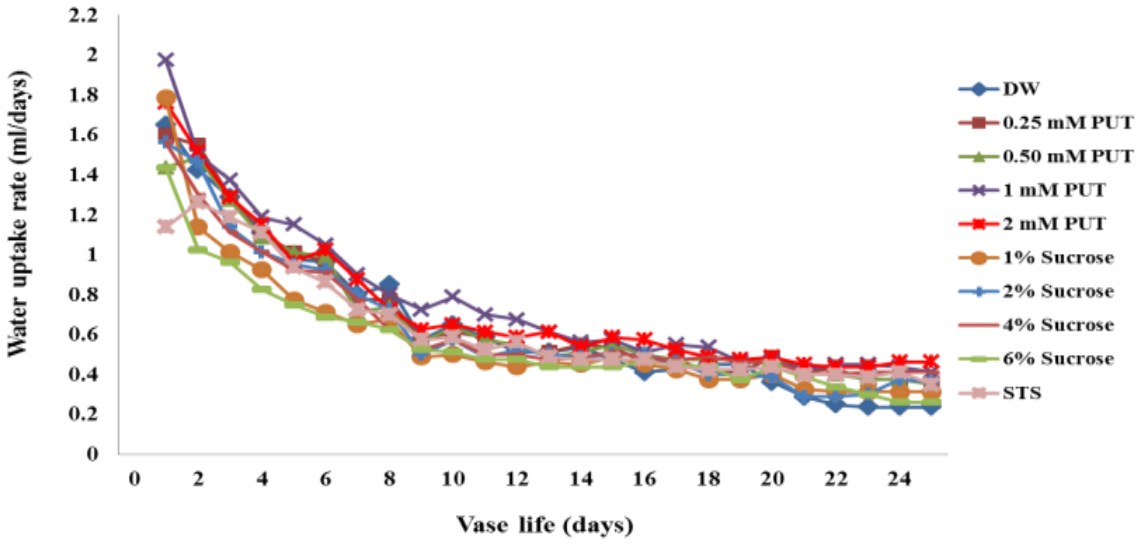

Figure 1. Water uptake rate of *Dendrobium* cv. 5N after treatment with putrescine and sucrose at different concentrations

Table 2 shows the effects of putrescine at 2 mM in combination with sucrose at various concentrations on the vase life and quality of *Dendrobium* cv. 5N flowers. The results indicated that putrescine at 2 mM and sucrose at 1% (w/v) prolonged the vase life of orchid flowers (20.28 days) comparable to flowers treated with STS or AVB, a commercial holding solution. This holding solution also promoted the blooming of flower buds, and delayed the abscission of the opened flowers (data not shown) compared to the other treatments. Our results were similar to Dantuluri et al. [10] who reported that polyamine in combination with sucrose improved the fresh weight, the uptake rate of vase solution, flower opening, and delayed the senescence of gladiolus spikes. Their study also showed that addition of sucrose at 6% (w/v) into putrescine solution at 2 mM caused a reduction in the vase life and increase in the abscission of opened flowers.

Sardoei et al. [4] reported that putrescine significantly improved the fresh weight, the uptake rate of vase solution, the flower opening, and the vase life of *gladiolus*. It also inhibited the synthesis of ethylene [11], and the activities of ACS and ACO enzymes [12]. Putrescine at 2 mM prolonged the vase life of lisianthus flowers [7], *Rosa hybrida* cv. Dolcvita [6]. Putresecine has also been reported to reduce the respiration rate, ethylene production, and maintain the firmness of mango [11], strawberries [13], and plums [14] during the ripening process.

Table 2. Effect of putrescine in combination with sucrose on vase life of *Dendrobium* cv. 5N

Treatment	**Vase life (day)[a]**
Distilled water (control)	17.14^{abc}
2 mM Putrescine	19.00^{abc}
4% sucrose	17.71^{abc}
6% Sucrose	19.42^{abc}
2 mM Putrescine + 1% Sucrose	20.28^{bc}
2 mM Putrescine + 2% Sucrose	18.57^{abc}
2 mM Putrescine + 4% Sucrose	16.85^{ab}
2 mM Putrescine + 6% Sucrose	16.14^{a}
STS	17.57^{abc}
AVB*	21.00^{c}
Coefficient of variation (CV%)	16.56

*commercial holding solution (AVB); [a] Means within the column not sharing the same letter were significantly different at $p = 0.05$ by Duncan's Multiple Range Test.

With respect to the water uptake rate, a mixture of 2 mM putrescine and 1% sucrose exhibited a greater water uptake rate than the other treatments, except for AVB treatment (Figure 2). Higher concentrations of sucrose caused a reduction in the water uptake rate. This might be because sucrose stimulates the growth of bacteria which cause vascular blockage [4].

Effects of putrescine and sucrose on gene expression in orchid flowers

The effect of putrescine on the expression of the *ACS* and *ACO* genes was determined by RT-PCR and the results are summarized in Figure 3. Putrescine reduced the expression of the *ACS* and *ACO* genes in *Dendrobium* cv. 5N flowers, similar to STS treatment. The transcript levels of the *ACS* and *ACO* genes in the flowers treated with putrescine were lower than the control flowers, suggesting that the expression of these two genes was suppressed by putrescine. Putrescine is involved in the production of ethylene, and can inhibit auxin-induced ethylene production and the conversion of methionine and ACC to ethylene in fruit protoplasts [12], and 'Angelino' plum [14].

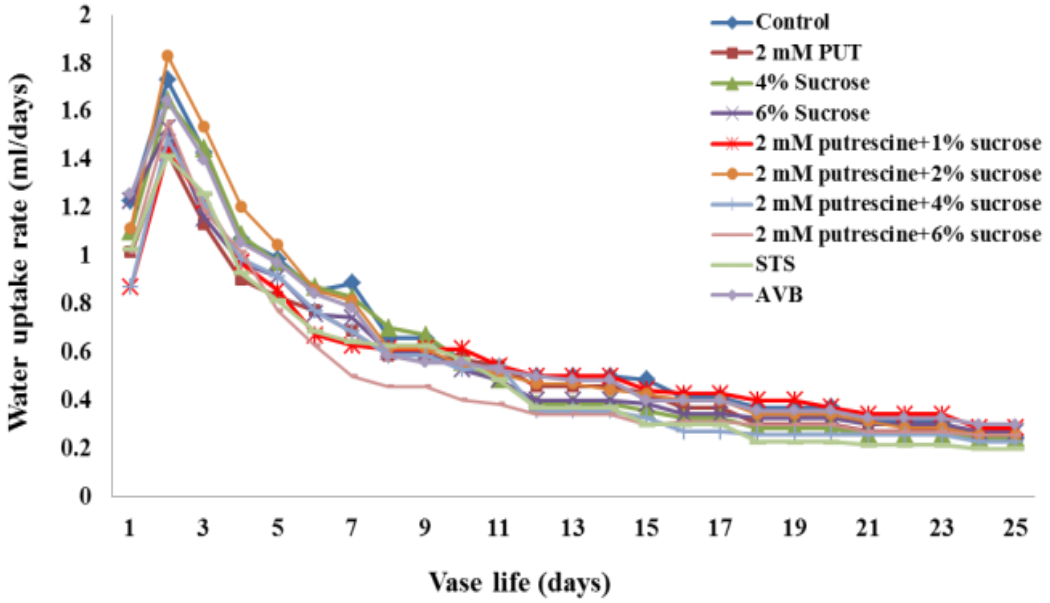

Figure 2. Water uptake rate of *Dendrobium* cv. 5N after treatment with putrescine in combination with sucrose at different concentrations

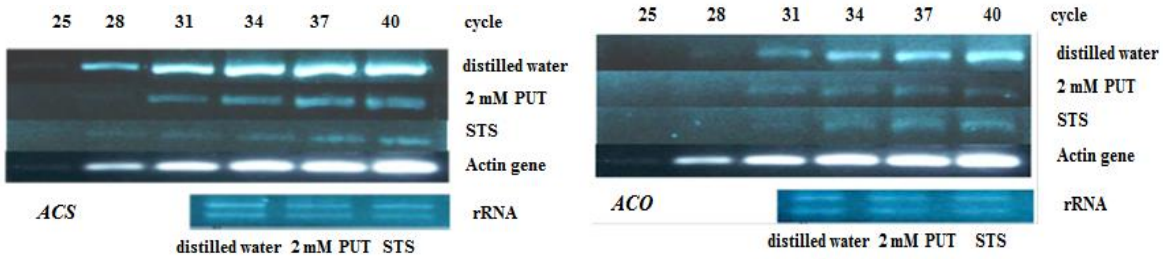

Figure 3. Expression levels of the *ACS* and *ACO* genes in *Dendrobium* cv. 5N treated with putrescine and STS for 7 days

Conclusions

Putrescine can be used as a holding solution for prolonging vase life and improving the quality of orchid flowers. It can prolong the vase life of orchid flowers by delaying senescence and promoting the blooming of the flower buds. Putrescine also promoted the uptake of the holding solution into the plant cells, and suppressed the expression of the *ACS* and *ACO* genes.

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