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Effects of sugars and amino-oxyacetic acid on the longevity of pollinated *Dendrobium* Pompadour flowers

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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 their combination in extending the vase-life of pollinated *Dendrobium* Pompadour flowers. 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 vase-life of up to 13 days, about 6 folds more than that of the control. Furthermore positive effects were also observed in a delay of weight loss, improved water uptake, change in petal 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 only prolonged the vase-life of flowers for just two days more compared to control. As has been studied in other experiments, pH of the holding solutions containing sugars alone maintained values of 5.0-7.0 while those with AOA and a combination of AOA and sugar at 3.0-5.0. Aminooxyacetic acid (AOA) and its combination with glucose extended the vase-life of pollinated *Dendrobium* Pompadour and also delayed the post-pollination symptoms.

Key words: Amino-oxyacetic acid, *Dendrobium* Pompadour, pollination, sugar, vase-life.

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 in vase life. In several species, flowers are long lived when not pollinated but show petal withering or abscission shortly following pollination (Ketsa and Narkbua, 9). *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 discolouration 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 sugars which leads to the death of flowers. 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 approaches can be employed, *i.e.* by inhibiting the production of pollination-induced ethylene (Ichimura *et al*., 7; Ho and Nicholas, 5), and by allowing a continues uptake carbon supply in order to prolong vase-life (Finger *et al.,* 4). 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) (Liao *et al*., 12). Maintaining continuous supply of exogenous sugars have been proven to prolong vase-life in many species, such as rose, chrysanthemum (Yakinova *et al.*, 16) and snapdragon (Ichimura and Hisamitsa, 6). Rattanawisalanon *et al*. (13) indicated that vase-life of *Dendrobium* "Jew Yuay Tew" flowers was extended when treated with a combination of sugar and AOA. 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

Dendrobium Pompadour flowers were obtained from the glasshouse of University of Malaya. Individual flowers were cut at the pedicles and placed in water vials containing 20 ml distilled water (control) or treatment solutions. Pollination was done by placing the pollen onto the stigma using hair brush. Experiments were carried out at ambient temperature (27 ± 2°C) under natural light conditions (600 lux) and a relative humidity of 75-85%. All experiments were done with three replicatcons.

Aminooxyacetic acid (AOA) was tested at 0.25 and 0.05 mM, glucose and sucrose were used at 2, 4 and 6%. Inclusion of 100 mg/l of chloramphenicol was used

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as an anti-bacterial agent for all treatments. pH measurements of all solution were taken daily using a pH meter (Hanna Instruments). All solutions were prepared at the beginning of the experiments and were not renewed.

Water uptake was calculated daily by subtracting the consecutive weights of the vials plus the solution (without the flowers). The weight of the flower was calculated by subtracting the weight of the vial with the solution and flower with the vial and solution only. The individual flowers were maintained at ambient conditions. The flowers were considered terminated once the perianth reached full closure. Thickness of a petal was measured daily using a micrometer which is held over the mid. of a petal. Flower colour was measured daily using a chromameter (Minolta) by placing the middle area of petals on the stage. Three replicates with a minimum of five flowers were used for each treatment and statistically tested using the Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The longevity of climacteric flowers is significantly reduced by pollination in orchids. Vase-life of unpollinated orchids which can last up to 6 months can be significantly reduced to a few days when pollinated (Doorn, 3). 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 (Lee *et al*., 10).

Senescence of flowers sensitive to ethylene can be delayed by treatment with inhibitors of ethylene. The effects of inhibitors of ethylene synthesis on the vase-life of cut flowers such as aminooxyacetic acid (AOA) and aminoethoxyvinylkglycine (AVG) have been studied. Wawrzynczak and Goszczynska (15) found AOA to be the most active ethylene inhibitor in "Dolca Vita" carnations. Our results (Table 1) show an agreement with the aforementioned study. Treatment with 0.05 mM AOA was found most effective for *Dendrobium* Pompadour. As shown in Table 1, vase-life of pollinated flowers was increased from 2 days (control) to 13 days. Furthermore, the flowers treated with 0.05 mM AOA also showed a delay in thinning and discolouration of petals (Fig. 1c-d). Water uptake was also improved as compared to that of control (Fig. 1b). This result however is not in agreement with the findings of Rattanawisalanon *et al.* (13) who concluded that the application of AOA alone resulted in a negative effect due to the toxicity of the chemical to the flowers. The role of pH value, however, is yet to be investigated in prolonging the vase-life of pollinated *Dendrobium* Pompadour. Previous studies have shown that low pH values are able to prevent and eliminate bacterial growth in holding solutions which improves water uptake by preventing stem blockage. 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 relations (Fig. 1b). In an earlier study on the fern *Adiantum raddianum,* the increase in vase solution pH containing AOA by inclusion of a *Hepes* buffer nullified the AOA effect (Doorn *et al*., 3).

Table 1. Longevity of pollinated *Dendrobium* pompadour flowers in different holding solutions of glucose, sucrose and AOA.

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
0.05 mM AOA	13.2f
6% glucose + 0.05 mM AOA + CHP	10.3e
2% sucrose + 0.05 mM AOA + CHP	4.9d

CHP = Cloramphenicol; Values shown are means of three replicates. Similar letters indicate that values are non-significant with each other as determined by Duncan's Multiple Range Test.

In the group of flowers where senescence is regulated by ethylene, exogenous sugars also considerably delay the increase in ethylene production and the time to visible senescence (Doorn, 1). Leon and Sheen (11) presented a model that shows that sucrose apparently inhibits the ethylene signal pathway through the production of low ABA concentration. 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 they are the main carbon-source utilized for energy in cut flower metabolism (Ho and Nicholos, 10). When detached from the mother plant, the energy supply of the flowers becomes limited due to the negligible photosynthesis. This limited supply of carbohydrates may lead to a decrease in flower longevity (Van der *et al*., 14). Furthermore, the loss of sugar can also result in the rupture of the semi-permeability of the cell membrane and eventually lead to an irreversible loss of membrane and structural destruction. Loss of compartmentalization causes the intermingling of previously separated components of the ethylene generating system, so there is an acceleration of ageing, senescence and death of flower tissue (Van der *et. al.,* 14). Our data shows that 4% glucose and 2% sucrose were able to increase the vase-life of pollinated *Dendrobium* Pompadour flowers to more than 5 days (Table 1). The inclusion of 100 mg/l of chloramphenicol into holding solutions containing sugars acted as an antimicrobial agent to prevent the growth of bacteria which cause blockage of the stem and disallow the uptake of sugar and water. Flowers held in these holding solutions maintain vase-life (Table 1), thickness of petal, colour 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 (Fig. 1a-d). This suggested that optimum concentration of sugars can effectively maintain the integrity of the cut flower structure. This finding suggests that optimal sucrose and glucose solutions might be required for cell wall synthesis and respiration. Similar results have been reported earlier by Liao *et al.* (12) with findings that indicated that optimal sucrose solution had a pronounced effect on vase life and quality of cut rose flowers. Our result, however, is not in agreement with the findings of Rattanawisalanon *et al*. (13), 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 no anti-microbial chemical was added into 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. Rattanawishalanon *et al*., (13) achieved the maximum increase in the vase-life of *Dendrobium* "Jew Yuay Tew" inflorences treated with a combination of 4% glucose and 0.05 mM AOA. The vase-life of cut roses was also prolonged with the treatment of sucrose pulse followed by pulsing with STS (Liao *et al*., 13). In our experiment, we also observed a prolonged vase-life of the

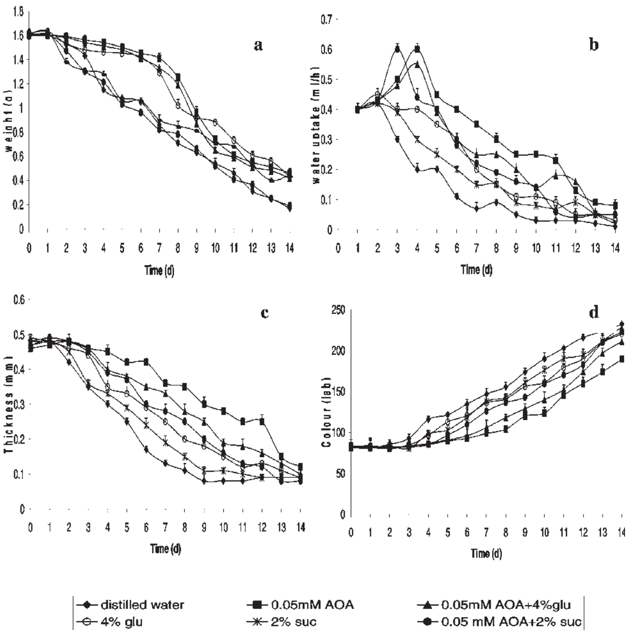

Fig 1a-d. Fresh weight changes (a), water uptake (b), petal thickness (c) and colour change (d) in pollinated *Dendrobium* Pompadour flowers held in different holding solutions.

Table 2. Change in pH of the holding solutions containing different concentrations of glucose, sucrose and AOA for pollinated *Dendrobium* Pompadour.

Treatment	pH value		
	Day 1	Day 4	Day 14
Distilled water + CHP	5.65de	6.56g	6.85g
2% glucose + CHP	5.74de	5.86e	6.32g
4% glucose + CHP	6.21f	4.77c	5.56d
6% glucose + CHP	6.63g	5.48d	5.86e
2% sucrose + CHP	6.12f	4.71c	5.43d
4% sucrose + CHP	6.87g	7.01h	7.38h
6% sucrose + CHP	6.66g	6.95gh	7.69g
0.025 mM AOA	4.15a	3.96a	4.12a
0.05 mM AOA	3.86a	3.95a	4.02a
6% glucose + 0.05 mM AOA + CHP	4.61bc	3.91a	4.15a
2% sucrose + 0.05 mM AOA + CHP	4.35ab	4.36ab	5.23d

CHP = Cloramphenicol; Values shown are means of three replicates. Similar letters indicate that values are non-significant with each other as determined by Duncan's Multiple Range Test.

Dendrobium Pompadour flowers treated with a combination of 4% glucose and 0.05 mM AOA (Table 1). 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 preferred respirable sugar is glucose rather than sucrose and is also cultivar and species dependant activity.

It is 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 discolouration. To further understand senescence and the extension of shelf-life of orchids, studies can be done to observe the effects of sugar and AOA treatments in ethylene production and the regulation of enzymes related to the physiological changes which occur as post-pollination symptoms such as thinning of petals and discolouration.

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