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Effects of Aminooxyacetic Acid and Sugar on the Longevity of Pollinated *Dendrobium* Pompadour

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Abstract: Experiments were carried out to investigate the effects of different types and concentration of sugars and Aminooxyacetic acid (AOA) and a combination of both sugar and AOA in extending the vase lives of pollinated *Dendrobium* Pompadour flowers. Post pollination symptoms such as weight loss, water uptake, petal thickness and discoloration were monitored. The results obtained showed that the most effective treatments in extending the vase life of pollinated *Dendrobium* Pompadour flowers and delaying the post pollination symptoms were 0.05 mM AOA and the treatment which combined 0.05 mM AOA with 4% glucose. Both treatments showed a longer vase life than that of the control. Furthermore positive effects were also observed in a delay of weight loss, improved water uptake, change in thickness and colour. A combination of 0.05 mM AOA and 2% sucrose extended the vase life of flowers up to 5 days. Inclusion of 4% glucose and 2% sucrose alone only prolonged the vase life of flowers two days more compared to that of the control. Holding solutions containing sugars alone maintained pH values of 5-7 while solutions containing AOA and a combination of AOA and sugars showed more acidic values of pH of 3-5 throughout. AOA and a combination of AOA and glucose can therefore extend the vase life of pollinated *Dendrobium* Pompadour flowers and also delay the post pollination syndrome.

Key words: Aminooxyacetic acid, *Dendrobium* Pompadour, pollination, senescence, sugar

INTRODUCTION

The life span in orchids varies according to cultivar and species, ranging from a day to as long as six months. Many factors can be attributed to the reduction of the vase lives of these orchids, cutting short a life cycle of several months to just a few days. In several species, the life span of flowers is cut short and senescence hastened following pollination (Van Doorn and Woltering, 2008). *Dendrobium* Pompadour is one such species of orchid that undergoes pollination-induced senescence. The visible cues of the pollination induced senescence are upward movements of the petals resulting in full closure of the perianth, thinning of petals and discoloration of petals.

The production of ethylene induced by pollination signals a cascade of events similar to that of natural senescence. One very crucial onset of event is the reduction of endogenous sugar which ultimately leads to the death of the flower. The loss of endogenous sugars following pollination is parallel to the reduction of endogenous sugar in cut flowers. To circumvent the problem of pollination-induced senescence and its symptoms, two areas can be approached. One is by inhibiting the production of pollination-induced ethylene (Liao *et al*., 2000) and second, is by allowing a continuous uptake carbon supply in order to prolong vase life. Ethylene inhibitors have been vastly studied and proven to be effective in prolonging vase life of cut flowers. Compounds such as aminooxyacetic acid (AOA) and aminoethoxyvinylkglycine (AVG) effectively delay senescence of climacteric flowers by inhibiting the action of 1-aminocyclopropane-1 carboxylate synthase (ACCS). Supplying cut flowers with exogenous sugars have been proven to prolong vase life in many cultivars such as rose, carnations and orchids. Rattanawisalanon *et al.* (2003) indicated that holding solutions containing sugars and AOA only did not manage to prolong vase life in *Dendrobium* AJew Yuay Tew flowers while a combination of both sugar and AOA successfully extended the vase life of these flowers.

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This experiment attempts to explore the effects of AOA and sugars on pollination-induced senescence and observe the visible cues that follow shortly after pollination.

MATERIALS AND METHODS

Plant material: *Dendrobium* Pompadour flowers were obtained from the glasshouse of University of Malaya on 5 May 2008. Flowers were hand-pollinated by placing the pollinia onto the stigma. Individual flowers were cut at the proximal end of the peduncles and placed in 20 mL water vials containing distilled water. Physiological changes were observed and recorded daily vase life of flowers was deemed terminated. To show the visual changes, photographs were also taken at each time point. Experiments were maintained at room temperature (26"2°C). All experiments were done in triplicates.

Chemicals and pH measurement: AOA (SIGMA) was tested at 0.25 and 0.05 mM. Glucose (SIGMA) and sucrose (SIGMA) were used at 2, 4 and 6%. Inclusion of 100 mg mL^{-1} of chloramphenicol (SIGMA) was used as an anti bacterial agent for all treatments. All solutions were prepared at the beginning of the experiments and were not renewed. pH measurements of all solution were taken daily using a Hanna Instruments pH Meter.

Water uptake and fresh weight: Water uptake was calculated by the subtraction of consecutive weights of the vials plus the solutions (without the flowers). The weight of the flowers was calculated by subtracting the weight of the vials with the solutions and flowers with the vials and solution only.

Vase life: Symptoms were recorded daily. The flowers were considered terminated once the perianth reaches full closure. Three replicates with a minimum of five flowers were used for each treatment and were statistically tested using the Duncan's multiple range test by an SPSS programme (Duncan, 1955).

Thickness: Thickness of the petal was measured using a micrometer. Measurements were done onto the middle area of the petals.

Colour measurements: The colour of the flowers were measured using a Minolta chromameter (CR-200) measurements were done by placing the middle area of the petals on the stage of the chromameter for measurement to be taken.

RESULTS

The results obtained showed that holding solutions containing 0.05 mM AOA and a combination of 0.05 mM AOA +4% were the best treatments in prolonging the longevity of pollinated *Dendrobium* Pompadour flowers (Table 1). The vase life was prolonged for up to 6 folds compared to that of the control. Treatments with 4% glucose and 2% Sucrose also managed to extend the longevity of the pollinated flowers from 2-5 days. The extensions of vase life by these treatments were not, however, as significant or as effective as the treatments with 0.05 mM AOA and the combination of 0.05 mM AOA +4% glucose. Inclusion of 0.05 mM AOA and 2% sucrose however did not have a significant effect in prolonging the vase life of the flowers. The rest of the treatments did not yield any positive effect in delaying or prolonging of vase life compared to that of the control.

Generally, all holding solutions maintained a pH value of the solution at around 5-6, while holding solutions containing AOA or a combination of AOA and sugars maintained the pH value at around 3-4 throughout the experiment (Table 2). The role of pH value however, is yet to be investigated in prolonging the vase lives of pollinated *Dendrobium* Pompadour in this experiment.

Table 1: Vase life of *Dendrobium* Pompadour flowers held in distilled water and different holding solutions

Treatment	Flower longevity (days)
Distilled water	2.0a
2% glucose+CHP	2.4ab
4% glucose+CHP	4.7d
6% glucose+CHP	2.5b
2% sucrose+CHP	4.7d
4% sucrose+CHP	2.0a
6% sucrose+CHP	2.0a
0.025 mM AOA	4.1c
0.050 mM AOA	13.2f
4% glucose+0.05 mM AOA+CHP	10.3e
2% sucrose+0.05 mM AOA+CHP	4.9d

Mean values with different letter(s) are significantly different

Table 2: pH values of holding solutions containing different concentrations of glucose, sucrose and AOA

	pH values		
Treatments	Day 1	Day 4	Day 14
Distilled water+CHP	5.64±0.01	6.56±0.02	6.85±0.03
2% Glucose+CHP	5.74±0.02	5.86±0.01	6.32±0.02
4% Glucose+CHP	6.21±0.01	4.77±0.03	5.56±0.04
6% Glucose+CHP	6.63±0.02	5.48±0.03	5.86±0.02
2% Sucrose+CHP	6.12±0.01	4.71±0.02	5.43±0.01
4% Sucrose+CHP	6.87±0.02	7.01±0.01	7.38±0.01
6% Sucrose+CHP	6.66±0.01	6.95±0.02	7.69±0.04
0.025 mM AOA	4.15±0.01	3.96±0.01	4.12±0.03
0.05 mM AOA	3.86±0.01	3.95±0.02	4.02±0.02
6% Glucose+0.05 mM AOA+CHP	4.61±0.02	3.91±0.01	4.15±0.04
2% Sucrose+0.05 mM AOA+CHP	4.35±0.01	4.36±0.03	5.23±0.01

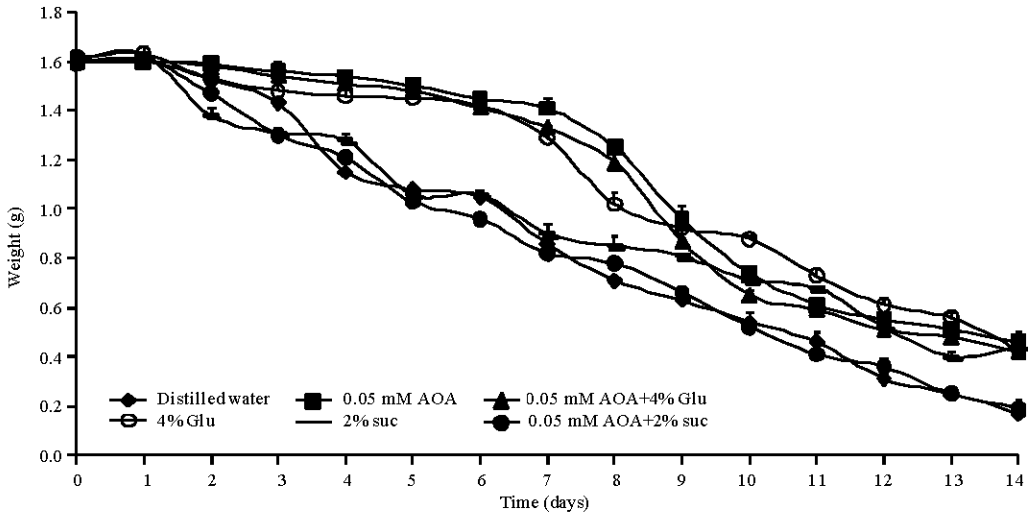

Fig. 1: Fresh weight changes of *Dendrobium* Pompadour flowers held in distilled water, 0.05 mM AOA, 4% glucose+0.05 mM AOA, 4% glucose, 2% sucrose and 0.05 mM AOA+sucrose

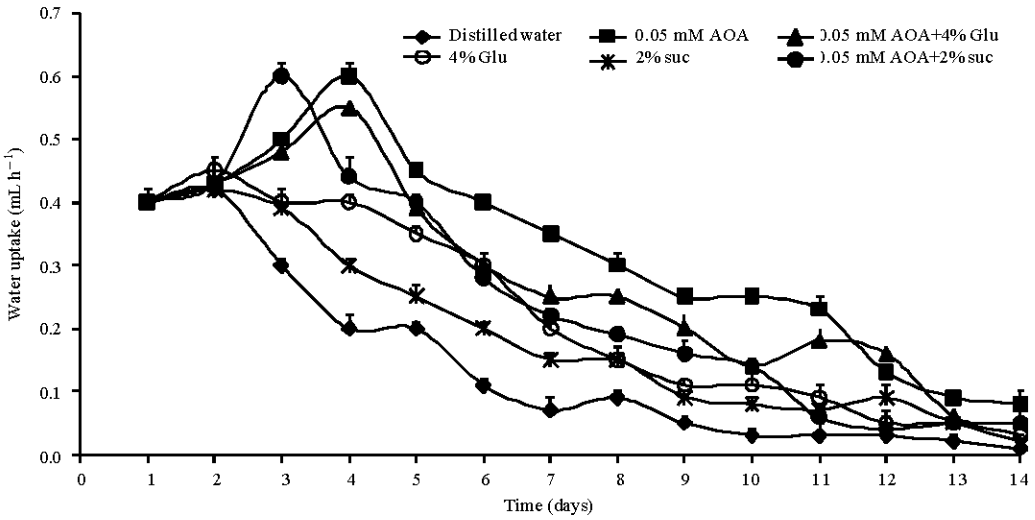

Fig. 2: Water uptake of pollinated *Dendrobium* Pompadour flowers held in distilled water, 0.05 mM AOA 4% glucose+0.05 mM AOA, 4% glucose, 2% sucrose and 0.05 mM AOA+2% sucrose

However, earlier studies have shown that low pH values are able to prevent and eliminate bacterial growth in holding solutions which ultimately allows for improved water uptake by preventing stem blockage.

The ability of 0.05 mM AOA and the combination of 0.05 mM AOA+4% glucose in extending the vase lives of the pollinated flowers was also concurrent with the delay in post pollination symptoms of the flowers. In both treatments, pollinated flowers showed delayed weight loss and a consistent maintenance of weight throughout the experiment compared to that of the control (Fig. 1).

In Fig. 2, data shows that the two treatments also managed to improve the water uptake of the pollinated flowers compared to the rest of the holding solutions. The uptake of water was maintained for the longest period of days and was highest until day 13 thereafter.

In this experiment, change in thickness of the petals of pollinated *Dendrobium* Pompadour flowers was also delayed in the treatments with 0.05 mM AOA and the combination of 0.05 mM AOA+4% glucose. In the presence of 0.05 mM AOA and the combination of 0.05 mM AOA and 4% glucose,

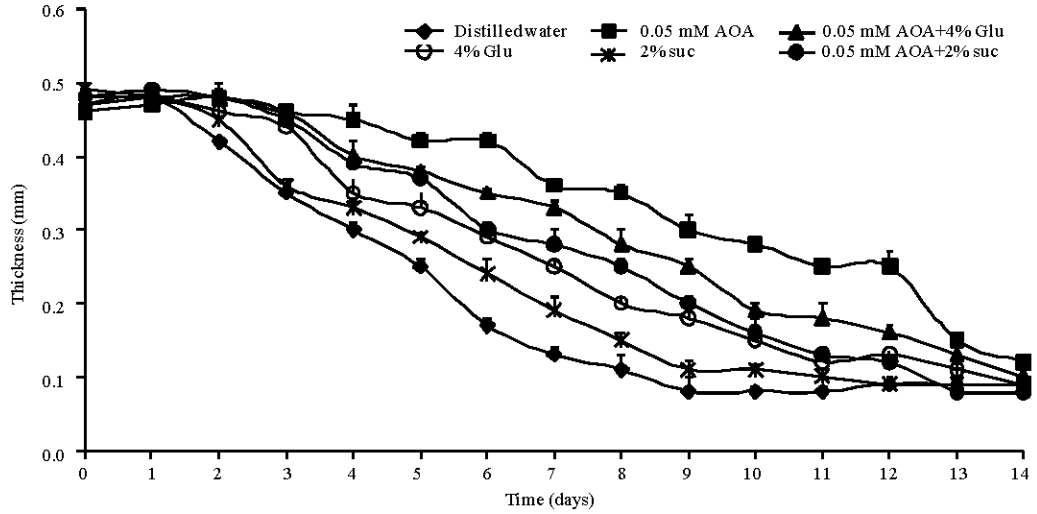

Fig. 3: Thickness of pollinated *Dendrobium* Pompadour flowers held in distilled water, 0.05 mM AOA 4% glucose+0.05 mM AOA, 4% glucose 2% sucrose and 0.05 mM AOA+2% sucrose

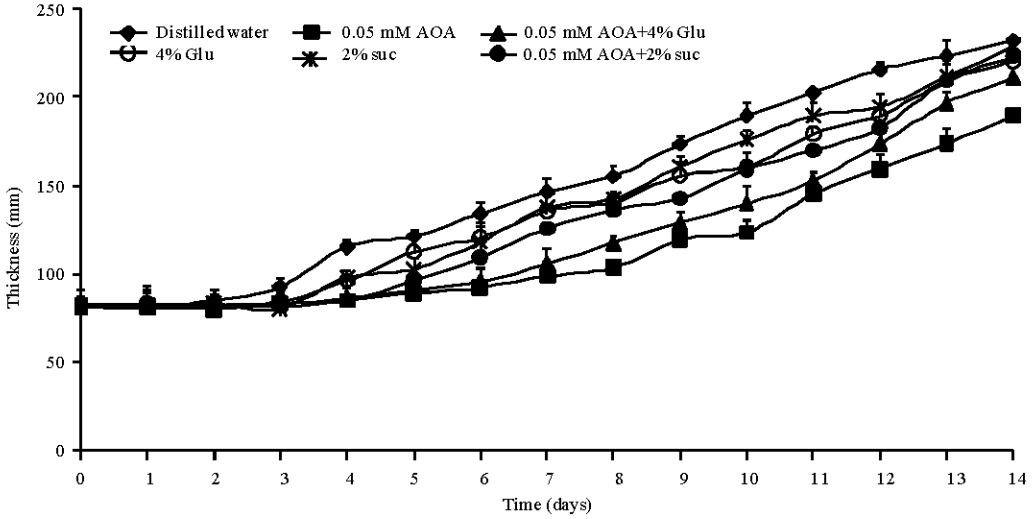

Fig. 4: Colour change of pollinated *Dendrobium* Pompadour flowers held in distilled water, 0.05 mM AOA and 4% glucose+0.05 mM AOA, 4% glucose 2% sucrose and 0.05 mM AOA+2% sucrose

discolouration of flowers were delayed for a significantly longer period compared to that of the control (Fig. 3).

The L.a.b value increases as the petal colour fades from a bright crimson purple to a lighter shade of purple (Fig. 4). Although the flowers held in the other solutions showed a similar pattern of discolouration the control, the colour change in flowers treated with 0.05 mM AOA is slowest compared to the rest of the folding solutions.

DISCUSSION

The longevity of climacteric flowers is significantly reduced by pollination in some cultivars. Vase life of unpollinated flowers which can last up to 6 months can be significantly reduced to a few days when pollinated (Van Doorn, 1997). The longevity of individual *Dendrobium* Pompadour flowers, when unpollinated, lasts for approximately two weeks. This vase life however is reduced to a mere 24 h when pollinated. (Zuliana *et al*., 2007).

The post-pollination effect of these climacteric flowers can be attributed to the production of endogenous ethylene, which leads to a cascade of events that mimic the events following natural death or senescence. In flowers, physiological changes that were observed during the pollination-induced senescence were

upward movements of the petals which eventually resulted in full closure of the entire perianth, thinning of petals and discoloration of petals.

The effects of inhibitors of ethylene synthesis on the vase life of cut flowers such as aminooxyacetic acid (AOA) and aminoethoxyvinylkglycine (AVG) have been have been vastly studied. In a study done by Wawrzynczak and Goszcynska (2003), AOA was found to be most active in inhibiting ethylene production in Dolca Vita carnations. Present results show an agreement with the mentioned study, achieving the most delayed vase life in pollinated cut *Dendrobium* Pompadour flowers treated with 0.05 mM AOA. Furthermore, the flowers treated with 0.05 mM AOA also showed a delay in thinning and discolouration of petals. Water uptake was also improved as compared to that of control. The maintenance of pH value of 0.5 mM AOA solution between 3-4 throughout the entire experiment may be a factor for such improved water relations. A low pH may inhibit blockage and allow for water uptake (Loubaud and Van Doorn, 2004).

In the group of flowers where senescence is regulated by ethylene, exogenous sugars also considerably delay the large increase in ethylene production and the time to visible senescence (Van Doorn, 2004). Leon and Sheen (2003) presented a model which shows that sucrose apparently inhibits the ethylene signal pathway through the production of low ABA concentration. Some studies have also shown that the sensitivity of flowers to ethylene is significantly reduced via treatment with exogenous sugars. Metabolic sugars play a crucial role in maintaining flower quality during the post-harvest period since sugars are the main carbon-source utilized for energy in the cut flower metabolism. When detached from the mother plant, the energy supply of the flowers becomes limited due to the negligible production of photosynthesis by the remaining foliage and the cut stem. This limited supply of carbohydrates may lead to a decrease in flower longevity (Van der Meulen-Muisers *et al*., 2001). Cut flowers are treated with sugars to increase the pool of respirable substrates, delay the onset of hydrolysis of structural components and improve water balance. Furthermore, the loss of sugar can also result in the degradation lipid and protein degradation substitutes for the lack of sugar as the main respiration substrate. This would then lead to a rupture of the semi-permeability of the cell membrane and eventually lead to an irreversible loss of membrane and structural destruction.

Our data shows that 4 and 2% sucrose were able to increase the vase life of pollinated *Dendrobium* Pompadour flowers. The inclusion of 100 mg L^{-1} of chloramphenicol into holding solutions containing sugars acted as an antimicrobial growth to prevent the growth of bacteria which may cause blockage of the stem and disallow the uptake of sugar and water. Flowers held in these holding solutions maintain vase life, thickness of petal and consistent water uptake for 3-4 days before the onset of senescence took place and petals started to move upwards and eventually reach full closure. This suggested that the optimum concentration of sugars can effectively maintain the integrity of the structure of cut flowers. This finding suggests that optimal sucrose and glucose solutions might be required for cell wall synthesis and respiration. Similar results have been reported by Liao *et al*. (2000) with findings that indicated that optimal sucrose solution had a pronounced effect on vase life and quality of cut rose flowers. The results in this study are not in agreement with the findings of Rattanawisalanon *et al*. (2003), who demonstrated that *Dendrobium* flowers held in 4% glucose had a shorter vase life compared to that of control. This however, can be due to the fact that bacterial growth occurred because of the inexistence of anti-microbial chemicals in the holding solutions used in that particular study.

Many studies have reported the effectiveness of treatments containing a combination of sugars and ethylene inhibitors in extending vase life of flowers. Maximum increase in the vase life of *Dendrobium* Heang Beauty inflorences treated with a combination of sugar and AOA (Chandran *et al*., 2006). The vase life of cut roses was also prolonged with the treatment of sucrose pulse followed by pulsing with STS (Liao *et al*., 2000). In this study, a prolonged vase life of the *Dendrobium* Pompadour flowers treated with a combination of 4% glucose and 0.05 mM AOA was also observed. The extension of vase life of flowers treated was not significantly different than flower treated with AOA alone. Therefore, it can be concluded that the more overriding factor that supersedes the effect of pollination-induced ethylene is AOA rather than glucose. Interestingly enough, flowers treated with a combination of 2% sucrose and AOA showed a shorter vase life than that of 4% glucose and 0.05 mM AOA. This could be due to the fact that the more preferred respirable sugar is glucose rather than sucrose. This preference is however cultivar and species dependant as shown in other studies using different sugar treatments. (Yakimova *et al*., 1996; Ichimura and Suto, 1999).

CONCLUSION

It is therefore concluded that 0.05 mM AOA and a combination of 4% glucose and 0.5 mM AOA effectively delayed pollinated-induced senescence and its symptoms

in *Dendrobium* Pompadour flowers. These solutions successfully improved water relations, maintained thickness of petals and delayed discoloration. Further studies can be done to observe the effects of the sugar and AOA treatments in ethylene production and the regulation of enzymes related to the physiological changes that occur as post pollination symptoms such as thinning of petals and discoloration.

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