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Effects of Sugars and Aminooxyacetic Acid on the Longevity of Pollinated *Oncidium* Gower Ramsey Flowers

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ABSTRACT The effects of sugars and aminooxyacetic acid (AOA) on the vase life of pollinated *Oncidium* Gower Ramsey flowers were investigated in this study. AOA was found to be an effective ethylene inhibitor as holding solutions containing 0.5 mM of AOA considerably prolonged the vase life of the flowers. The best treatment in delaying the senescence of pollinated *Oncidium* Gower Ramsey flowers were solutions of 4% sucrose + 0.5 mM AOA and 0.5 mM AOA. Both these treatments managed to delay the discolouration, furrowing and appearance of the veins up to nearly twice the length of time it took for the control flowers held in distilled water, to start senescing. The addition of glucose to the holding solution was not as effective as sucrose in delaying the senescence and prolonging the vase life of the flowers. Signs of senescence such as discolouration were measured using the Minolta chroma meter. Weight loss and pH of the holding solutions were also measured daily. AOA added in the solutions had a positive effect of lowering the pH of the holding solution thus inhibiting bacterial growth in the vial. A low pH also accounted for better water uptake by the flowers which delayed turgor loss and thus also delaying the wilting of the flowers.

ABSTRAK Satu kajian tentang kesan gula dan asid aminoasetik (AOA) ke atas jangka hayat bunga *Oncidium* Gower Ramsey yang didebungakan telah dikaji. Penambahan glukosa ke dalam larutan didapati tidak sebaik sukrosa dalam memperlambatkan kelayuan dan memanjangkan hayat bunga yang dikaji. AOA didapati perencat etelina yang berkesan apabila larutan yang mengandungi 0.5 mM AOA sangat berjaya memanjangkan hayat bunga. Secara keseluruhannya, larutan yang paling berkesan dalam memanjangkan hayat *Oncidium* Gower Ramsey yang telah didebungakan adalah larutan yang mengandungi kombinasi 4% sukrosa + 0.5 mM AOA dan 0.5 mM AOA sahaja. Kedua-dua larutan ini berjaya memperlambatkan kehilangan warna kelopak bunga, kedutan dan dan kewujudan urat daun sebanyak dua kali ganda masa yang diambil oleh bunga kawalan (air suling). Tanda-tanda kelayuan seperti kehilangan warna, diukur menggunakan kroma meter Minolta. Berat yang hilang dan pH larutan turut diukur setiap hari. AOA yang ditambah ke dalam larutan memberikan kesan positif dalam menurunkan pH larutan sekaligus merencat pertumbuhan bakteria. pH yang rendah turut memperbaiki pengambilan air oleh bunga yang menyebabkan kesegahan bunga dapat dipanjangkan sekaligus memperlambatkan kelayuan dan memanjangkan jangka hayat bunga.

(Aminooxyacetic acid, *Oncidium* Gower Ramsey, senescence, sugar, vase life)

INTRODUCTION

Orchids are commercially viable because of their highly valued flowers. The great variety in colour, scent, size and shapes plus the exotic ambience of the flowers make them especially interesting and attractive to have. The popularity of the orchids is proven by the rapidly growing orchid industry worldwide. In Thailand, the orchid cut flower industry has been a major

foreign-exchange earner as it accounted for US $20 million in the 1990's and nearly $40 million in 2001 [1].

Malaysia is a relatively new player in the world cut flower industry. Nevertheless, the market is growing steadily and its economic importance has been recognized by the Malaysian government. In the National Agricultural Policy (1992 - 2010), cut flowers, including orchids have been identified as a priority group of crops with the potential to meet domestic and international demands [2]. Orchid is an important export industry in Malaysia with production value of approximately RM150 million per year [3]. Orchid production in Malaysia covers 300,000 hectars involving 36 growers. The export value of cut orchid flowers for Malaysia was estimated at RM40 million (worth of orchids) per annum, while the value for the domestic market was estimated at RM20 million [4].

Among the most popular orchid groups produced for the mass market are the *Dendrobium, Cymbidium, Paphiopedillum, Cattleya, Phalaenopsis* and *Oncidium*. *Oncidium* Gower Ramsey is a hybrid cross of *Oncidium* Goldiana and *Oncidium* Guinea Gold. This epiphytic orchid is very well received in both the Asian and European markets.

However, two major problems are faced in maintaining the quality and longevity of cut flowers and these are the loss in carbon source and the exposure to exogenous and endogenous ethylene. Changes in sink-source relationships occur when cut flowers are detached from the main plant. The inexistence of the main plant as a source of energy forces cut flowers to function as a source rather than a sink organ. In order to fulfill this function, the recycling of nutrients and energy reserves occurs by breaking down and degrading lipids and protein as an alternative energy [5]. Cut flowers suffer from energy deficiency and are susceptible to different stresses. In these situations, cells require more sugar to fulfil energy needs and carbon sources for the defensive response to stresses [6]. In addition, ethylene, an odourless and colourless gas, decreases the longevity of cut flowers by hormonally causing them to wilt rapidly [7]. It is not only found in the atmosphere as a product of incomplete combustion, is also produced by aging plant tissues such as ripening fruit and senescing flowers [8]. Orchids, such as the *Oncidium* Gower Ramsey are among the flowers that suffer from the post pollination effect signalled out by endogenous ethylene which eventually results in genetic and physiological changes of the flower. These changes are very similar to that of natural senescence involving weight loss, water loss and discoloration which eventually leads to death.

This study aims to determine a suitable holding solution to prolong the vase life of *Oncidium* Gower Ramsey flowers. The effectiveness of aminooxyacetic acid (AOA) in inhibiting the pollination-induced ethylene and the effects of exogenous sugars in delaying senescence of the *Oncidium* Gower Ramsey flowers were studied.

MATERIALS AND METHODS

Plant Material

Oncidium Gower Ramsey flowers were obtained from the glasshouse in University of Malaya. The flowers selected were 3 - 6 days post full anthesis, weighing between 0.30 to 0.35 g each. All the flowers were cut at the pedicles and pollinated before being placed into vials with 20 ml of the prepared solutions. Pollination was done by removing the pollinia from the anther onto the stigma of the flowers. The flowers were maintained at room temperature (26 ± 4°C) under natural light conditions (600 lux).

Vase life

The start of senescence was indicated by initial browning of the flowers and peduncles and the flowers were considered dead when there was a rapid quantitative and physical deterioration in the flower, indicated by total discolouration of the labellum and necrotic peduncles [7]. Three replicates of five flowers were used for each treatment and were statistically tested by using the Duncan's Multiple Test.

Chemicals and Reagents

Glucose (Sigma) and sucrose (Sigma) were used at 4% concentration while AOA (Sigma) was tested at concentrations of 0.25 mM and 0.5 mM. Combinations of 0.5 mM AOA + 4% glucose and 0.5 mM AOA + 4% sucrose were also used as chemical treatments. All solutions were prepared in the beginning of the experiment and were not renewed.

pH Measurement
pH of solutions were measured daily using a pH meter (Hanna pH 209, Germany).

Water Uptake and Fresh Weight
The difference between consecutive weighing of the vials plus solution (without the flower) was used to determine the water uptake. Evaporative water loss was deemed negligible. The weight of each flower was determined by subtracting the weight of the vial and solution from the weight of the flower, vial and solution. All measurements were taken at the same time daily.

Measuring Colour Difference (L*a*/b*)
L*a*/b* was measured daily using a Minolta Chroma Meter (CR-200, Japan). The L*a*/b* colour system closely represents human sensitivity to colour. Thus, it can be used to measure the perceived colour difference of the flowers as they senesce. The L*a*/b* colour system represents the following:

L* = Value/ Lightness (Brightness)
a* = Hue (Variety or Shade of Colour)
b* = Chrome (Saturation or Vividness)

The chroma meter was calibrated against a standard white calibration plate. The colour of petals was measured by placing the middle part of the flower petal onto the stage.

RESULTS

The vase life of the *Oncidium* Gower Ramsey flowers can be divided into three phases. At Phase 1, the labellum or large lip of the flower is bright yellow and smooth textured, while the base is distinctly speckled red. During Phase 2, signs of senescence start to appear. This is indicated by initial browning, furrows and prominently displayed veins on the labellum. At Phase 3, the base of the perianth together with the column is dark brown and the peduncle is necrotic. The labellum is also fully discoloured (browned). At this stage, the flower is considered dead [7].

As shown in Table 1, in this study, control Gower Ramsey flowers exhibited a short vase life compared to other treatments. Control flowers placed in distilled water only lasted for an average of 5 days before displaying any signs of senescence. On the other hand, either treatments of 4% sucrose + 0.5 mM AOA and 0.5 mM AOA were the most successful in extending the vase life to an average of 10 and 9 days, respectively (Table 1). The vase life of *Oncidium* Gower Ramsey held in the other holding solutions were not significantly prolonged compared to the control.

The extension of vase life in both the 4% sucrose and combination of sucrose+ 0.5 mM AOA coincided with the delay in the onset of weight loss (Figure 1). Although eventually all the flowers showed weight loss in this experiment, but the onset of weight loss in the above mentioned treatments only began at day 7 and day 5 (respectively) compared to the control which started experiencing weight loss as early as day 3. The occurrence of weight loss can be attributed to the loss of turgor caused by the decrease in the rate of water uptake in the flowers. In Figure 2, the data shows that water uptake also improved in holding solutions containing 4% sucrose and a combination of 4% sucrose + 0.5 mM AOA.

The L*a*/b value was used as an objective measurement of petal colour changes. This method was developed by Chandran *et al.*, (1997) in determining banana peel colour change. Throughout the experiment, it was observed that as the petal colour progresses from a bright yellow (negative value) to brown (positive value) the L*a*/b value increased (Figure 3). In this study, the control showed change in colour as early as day 4 while flowers held in the 4% sucrose and a combination 4% sucrose + 0.5 mM AOA only started experiencing colour change on day 9 and 11 respectively.

Throughout the experiments, holding solutions containing AOA maintained pH values of 3.0-4.0 whilst solutions containing sugars only had pH values of 5.0 - 6.0 (Table 2). The pH values of solutions containing AOA remained consistent throughout the experiment compared to the solution without AOA which showed a rapid decline in pH towards the end of the experiment.

Table 1. The vase life of pollinated *Oncidium* Gower Ramsey flowers held in different holding solutions. Values are means of three replications.

NO	HOLDING SOLUTIONS	VASE LIFE (DAYS)
1.	Distilled water	5.3a
2.	Glucose 4%	5.7b
3.	Sucrose 4%	5.3a
4.	0.25mM AOA	5.0ac
5.	0.5mM AOA	9.7d
6.	4% glucose + 0.5mM AOA	6.2e
7.	4% sucrose + 0.5mM AOA	10.1df

Table 2. pH values of holding solutions of *Oncidium* Gower ramsey Flowers at day 0 and vase life termination day

NO	TREATMENT	pH OF SOLUTION AT DAY 0	pH OF SOLUTION AT VASE LIFE TERMINATION DAY
1	Distilled Water	6.9 + 0.02	7.7 + 0.02
2	4% Glucose	4.8 + 0.01	5.6 + 0.01
3	4% Sucrose	5.3 + 0.02	5.9 + 0.02
4	0.25 mM AOA	3.4 + 0.03	3.6 + 0.03
5	0.5 mM AOA	3.2 + 0.01	3.3 + 0.01
6	4% Glucose + 0.5 mM AOA	2.9 + 0.04	2.9 + 0.04
7	4% Sucrose + 0.5 mM AOA	3.1 + 0.02	3.3 + 0.02

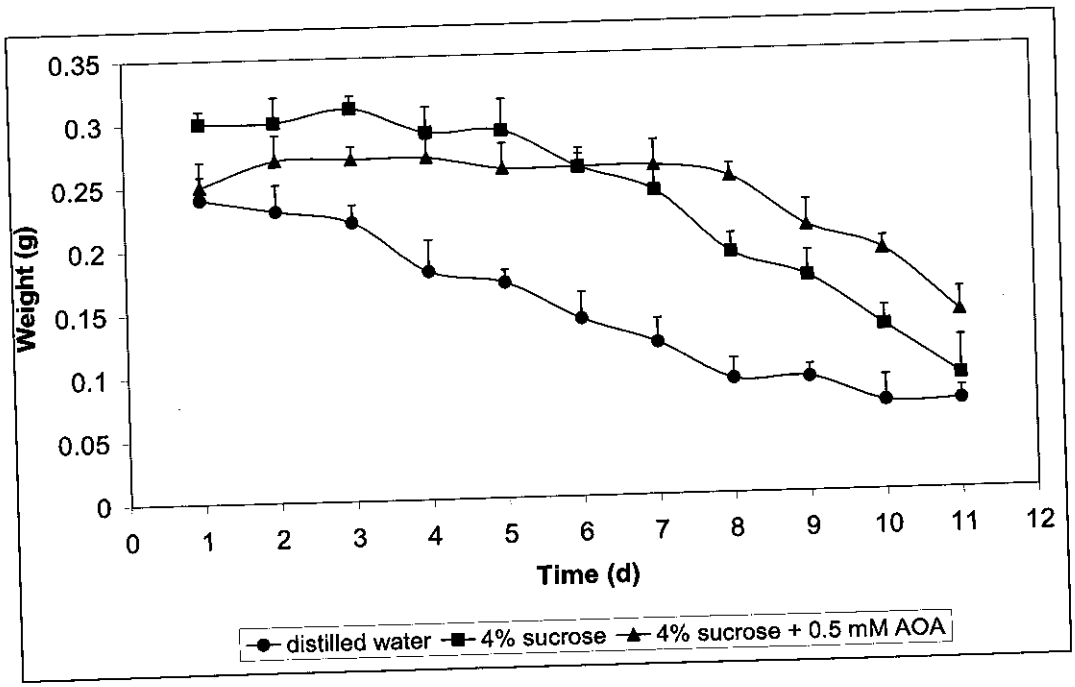

Figure 1. Weight loss of detached pollinated *Oncidium* Gower Ramsey in the different treatment solutions. Values are means of three replications with ± standard errors.

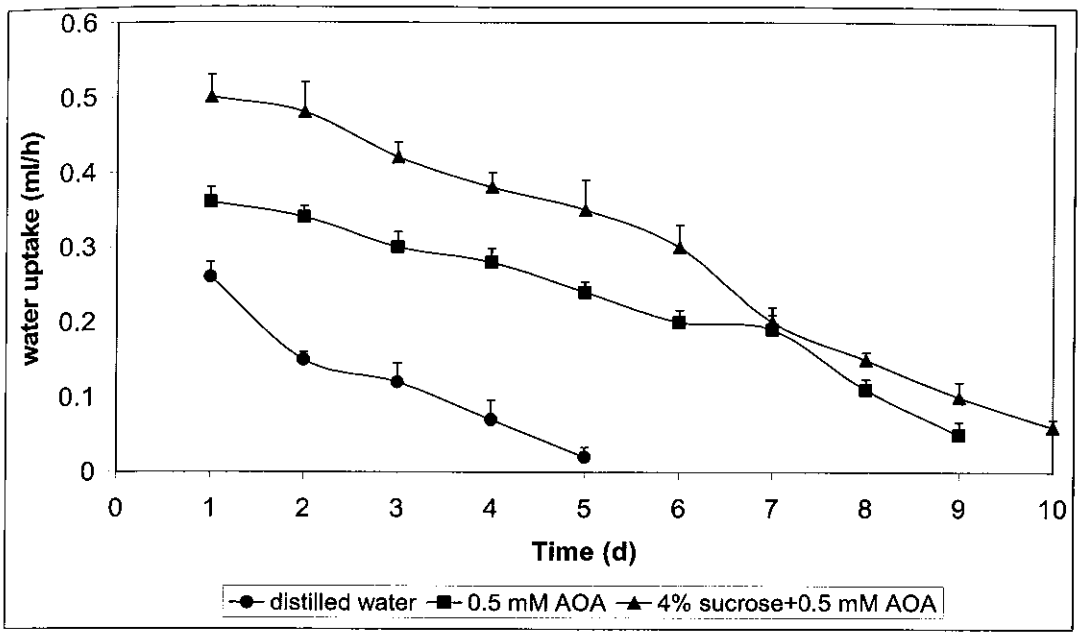

Figure 2. Water uptake of detached pollinated *Oncidium* Gower Ramsey in the different treatment solutions. Values are means of three replications with ± standard errors.

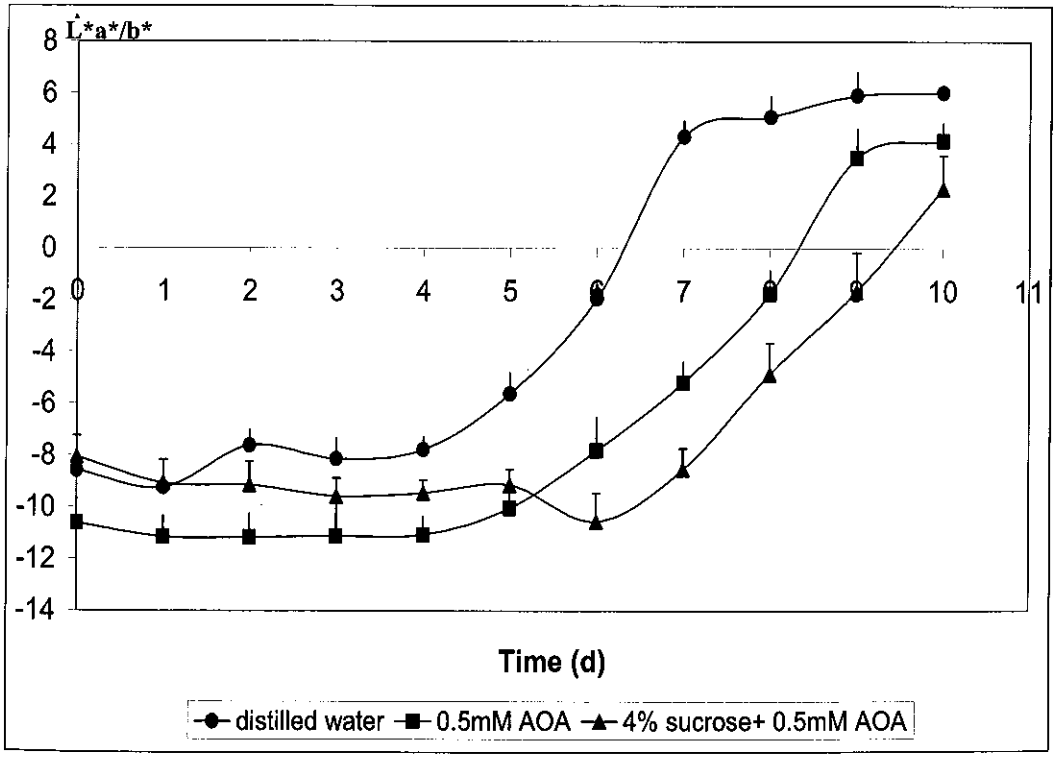

Figure 3. L*a*/b* values of pollinated *Oncidium* Gower Ramsey in the different treatment solutions. Values are means of three replications with ± standard errors.

DISCUSSION

It has been well documented that pollination of orchid flowers induces a dramatic increase in ethylene production which subsequently causes senescence symptoms such as rapid petal wilting and discolouration [9]. Nair and Tung [7] reported that the climacteric peak in ethylene production correlates with the time of initial wilting for the flowers (Phase 2). Thereafter, the flowers go through a series of phytogeriatric changes thus indicating that the production of ethylene is indeed one of the main causes of senescence.

Both sugars and AOA in the treatment solutions play multiple roles in preventing or delaying senescence. Sugar is an important aspect of plant life and is used as the main respiratory substrate for the generation of energy and metabolic intermediates that are then used for the synthesis of macromolecules and other cell constituents. It is also an important component of the DNA and RNA structure besides being an integral part of plant cell walls [10]. Sugars may also be important in helping to maintain water balance and thus also delays turgor loss as the flowers senesce. In this study, all the flowers in the different treatments exhibited a decrease in the fresh weight. In some flowers such as cut rose [11], carnations [12] and the flower tissue of morning glory [13] an infiltration of the intracellular spaces of the petals has been shown to be apparent suggesting that there may have been a loss of membrane integrity, causing an increase in permeability that leads to a rapid breakdown of cellular compartmentalization together with a parallel increase in the synthesis of ethylene.

When attached to the plant, the flowers are supplied with a constant food source in the form of carbohydrates produced by photosynthesis. These carbohydrates will then be converted to sugars which will be used as an energy source for cell development and maintenance. Cut flowers suffer from energy deficiency and are susceptible to different stresses. In these situations, cells require more sugar to fulfill energy and carbon requirements for the defensive response to stresses [6]. Sugars are also known to reduce ethylene sensitivity and thereby delay the time to senescence in flowers where petal senescence is regulated by endogenous ethylene [14]. As the flowers have been removed from the plant, carbohydrates reserves in the flowers must be used to maintain its metabolism.

In the event of sugar starvation, new cell components would not be able to be generated and osmotic pressures/ water balance would not be maintained and this will inevitably lead to cell death and senescence of the flowers. Sugar starvation also causes the loss of membrane integrity with a concomitant increase in permeability leading to a rapid breakdown of cellular compartmentalization. Loss of compartmentalization causes intermingling of previously separated components of the ethylene generating system, causing an acceleration of ageing, senescence and death of flower tissue [13]. This may be the reason why the combination treatment of 4% sucrose + 0.5 mM AOA works better than 0.5 mM AOA alone. Keeping the flowers in vase solutions containing sucrose has been shown to extend the vase life of flowers [15] and this has been proved as results of *Oncidium* Gower Ramsey flowers treated with 4% sucrose solution showed a longer vase life (8 days) compared to flowers treated with 4% glucose (seven days). Treatments with 4% glucose generally did not do as well as treatments with 4% sucrose in prolonging the vase life of the flowers. The reason for the preference of sucrose over glucose is unknown but could be due to the form of metabolic carbohydrates most preferably used by the flowers was sucrose and not glucose (in mobilization related to sugar starvation and senescence) [16].

AOA is used as a senescence retarding agent at concentrations of 0.5 mM, 0.25 mM and also in combinations of 4% sucrose + 0.5 mM AOA and 4% glucose + 0.5 mM AOA. In contrast to the results reported by Rattanawisalanon *et al.*[17]. AOA was not found to be toxic to the flowers but was instead found to be very effective in delaying senescence especially in treatments of 0.5 mM AOA and 4% sucrose + 0.5 mM AOA. Flowers treated with 0.5 mM AOA and 4% sucrose + 0.5 mM AOA showed an increased vase life, a delay in discolouration and browning, an onset of weight loss and an improved water uptake compared to the control. It was observed that the addition of 0.5 mM AOA to 4% glucose solution did not yield the same results as 4% sucrose + 0.5 mM AOA. This could indicate that glucose interferes with the effectiveness of ethylene inhibition by AOA. Treatments with 4% sucrose + 0.5 mM AOA yielded the best results. This is

probably because exogenous sucrose (the form of metabolic carbohydrate most preferred by the flowers) can act as a continuous supply of energy retaining the turgidity of the flowers and thus also enhancing the effects of AOA. In a cDNA microarray analysis of gene expression during carnation senescence, it was found that sugar feeding delayed expression of virtually the same group of genes (several hundred) as silver thiosulphate which blocks the ethylene receptor [16], thus this proves that exogenous sugars does enhance the effects of AOA and it also explains why weight loss only occurred on day 7.

Bacterial contamination of the treatment solutions would be one of the main causes of decreased water uptake in flowers. Sugars in holding solutions promote bacterial growth that may cause clogging of the xylem vessels and thus inhibit the efficient uptake of water and dissolved sugars which will then lead to the death of the flowers [17]. Treatments of 0.5 mM AOA and combinations of 4% sucrose + 0.5 mM AOA and 4% glucose + 0.5 mM AOA all managed to maintain a pH of 3.0 - 4.0 throughout the experiment. At this low pH, bacterial growth was inhibited therefore preventing occlusion of the xylem vessels and an improved water uptake.

From the study, it can be concluded that holding solutions containing aminooxyacetic acid (AOA) and sucrose effectively delayed the senescence of the affected *Oncidium* Gower Ramsey flowers due to its ethylene inhibition properties. This is shown by the delayed weight loss and discolouration experienced by the affected flowers and an improved water uptake. In addition a low pH provided by the addition of AOA further delayed senescence as a low pH prevented the growth of bacteria.

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