

**EFFECTS OF HARVESTING STAGES AND PRESERVATIVE
SOLUTIONS ON VASE-LIFE OF *MOKARA* MADAME PANNE
CUT ORCHID**

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Title

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Title	Effects of Harvesting Stages and Preservative Solutions on Vase-life of <i>Mokara</i> Madame Panne Cut Orchid
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ABSTRACT

Four harvesting stages and five preservative solutions were tested to identify their effects on extending the vase-life and improving the bud-opening of *Mokara* Madame Panne cut orchid grown in Chiang Mai province, Thailand. Two major experiments were conducted at the Department of Horticulture, Maejo University.

In the first experiment, the inflorescences of *Mokara* Madame Panne with 3-4, 5-6, 7-8 and 9-10 opened florets were used as harvesting stage-1 to harvesting stage-4, respectively. The changes in respiration rate, ethylene production, water relation, and total sugar content in florets and flower-stems were studied. Results showed that there were not significant differences in the vase-life (11-13 days) and bud-opening (49-60 %) among the four harvesting stages.

In the second experiment, only harvesting stage-3 (7-8 opened florets) was used. The preservative solutions containing 150 ppm 8-hydroxyquinoline sulfate (8-HQS) + 2% sucrose, 50 ppm aluminium sulfate $[Al_2(SO_4)_3]$ + 2% sucrose, 150 ppm 8-HQS + 50 ppm $Al_2(SO_4)_3$, 150 ppm 8-HQS + 50 ppm $Al_2(SO_4)_3$ + 2% sucrose, and only reverse osmosis (RO) water were evaluated for the same parameters as in the first experiment, except respiration rate and ethylene production. In addition, the change in floret color was also determined. Results revealed that the preservative solution containing 150 ppm 8-HQS + 50 ppm $Al_2(SO_4)_3$ can extend the vase-life of *Mokara* Madame Panne cut orchid significantly for 26 days as compared to the control treatment (19 days). The other preservative solutions, 150 ppm 8-HQS + 2% sucrose, 50 ppm $Al_2(SO_4)_3$ + 2% sucrose and 150 ppm 8-HQS + 50 ppm $Al_2(SO_4)_3$ + 2% sucrose provided the vase-life for 19, 18 and 22.5 days, respectively.

Key words: *Mokara* Madame Panne orchid, harvesting stages, preservative solutions, vase-life

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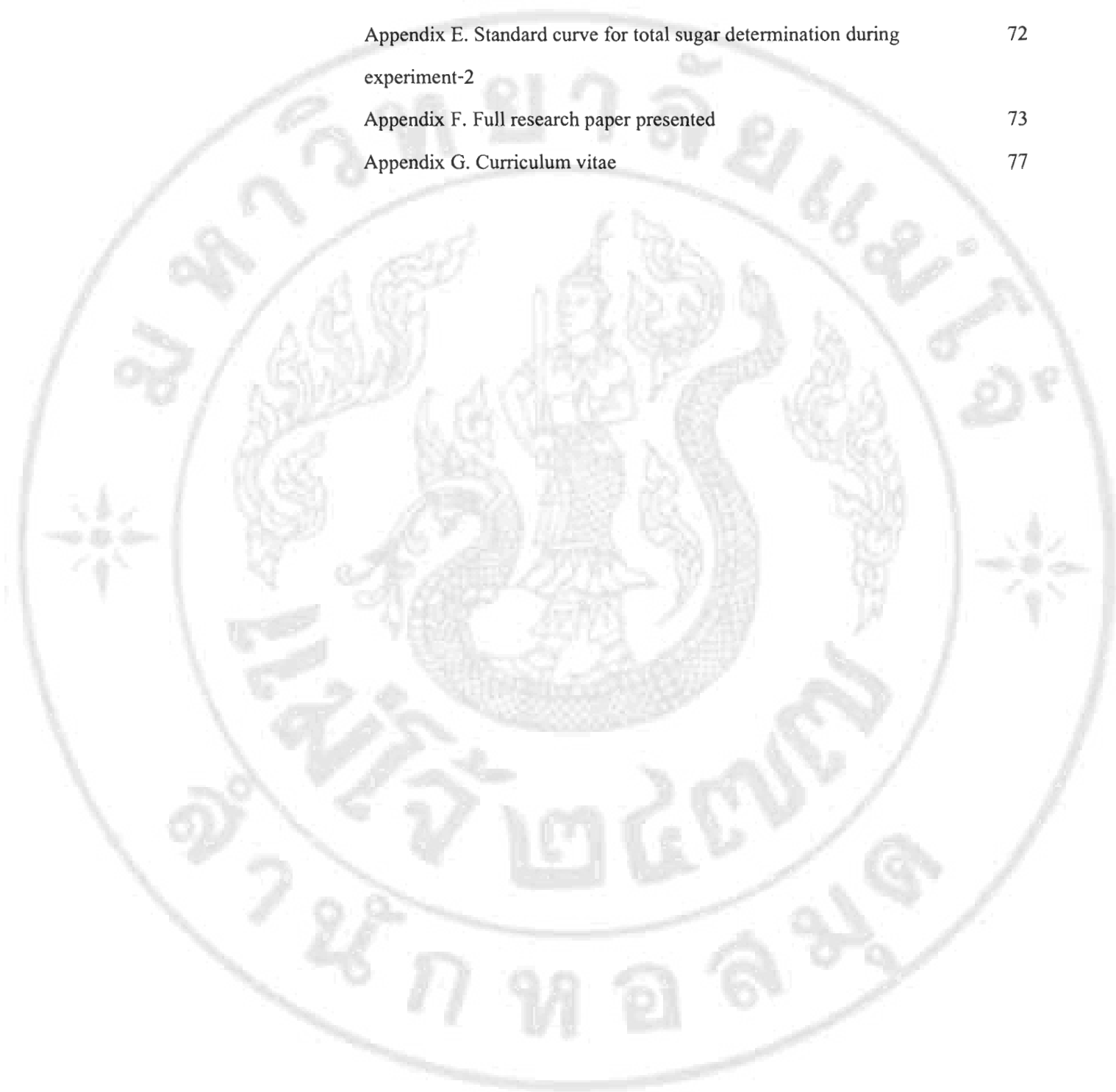
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Abbreviations

ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	Analysis of variance
AOA	Aminooxyacetic acid
AS	Aluminium sulfate
Assist.	Assistant
Assoc.	Associate
AVG	Aminoethoxyvinylglycine
CA	Controlled atmosphere
CRD	Completely Randomized Design
DMRT	Duncan's Multiple Range Test
EFE	Ethylene forming enzyme
e.g.	For example
et al.	And other people
FID	Flame ionization detector
FW	Fresh weight
g/gm	Gram
GA ₃	Gibberellic acid
GC	Gas chromatograph
h/hr	Hour
HS	Harvesting stage
8-HQC	8- hydroxyquinoline citrate
8-HQS	8- hydroxyquinoline sulfate
i.e.	That is
Kg	Kilogram
L/Lit.	Liter
mA	Milli ampere
1-MCP	1-methylcyclopropene
Met / MET	Methionine



min	Minute/minutes
mg	Milligram
mM	Milli molar
mm Hg	Milli meter mercury
nm	Nano meter
ppm	Parts per million
Prof.	Professor
RH	Relative humidity
RO	Reverse osmosis
sp	Specie
SAM / AdoMet	S-adenosyl methionine
STS	Silver thiosulfate
TCD	Thermo conductivity detector
USA	United States of America
Wt.	Weight

Chapter 1

Introduction

Mokara is one of the orchid genera which is considered very successful for cut flower production. It is a man-made genus and multigeneric (*Arachnis* x *Ascocentrum* x *Vanda*) hybrid created in Singapore (Yew-Hwa, 1995; Yam and Thame, 1999). Since it has monopodial growth habit, it can be planted in high density, at least 60,000 plants per hectare. Its commercial cultivars can produce 15 salable inflorescences per plant annually (Yam and Thame, 1999). Further more, it bears unique star-shaped florets, 12-18 in each inflorescence and found in many colors. It is also one of the exported cut flowers from Singapore (Yam and Thame, 1999).

Mokara Madame Panne which contains peculiar red spotted yellowish florets is very popular in Thailand (Figure 1.1). Its major flowering period is May to October. In general, its inflorescences are harvested at half or more blooming stages according to market demand (personal communication with growers). However, it has not been found scientifically studied which stage is the best for its maximum vase-life.

Besides temperature and humidity management throughout the handling chain, there are two major factors by which the vase-life and bud-opening of cut flowers can be extended. One is appropriate harvesting stage and another one is suitable preservative solution mixture. Different kinds of flowers have different harvesting/maturity stages to be used as cut flowers. In some varieties of *Dendrobium* orchid, researchers had used inflorescences having 37.5-66.7 % opened florets (Ketsa and Wongs-Aree, 1995; Rattanawisalanon et al. 2003) which showed the highest vase-life and bud-opening. Ketsa and Wongs-Aree (1995) also reported that the number of open florets in each individual inflorescence of *Dendrobium* must be adequate because they ensure the maximum water supply even in the presence of preservatives in order to maximize bud-opening with less defects.

Similarly, five different harvesting stages of *Leucocoryne* flower, Liliaceae (Elgar et al., 2003) and four harvesting stages of *Narcissus tazetta* (Jowkar and Kafi, 2005) were tested to determine their postharvest life. While selecting, any harvesting stage which can give the longest vase-life and in which remaining buds can open satisfactorily, should be considered. Therefore,

four various harvesting stages of *Mokara* Madame Panne cut orchid / inflorescences were applied with 3-10 (26- 59 %) opened florets in this study.

For extending the vase-life and bud-opening of cut flowers, various preservatives should be included in holding solutions. Most preservatives used in holding solutions are - sugars (sucrose, glucose), citric acid, germicides (8-hydroxyquinoline sulfate/8-HQS, aluminium sulfate/ $\text{Al}_2(\text{SO}_4)_3$) and ethylene inhibitors (amiooxyacetic acid/AOA, silver thiosulfate/STS, 1-methylcyclopropene/1-MCP). Sugars are mainly applied for the supplement of food reserves. Citric acid lowers the pH of vase solution and helps in solution uptake into the flower-stems. Similarly, different germicides can control different types of microbes specially bacteria; so, water quality of vase and its uptake improve. The problem of ethylene-induced flower senescence can be overcome by adding ethylene inhibitors into the vase solution which can control either ethylene synthesis or ethylene action according to their nature of work.

The preservative solution containing 2 % sucrose and 200 ppm 8-HQS significantly extended the vase-life (9.5 days) of cut 'Sonia' rose flowers (Ichimura et al., 2003). While in different varieties of *Dendrobium* orchid, the holding solution with 225 mg l⁻¹ 8-HQS + 50 mg l⁻¹ $\text{Al}_2(\text{SO}_4)_3$ + 4 % glucose increased vase-life and bud-opening the greatest and significantly, 23-34 days and 98-100 % respectively (Ketsa and Kosonmethakul, 2001). It indicated the potentiality for further improving in vase-life and bud-opening of *Mokara* Madame Panne flower, although its average vase-life is considered to be already higher, 14 days (Yam and Thame, 1999). On the other hand, there was not found either clear response to ethylene, or effect of inhibitors of ethylene synthesis or ethylene action in some *Dendrobium* species tested (Goh et al., 1985 cited by Rattanawisalanon et al., 2003; Ketsa and Thampitakorn, 1995). However, there is not available supporting data about it on *Mokara* cut orchid.

On the basis of these reviews and preliminary experiment, it was decided to test only sucrose, 8-HQS and $\text{Al}_2(\text{SO}_4)_3$ in this study. Thus, two experiments were carried out: one for identifying the best harvesting stage to be used as cut flower and another one for finding out the suitable preservative solution mixture for further improving the vase-life and bud-opening of *Mokara* Madame Panne cut orchid.

Objective of the Study

General objective

The general objective of this study was to find out the best harvesting stage/stages and preservative solution for maximum vase-life and bud-opening of *Mokara* Madame Panne cut orchid grown in Chiang Mai province of Thailand.

Specific objectives

1. To determine the effects of different harvesting stages on vase-life and bud-opening of *Mokara* Madame Panne cut orchid.
2. To investigate the roles of various possible preservative solutions for extending the vase-life and for improving the bud-opening of *Mokara* Madame Panne cut orchid.



Figure 1.1 *Mokara Madame Panne* Orchid.

Chapter 2

Literature Review

2.1 Orchids and genus *Mokara*

2.1.1 Orchids

Genera, species and distribution

Orchids are the special kind of flowering plants belonging to the family Orchidaceae. They are composed of more than 800 genera, around 25,000 species and more than 110,000 registered hybrids; a number that continues to rise (Sheehan, 2003). Wild orchids exist in all parts of the world, except the Antarctic and major deserts (Stewart, 1988), due to their incredible diversity and adaptability. However, cool, moist mountain valleys of South America are considered their richest habitats (Stewart, 1988).

In the view of commercial importance, for cut-flowers and/or potted plants, *Dendrobium*, *Phalaenopsis*, *Cymbidium*, *Cattleya*, *Oncidium*, *Paphiopedilum*, *Vanda* and vandaceous genera of orchid are the most common. Today's growing most of orchids are man-made hybrids, not wild species.

Growth habit

Orchid plants are perennial and mostly herbaceous in nature. There are found two major growth habits: sympodial and monopodial (Orchid Society of South East Asia, 1993; Sheehan, 2003). In sympodial orchids (e.g. *Cattleya*), the succession growth pattern of shoots or bulb-like stems (pseudo-bulbs) occurs. They grow for a limited time (determinate growth) and when mature, usually bear terminal flowers. In monopodial orchids, such as in *Vanda* and *Mokara*, the indeterminate growth of usually single stem is found which is always vegetative at the apex. The inflorescence grows from the side of the stem, not from the crown.

Moreover, on the basis of their natural habitats, orchids can be terrestrial (growing in the ground), epiphytic (growing on trees), and some are even found growing on rocks, called lithophytes (Stewart, 1988; Orchid Society of South East Asia, 1993; Sheehan, 2003). But, more than half the known species grow as epiphytes (Stewart, 1988), deriving their nutrients from

substances dissolved in the rain water (not parasite to host plants). There are no truly aquatic and desert-loving orchids (Stewart, 1988).

Many orchids have special vegetative features- swollen stems/pseudo-bulbs, succulent leaves, and aerial roots with a thick protective and absorptive covering (called velamen) for survival. There are several main characteristics that a flower must bear to be called an orchid (Sheehan, 2003). They are:

Zygomorphic flower	: special type of irregular flower.
Gynandrium (column)	: fused both male and female parts.
Rostellum	: stigmatic tissue
Pollinia	: little packet of pollen grains.
Labellum	: lip, highly modified petal.
Unusual seed	: minute like dust particle, does not contain endosperm

2.1.2 Genus *Mokara*

Origin

Mokara orchid is an artificial (man-made) genus and a trigeneric (multigeneric) vandaceous hybrid. It was developed in Singapore by involving *Arachnis*, *Ascocentrum* and *Vanda* orchids (Yew-Hwa, 1995; Yam and Thame, 1999). The name '*Mokara*' is given in the honor of "Mr. Mok Chow Yew", an originator of new orchid genus *Mokara* (Yam and Thame, 1999). The first hybrid, *Mokara* Wai Liang (*Arachnis* Ishbel X *Ascocenda* Red Gem) was registered in 1969 (Yew-Hwa, 1995; Yam and Thame, 1999); and by the mid-1998, there had already been registered 110 mokaras (Yam and Thame, 1999). Additionally, 32 more hybrids of *Mokara* have been included till mid-2007 in the list of 'new orchid hybrids' (The Royal Horticultural Society, 1998; 1999; 2000; 2001; 2002; 2003; 2004; 2005; 2006; 2007).

Types of hybrids

There are 3 types of hybrids in *Mokara* according to crossing systems:

First-generation hybrids. They are crosses between either an *Arachnis* and an *Ascocenda* (*Ascocentrum* x *Vanda*) or an *Aranda* (*Arachnis* x *Vanda*) and an *Ascocenda*. Out of the 110 hybrids, more than half are first generation hybrids. The most commercially successful varieties are *Mokara* Chark Kuan, *Mokara* Khaw Phaik Suan, *Mokara* Bangkok Gold, *Mokara* Bibi, *Mokara* Kelvin, *Mokara* Sayan, and *Mokara* Mak Chin On (Yam and Thame, 1999).

Second-generation hybrids. These are developed by crossing between first-generation *Mokara* and either a *Vanda* or an *Ascocenda*. Yam and Thame (1999) have noted that, although technically *Mokara* can also be crossed between first-generation *Mokara* and *Aranda* or between *Mokaras*, but these crosses are rarely made because no viable seeds are formed.

Most second-generation hybrids tend to have rounder, bigger flowers with broader floral parts. These plants also have longer and broader leaves. The better-known second-generation hybrids are *Mokara* Willie How (not registered), *Mokara* Dickson How (*Mokara* Khaw Phaik Suan x *Ascocenda* Yvonne Rowse) and *Mokara* Zaleha Alsagoff (*Mokara* Khaw Phaik Suan x *Vanda* Rasri Gold) (Yam and Thame, 1999).

Most *Mokara* hybrids are either diploid ($2n=38$) or triploid ($2n=57$) (Yew-Hwa, 1995; Yam and Thame, 1999). So far studied in second-generation *mokaras*, all of them have been found to be triploids. Triploid *mokaras* are generally more vigorous than the diploids, with rounder and larger flowers (Yam and Thame, 1999).

Third-generation hybrids. These are the hybrids crossed between second-generation *Mokara* and either a *Vanda* or an *Ascocenda*. Only a few third-generation *Mokara* hybrids have been registered, an example is *Mokara* Aowc (Yam and Thame, 1999).

Importance

Mokara orchids grow well in the low-land tropics. As a monopodial growth habit, mokaras allow high-density planting, with 60,000 or more plants per hectare. A commercially successful *Mokara* can produce more than 15 salable sprays / inflorescences per year per plant (about 1,000,000 per hectare per year) (Yam and Thame, 1999).

One can find many colored mokaras from yellow to orange and pink to dark purple. They have long vase life, most commercial varieties last for at least two weeks. So, *Mokara* has been one of the key cut-flowers exported from Singapore (Yam and Thame, 1999).

Mokara orchids are also one of the favoring plants used for landscaping, because they can be grown in the open area, easy to maintain and colorful. First-generation mokaras can be grown under full sun while most second-generation mokaras require little shade. In the National Orchid Garden and Mandai Orchid Garden of Singapore, one can see the rainbow of colors exhibited in mokaras (Yam and Thame, 1999).

2.2 Factors affecting vase-life of cut flowers and their management

There are several factors that affect the postharvest quality and the vase-life of cut flowers. These factors are: harvesting stages of cut flowers, water quality of vase, food supply, temperature and relative humidity, physiological and bacterial plugging, respiration and ethylene.

2.2.1 Harvesting stages of cut flowers

Every type of flower has its harvesting (maturity) stages for using as cut flowers. Some flowers are cut at still in bud or partly open, e.g. rose while others are harvested at various stages. The ideal phase of flower development for harvest depends on the plant species, cultivars, seasons, distance to market place, and consumers' preferences. In general, if flowers are cut at more advanced phases of development, they have shorter vase-life than younger flowers, and if flowers are harvested too early, they do not develop properly showing lower quality and shorter life (Nowak and Rudnicki, 1990).

Five harvesting stages of *Leucocoryne* (Liliaceae) that were 'tight bud', 'slight calyx', 'first flower unfurling', 'one flower open' and 'three flowers open', were used by Elgar et al. (2003). According to their experiment, latter 3 stages showed the satisfactory flower opening (>

70 %), however vase-life of all harvesting stages was not significantly different. For different varieties of *Dendrobium* orchid, Ketsa and Kosonmethakul (2001) used inflorescences of with 8 ± 1 (57 %) opened florets and 6 ± 1 flower buds; while inflorescences having 5-10 (42-66.7 %) opened florets and 5-7 flower buds were applied by Rattanawisalanon et al. (2003) in their experiments.

Ketsa and Wongs-Aree (1995), mentioned that the number of open florets in each individual inflorescence of *Dendrobium* must be adequate before harvest. Because they will ensure the maximum water supply even in the presence of preservatives for maximizing bud-opening with less defects (twisting and half opening). They had used a long export-grade inflorescences of six *Dendrobium* varieties with 9-10 opened florets (37.5- 43.5 %) and 13-15 flower buds. By the end of vase-life (14 days), 96 % buds had opened successfully in average.

There was lack of information about the harvesting stages of *Mokara* orchid in the reviewed literatures. However, one can obviously distinguish among them on the basis of number or percentage of opened florets on the inflorescences (Personal communication with growers). Growers and traders think that half and more than half blooming stages of *Mokara* inflorescences are the best for their vase-life, though these have not been scientifically proven.

The important thing is any type of cut flower should be harvested at that maturity stage after which the remaining unopened buds can open fully in highest number with maximum vase-life.

2.2.2 Water quality of vase

Most flower and foliage types are very sensitive to drying out, especially those with a large leaf area. Many flowers which have woody stems tend to need more water. The water used in containers and vases must be clean and free of bacterial contamination. It also should have an acidic pH (3 to 4), as this improves the flow rate of the solution up the stems (Nowak and Rudnicki, 1990; Gollnow and Wade, 2002). Citric acid can be used to bring the pH down and germicide should be added to prevent rapid multiplication of bacteria in the solution. It is important because bacteria cause bacterial plugging in the cut flower-stems and water uptake is restricted, resulting in early wilting of flowers and flower buds (Nowak and Rudnicki, 1990; Gollnow and Wade, 2002).

A good supply of high quality water is particularly necessary for opening flower buds (Reid and Evans, 1986 cited by Ketsa and Wongs-Aree, 1995). A research conducted by Jowkar and Salehi (2005), showed that 300 mg l⁻¹ citric acid included holding solution significantly increased the vase-life (7-8 days) and florets opening (10.5/ spike) in cut tuberose. This may be due to reducing the solution viscosity and the micro-organism growth via lowering the pH of vase solution (Alvarez et al., 1994; Reddy et al., 1995b; Van Doorn and Peirik, 1990 cited by Jowkar and Salehi, 2005).

But, Rattanawisalanon et al. (2003) reported that 0.5 mM aminooxyacetic acid (AOA) together with 4 % glucose considerably increased vase-life (20 days) and bud-opening of *Dendrobium* Jew Yuay Tew without pH adjustment by citric acid. It was concluded that AOA exerted its effect by improving water relation, not by decreasing ethylene; since it is thought that AOA inhibits ethylene synthesis. Sugars may improve water relation by increasing the level of osmotic salutes and reducing toxicity of germicides (Van Doorn, 2001).

In another experiment of Rattanawisalanon et al., 2003, sterilization of the vase water increased the vase-life of *Dendrobium* Jew Yuay Tew cut flowers by 2 days but this effect was largely absent in the presence of 0.5 mM AOA and 4 % glucose in vase water. Warming the water at 38-40°C also improves water absorption, since warm water moves more easily through the stem than cold water (Nowak and Rudnicki, 1990). In contrast, Slootweg (1995) noted that most flower crops (rose 'Sonia', Eustoma and Bouvardia) showed the faster water uptake in cold water (0-4°C), however very little effect was found on the length of the vase-life and bud-opening.

2.2.3 Food supply

Stored foods (especially sugars) in flower-stems, leaves and flowers are needed to sustain the cut flowers after harvest, and allow flower buds to open. Applied sugars to cut flowers can supplement the food reserves. This is a common practice for many commercial cut flowers. It is known that exogenous supply of sugars extends the postharvest life of cut flowers. The applied sugars may serve to improve water relations as they may increase the levels of osmotic solutes, and they can maintain respiration in case of lack of adequate substrate (Van Doorn, 2001). It is also known that sugars reduce ethylene sensitivity, and thereby delay wilting in ethylene-sensitive flowers (Van Doorn, 2001).

Sugar can prevent flowers (particularly those with woody stems) from dehydrating when placed in vase water, while high concentrations (more than 1-2 %) can cause excessive nectar production or make leaves dry out (Gollnow and Wade, 2002). It can also facilitate for microbial growth. Therefore, a germicide is always added to the sugar solution to prevent microbial growth.

Jowkar and Salehi (2005) reported that higher dose than 1 % sucrose decreased vase-life of cut tuberose. It may be due to probable increase of microbial agents in vase solution in the presence of sucrose. Similarly, Rattanawisalanon et al. (2003) mentioned that glucose or sucrose alone in the vase water had no effect on vase-life of *Dendrobium* Jew Yuay Tew inflorescences, but the combination of 0.5 mM aminooxyacetic acid (AOA) and 4 % glucose considerably increased it. This treatment suppressed bud drop, promoted bud-opening, and delayed the time to senescence of open flowers due to continuous uptake of water and dissolved sugar by the flowers.

The study of Ichimura et al. (2003) revealed that 2 % sucrose plus 200 ppm / mg liter⁻¹ 8- hydroxyquinoline sulfate (8-HQS) significantly extended the vase-life (9.5 days) of cut 'Sonia' rose flowers. And sucrose alone was found more effective (vase life 8.7 days) than 8-HQS alone (vase life 6.2 days). These results showed that decrease in soluble carbohydrate concentration in petals was more important than vascular occlusion in determining the vase-life of cut 'Sonia' roses.

Continuous treatment of cut snapdragon (*Antirrhinum majus* L. cv. Yellow butterfly) with sucrose at 50 g liter⁻¹ (5 %) in combination with 200 mg liter⁻¹ 8-HQS markedly promoted floret opening and extended the vase-life of flower spikes (Ichimura and Hisamatsu, 1999). This treatment suppressed the decrease in fresh weight of florets. The onset of climacteric ethylene production was also delayed by this sucrose treatment which suggests that the effects of sucrose for promoting bud-opening and inhibiting flower senescence is attributed to an increase in sugar concentrations and the inhibition of ethylene synthesis (Ichimura and Hisamatsu, 1999).

Postharvest life of cut *Eustoma grandiflorum* flowers is limited by poor bud-opening and bend neck in open flowers. Vase solutions containing up to 6 % sucrose or glucose improved the quality and vase-life of the flowers (Cho et al., 2001). The additional carbohydrate improved petal color, increased bud-opening, strengthened pedicels, and extended overall inflorescence longevity up to 8 days (Cho et al., 2001). The principal sugar in the petals of opened flowers was

glucose, with lower concentrations of sucrose and fructose. Presence of sugar in the vase solution greatly increased the concentrations of sugars in the perianth of buds and opened flowers (Cho et al., 2001). In contrast, in cut *Narcissus tazetta* var. *chinensis*, Ichimora and Goto (2002) reported that sucrose (pulsing with 5 and 10%) could not extend its vase-life, possibly due to already presence of high level of sugars / carbohydrates in various floral organs (flower stem, petal and leaf etc.).

Sing and Sharma (2003) described that combination of 5 % sucrose plus 600 mg l⁻¹ 8-hydroxyquinoline citrate (8-HQC) increased the vase-life of pulsed (20 % sucrose for 24 hrs.) gladiolus spikes cv. White Prosperity from 8.5 days in control to 13 days. Vase-life of 12.5 days was found in 5 % sucrose plus 300 mg l⁻¹ aluminium sulfate [Al₂ (SO₄)₃] treatment. Similarly, maximum number of fully opened florets (8.4) was observed in spikes treated with 8-HQC plus sucrose followed (8.1 florets) by Al₂ (SO₄)₃ in combination with sucrose.

Pulsed Asiatic hybrid lily with 10 % sucrose and 1 mM silver thiosulphate (STS) for 6 hrs significantly increased the vase-life and improved floral attributes such as freshness, color intensity and flower size (Sindhu and Pathania, 2003). Its further improvement in vase-life (6.6 days) was achieved by keeping pulsed flowers in a preservative solution containing 2 % sucrose and 200 ppm 8-HQC (Sindhu and Pathania, 2003).

2.2.4 Temperature and relative humidity

Temperature

Temperature is the environmental factor that most influences the deterioration rate of harvested commodities. For each increase of 10°C above optimum, the rate of deterioration increases by two to three folds (Kader, 1992).

Higher temperature affects the rate of transpiration (water loss from the plant parts including flowers), and there after accelerates postharvest decline (shriveling and wilting). Besides, it also increases the effect of ethylene and respiration rate up to the level of 30 or 35°C. Spore germination and growth rate of pathogens are greatly influenced by temperature.

To ensure a good vase life, field heat need to be removed from the flowers rapidly after picking by means of pre-cooling. Ketsa et al. (2005) reported that pre-cooling of *Dendrobium*

Pompadour cut orchid at 10°C (85-95 % RH) for 60 min gave longer vase life and in most experiments it promoted bud-opening.

After pre-cooling flowers also must be stored or handled at low temperature between 1- 4°C. However, species that grow naturally in tropical climate (e.g. orchids) need warmer storage temperatures (minimum 10°C) to avoid chilling injury. Fluctuating temperatures during transport is one reason that cut flowers may reach market in poor condition, even though they may have packed in a top-quality condition (Gollnow and Wade, 2002).

Relative humidity

Relative humidity (RH) is defined as the capacity of air to hold the water vapor at specific temperature in relation to the maximum capacity. At a given temperature and rate of air movement, the rate of water loss from the commodities depends on the relative humidity. Since, fresh horticultural commodities are the 'unique packages of water', water loss severely degrades their postharvest quality (desiccation) and life. Therefore, like temperature, RH is also important to be managed, 95-98 % in the cool room and package (Kader, 1992; Gollnow and Wade, 2002).

Water loss from warm products to warm air is particularly serious under windy conditions or during transport in an open vehicle. On the other hand, maintaining high RH is sometimes difficult because refrigeration removes moisture. However, humidification devices can be used in storage. Similarly, commodities that can tolerate direct contact with water can be sprinkled to promote higher relative humidity (Kader, 1992). Even buckets of water can increase humidity as the fans blow air across the water's surface and increase evaporation. Wrapping and enveloping / packing commodities in plastic films will also help in maintaining higher RH. Frequent sanitary measures should also be employed in storage, because high RH can also facilitate for disease growth (Nowak and Rudnicki, 1990; Kader, 1992).

2.2.5 Physiological and bacterial plugging

Physiological plugging

When cells of flower stem are wounded by cutting, they naturally produce substances to seal the wound. These substances can block the water flow up to the stem; this condition is called 'physiological plugging'. Due to this, flowers and flower-buds wilt earlier than natural senescence.

To avoid this situation: cut flowers should be held continuously in clean water after harvest, containers should also be used clean, flower stems should be re-cut slanting under the water, and vase solution should be acidic (having low pH).

Bacterial plugging

Bacteria and other microbes are responsible for 'bacterial plugging'. These micro-organisms may present in tap water, vase, and other containers that are not cleaned properly.

According to Put (1990), a wide variety of bacteria and fungi was isolated from freshly cut flower stems and vase solution of chrysanthemum 'Spider', gerbera 'Appelbloesem' and 'Fleur', and rose 'Sonia'. Bacterial genera present on the stems, were mainly also present in the corresponding vase water. Initially *Enterobacter* and *Bacillus species* and fungi were found dominantly while after 3 days of vase life, *Pseudomonas species* showed dominance. The longer the vase-life, the greater were changes in the micro-flora of the vase water; later again showed predominance of *Enterobacter* and *Bacillus species* (Put, 1990).

After 10 days of vase life, fungal growth increased dramatically in chrysanthemum and gerbera vase water. Microbial load on stems of cut rose was found to be much lower than those on chrysanthemum and gerbera stems. The end of vase-life of rose flowers was characterized by normal senescence symptoms or by weak wilting of leaves and flowers. In chrysanthemum and gerbera, however, an extensive water stress developed (Put, 1990).

Similarly, Ketsa et al. (1995) reported that on day-12 of holding, *Bacillus sp* was identified in vase water of *Dendrobium* 'Pompadour' flower which was present in all treatments except one containing 8-HQS + AgNO₃ (silver nitrate) + glucose. In contrast, both *Pseudomonas sp* and *Erwinia sp* were not detected in vase solution of any treatments containing AgNO₃ or 8-HQS alone and with glucose. However, few unidentified micro-organisms were also observed

showing different characteristics of colony types. It indicated that *Bacillus sp* may be one of the genera of bacteria that causes plugging in orchid flower-stems during holding in water.

Role of biocides / germicides

To protect cut flowers from bacterial plugging, a few effective germicides can be applied in vase solution. Additionally sanitary measures and changing vase water regularly may also be useful in reducing the population of microbes.

According to Ketsa and Kosonmethakul (2001), when a study was conducted in 3 varieties of *Dendrobium* orchid (Sonia Red Joe, Sonia Bom Joe and Walter Oumae Taba 4N), the holding solution containing 225 mg l⁻¹ 8-HQS + 50 mg l⁻¹ Al₂ (SO₄)₃ + 4 % glucose increased their vase-life and bud-opening the greatest and significantly, 23-34 days and 98-100 % respectively.

Although 8-HQS and Al₂ (SO₄)₃ both are the effective germicides, the combination of Al₂ (SO₄)₃ and 8-HQS was more effective in increasing bud-opening and vase-life than Al₂ (SO₄)₃ or 8-HQS was used alone with glucose. 8-HQS and Al₂ (SO₄)₃ may have specific antimicrobial properties that are specific for different organisms, that's why they can not be substituted for each other. This formulation should have also less harmful impact to humans and environment and may be less costly than one containing AgNO₃ (Ketsa and Kosonmethakul, 2001).

In fact, in the holding solution containing 8-HQS + AgNO₃ + glucose used for *Dendrobium* Pompadour cut flowers, various species of bacteria such as *Bacillus sp.*, *Pseudomonas sp.* and *Erwinia sp.* were absent (Ketsa et al., 1995). Where as, chemicals either 8-HQS or AgNO₃ with or without glucose could not suppress *Bacillus sp.* of vase solution (Ketsa et al., 1995). And only combination of both chemicals effectively maintained water uptake and prolonged vase-life of *Dendrobium* cut orchid. Thus, same as 8-HQS + AgNO₃, 8-HQS+ Al₂ (SO₄)₃ might exert their synergetic effect for orchid cut flowers.

In tuberose cv. Double, it was also found that the solution containing 500 ppm aluminium sulfate or calcium nitrate increased vase-life (10 days) and bud-opening (57-58 %) significantly (Bhaskar et al., 1999).

2.2.6 Respiration

Respiration is the physiological process by which stored organic materials (carbohydrates, proteins and fats) are broken down into simple end products with a release of energy as heat. In this process oxygen (O_2) is used and carbon dioxide (CO_2) is produced.

Due to respiration, there is continuous loss of stored food reserves in the horticultural commodities and thus it hastens their senescence. The rate of deterioration of harvested commodities is generally proportional to the respiration rate (Kader, 1992).

Horticultural commodities are classified according to their respiration rates, e.g. most of the cut flowers are considered as having very high (40-60 mg CO_2 /kg/hr) respiration rates (Kader, 1992). So, it is also important to reduce the respiration rates of cut-flowers for extending their vase-life by temperature management. Applied sugars can maintain respiration by providing respiratory substrate.

2.2.7 Ethylene

Ethylene (C_2H_4) is a natural volatile hormone which is produced by all tissues of higher plants and by some micro-organisms. It is active physiologically in trace amount (less than 0.1 ppm), and regulates many aspects of growth and development and senescence (Salisbury and Rose, 1992; Kader, 1992). It also plays a major role in the abscission of plant organs including flowers and flower buds.

Ethylene production

Ethylene (C_2H_4) is derived from amino acid, methionine and amino-acid-like compound 1-aminocyclopropane-1-carboxylic acid (ACC). Methionine is converted into S-adenosyl methionine (SAM) and SAM changes into ACC with the help of ACC synthase enzyme. In The final reaction ACC is converted into ethylene (C_2H_4) which is catalyzed by ethylene forming enzyme /EFE or ACC oxidase (Figure 2.1)

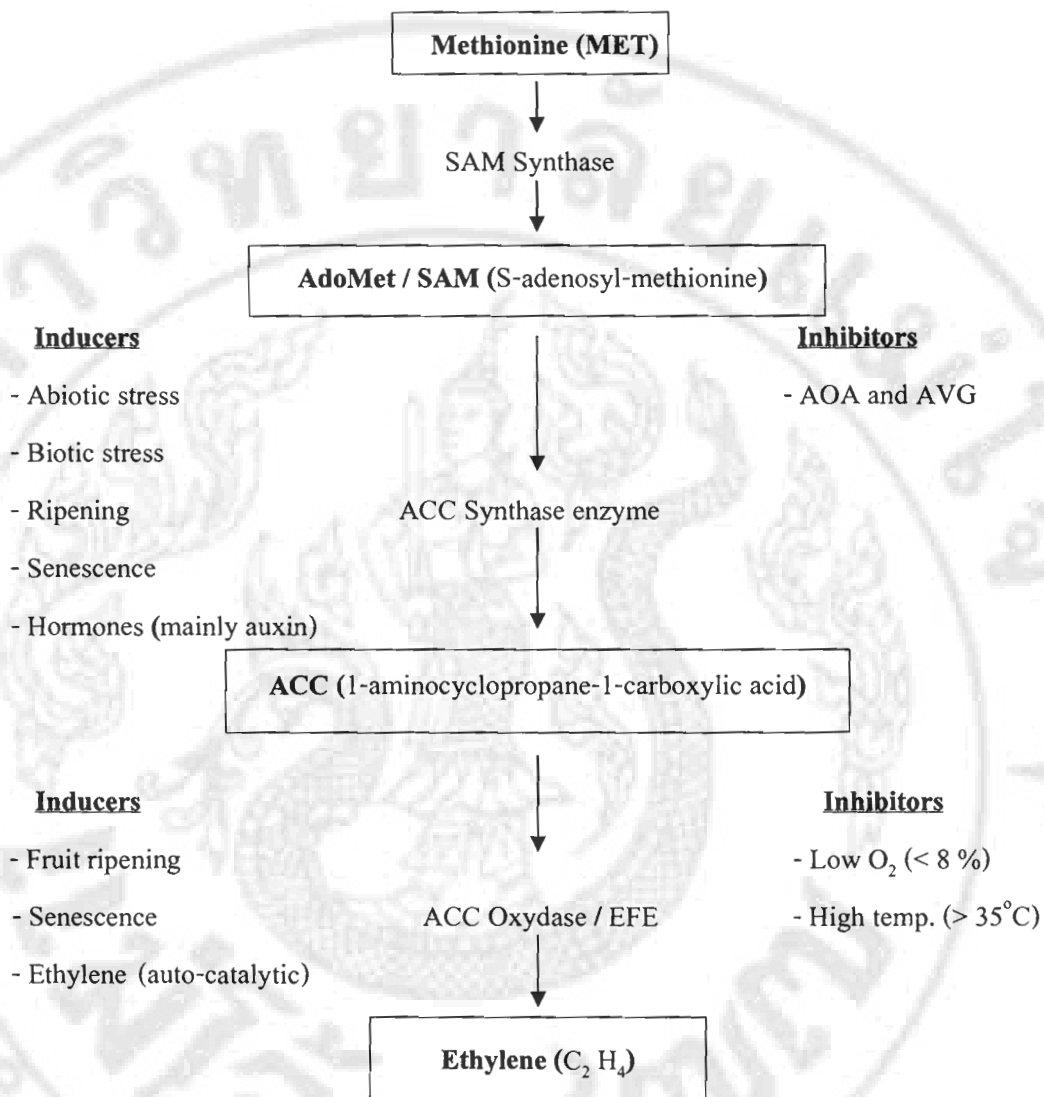


Fig. 2.1 Pathway of ethylene formation and its regulation in higher plants

(Yang and Hoffman, 1984, and Abeles et al., 1992 cited by Hew and Yong, 2004;
Salisbury and Rose, 1992; Kader, 1992)

Generally, ethylene (C₂H₄) production rates increase with maturity at harvest, physiological injuries, disease incidence, increased temperatures up to 30°C and water stress (Kader, 1992). It is generally accepted that ethylene production increases during flower senescence and ethylene can accelerate flower senescence (Reid, 1989 cited by Ketsa and Thampitakorn, 1995).

In *Vanda* Miss Joaquim and *Paphiopedilum* flowers, a high rate of ethylene production was detected when the flowers senesced (Goh et al., 1985 cited by Ketsa and Thampitakorn, 1995), while the flower buds of *Dendrobium* Jacquelyn Hawaii produced higher levels of ethylene than opened and senescent florets (Ketsa and Thampitakorn, 1995).

The rate of ethylene production of buds and young flowers of *Vanda* Christine increased with bud growth, reaching a high value in half open florets. This was followed by a gradual decline but it increased again when the flowers showed sign of senescence (Yip and Hew, 1988 cited by Ketsa and Thampitakorn, 1995).

Ethylene sensitivity

Not only the level of ethylene, but also the sensitivity of various flowers to ethylene can control their senescence and other effects. Several flowers are sensitive to ethylene, which are adversely affected by ethylene. Variation in sensitivity to ethylene has been reported among different flower species (Woltering and Van Doorn, 1988 cited by Porat et al., 1995), and in the same flower itself with age and following pollination (Porat et al., 1995).

In some *Dendrobium* species tested neither there was clear response to ethylene, nor effect of inhibitors of ethylene synthesis or ethylene action (Goh et al., 1985; cited by Rattanawisalanon et al., 2003; Ketsa and Thampitakorn, 1995), which suggested that their petal senescence is not regulated by ethylene. The ethylene sensitivity in *Dendrobium* may depend on the species or cultivars since the time to senescence in *Dendrobium* and *Phalaenopsis* was rather sensitive to exogenous ethylene (Woltering and Van Doorn, 1988 cited by Rattanawisalanon et al., 2003).

Chapter 3

Materials and Methods

3.1 Experiment – 1

Effects of different harvesting stages on vase-life and bud-opening of *Mokara Madame Panne* cut orchid

3.1.1 Plant material

Inflorescences/cut flowers of *Mokara Madame Panne* orchid were purchased at four harvesting /maturity stages (Figure 3.1) in Maejo Orchid Farm, Maejo, Chiang Mai. They were transported in dry condition and reached the laboratory within one hour of harvest. The four stages of maturity used as treatments were:

- 3-4 opened florets (harvesting stage-1 or HS-1)
- 5-6 opened florets (harvesting stage-2 or HS-2)
- 7-8 opened florets (harvesting stage-3 or HS-3)
- 9-10 opened florets (harvesting stage-4 or HS-4)

After proper selection, flower-stems (peduncles) of all selected inflorescences were recut under water keeping 13 cm length from the lowermost opened floret for uniformity. Individual inflorescences (total 10) were placed in 50 ml glass cylinders containing 40 ml 'reverse osmosis (RO)' water. There were 30°C ($\pm 2^\circ\text{C}$) mean temperature, 65 % ($\pm 5\%$) relative humidity and natural light (12 hr /day) during this experiment.

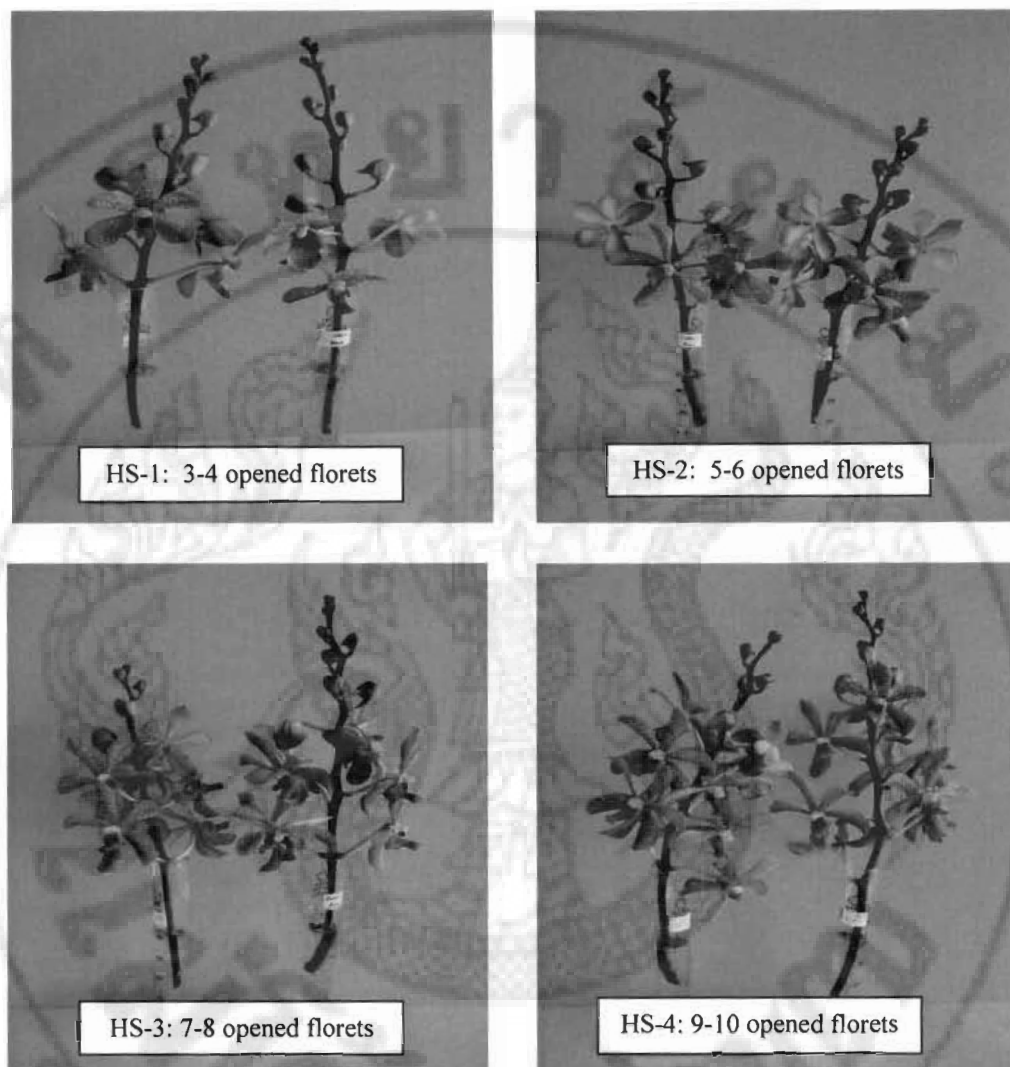


Figure 3.1 Four different harvesting stages of *Mokara* Madame Panne cut orchid, HS-1 to HS-4 used in experiment-1.

3.1.2 Measurement of respiration rate and ethylene production

To measure the respiration rate and ethylene production, 3 replications each containing 3 inflorescences of *Mokara* Madame Panne were prepared. Inflorescences of each replication were enclosed in 8 liter air-tight plastic container for 30 minutes. The experiment was conducted on days 0, 7 and 12 after harvest. For each day of sampling, the 1 ml of headspace-air was withdrawn by using plastic syringes to determine both CO_2 (carbon dioxide) and C_2H_4 (ethylene) gases released by the inflorescences. Similarly for standard sample, 1 ml gas samples of CO_2 and C_2H_4 both containing 1% were withdrawn from standard gas cylinder with the help of another

plastic syringes. The concentration of ethylene was assessed by using a gas chromatograph/GC (SHIMADZU GC-14 B, Japan) equipped with a flame ionization detector, FID (Figure 3.2 A). There was a 4 m length stainless steel column with 3 mm inner diameter, filled with 80/100 mesh activated alumina in that GC. The other conditions of GC were: carrier gas N₂ (Nitrogen), column oven temperature 60°C, injector temperature 90°C, detector temperature 100°C and flow rate of carrier gas 45 ml/min. The result was expressed as micro liter ethylene production per kg fresh weight per hour ($\mu\text{l C}_2\text{H}_4/\text{kg/hr}$).

In the same way, CO₂ concentration was determined by another gas chromatograph (SHIMADZU GC-14 B, Japan) equipped with thermal conductivity detector, TCD (Figure 3.2 B). The some conditions of GC used for CO₂ determination (column oven temperature, injector temperature, detector temperature and flow rate of carrier gas) were the same as mentioned for ethylene. Whereas there were 2 m length and 3 mm inner diameter column filled with 80/100 mesh porapak, carrier gas He (Helium), auxillary temperature for TCD 100°C and current for TCD 100 mA for CO₂ detection. The following formula was used for the calculation of respiration rates. In order to calculate the respiration rates, fresh weight (FW) of inflorescences, free-space/headspace of containers as well as temperature of lab room were recorded during the experiment. Respiration rate was expressed as milligram CO₂ per kg fresh weight per hour (mg CO₂/kg/hr).

The formula used for the calculation of respiration rates:

$$V_1 = \frac{P_2 V_2 \times T_1}{P_1 \times T_2}$$

Here,

V_1 = Calculated volume of CO₂ in liter

V_2 = Volume of CO₂ in liter released by applied commodity (in diff. treatments)

P_1 = Standard pressure of ideal gas (i.e. 760 mm Hg)

P_2 = Pressure of air in GC lab (i.e. 720 mm Hg)

T_1 = Temperature of ideal gas (i.e. 273°C)

T_2 = Temperature of GC room (i.e. 273 + 28°C = 301°C)

Before using this formula, the volume of CO₂ (V₂) produced by the inflorescences of *Mokara Madame Panne* was calculated. For this, firstly average area/value of headspace-air obtained from the gas chromatography in a specific time-line was converted into percentage (%) by divided it by the average area of standard sample. Then, to get the real % of CO₂ released by the inflorescences, the percentage of CO₂ in the air of GC lab (0.035 %) was subtracted from the total % of CO₂. At last, the percentage of CO₂ was converted into liter on the basis of volume of headspace available for each treatment and replication.

$$\text{Weight of CO}_2 = \frac{V_1 \times 44}{22.4}$$

Here,

44 = Molecular weight of CO₂ (in gm)

22.4 = Volume of 1 mole of CO₂ at 0°K (in Lit)

Value was in mg CO₂ produced by mass of applied commodity (after multiplied by 1000)

There after, the rate of respiration was calculated as mg CO₂ per kg FW per hr.

For the calculation of ethylene production, the same process as mentioned above for calculating the volume of CO₂ (V₂) was followed. After that, the value of ethylene production was converted into micro liter produced by one kg fresh weight of inflorescences per hour basis. There is no need to use any specific formula for ethylene production as for respiration rate.



Figure 3.2 Gas chromatographs (SHIMADZU GC-14 B, Japan) equipped with a flame ionization detector, FID (A) and with thermal conductivity detector, TCD (B) used for C_2H_4 and CO_2 detection, respectively.

3.1.3 Water uptake and water loss measurement

Each individual inflorescences of *Mokara Madame Panne* was put in 50 ml glass cylinder which was filled with 40 ml RO water. Paraffin oil at the rate of 0.5 ml was also added to prevent evaporation loss. The weight of cylinder + RO water + inflorescence before and after adding water was taken every day (after 24 hrs.), and the amount of added water to the original level (ml water) was recorded from each treatment and replication. Each harvesting stage (treatment) contained 10 replications and each replication had one inflorescence.

Water uptake, water loss and water balance were calculated as following:

Wt. loss of inflorescence and water (g/ inflo./day) = wt. of cylinder + water + inflorescence before refill (I)

Water uptake (ml/ inflo. /day) = amount of added water to the original level (II)

Wt. loss of inflorescence (g/ inflo./day) = wt. of cylinder + water + inflorescence after refill to the original level – wt. of previous day (III)

Wt. loss of water/water loss (g/ inflo. /day) = I – III (IV)

Water balance (g/ inflo. /day) = II – IV

3.1.4 Total sugar determination

Total sugar content in florets and flower-stems of *Mokara Madame Panne* cut orchid at different harvesting stages was determined by colorimetric method (Dubois et al., 1956) (Appendix A, B) considering 4 steps as follow:

Step 1 Sample preparation

Three replications of floret (including pedicel) and flower-stem (peduncle) samples were prepared separately from 3 inflorescences of *Mokara Madame Panne*. These samples were taken from each treatment on days 0, 5, 10 and 13 after harvest. For floret's sampling, 2 gm tissues were taken by chopping florets and buds from 3 selected inflorescences situated in the middle 8 cm length of peduncles. Similarly 1-2 gm samples of flower-stems were prepared from the same 8 cm middle parts. Each sample was heated in microwave oven (SAMSUNG TDS, M183GN, Japan) for 15 seconds. There after, 9 ml of 80 % ethanol was added and homogenized two times each of 30 seconds by homogenizer (KIKA WREKE ,T8.01, Germany). Then transferred to new plastic bottles with additional 9 ml of 80 % ethanol and stored at 5°C until the final sugar determination was carried out.

Step 2 Making standard curve of sugar

Firstly, 0.1 % stalk solution of reducing sugar (D-fructose) was prepared (Appendix C) by using RO water. In order to make standard curve 5, 10, 15 and 20 µl of sugar stalk solution with 3 replications were used. Each standard solution was prepared 100 µl by adding required amount of 80 % ethanol with pipette, and mixed well with vortex (VORTEX-GENIE 2, G-560E, USA). Then, 100 µl of 5 % phenol and 1 ml of 98 % H₂SO₄ (sulfuric acid) were added in each standard solution.

Before taking absorbance, standard solution was mixed well with vortex and left for 20 minutes for complete color development. Then, absorbance value of each standard solution was measured by spectrophotometer (HITACHI, U-1500, USA) (Fig. 3.3) setting at wave length of 490 nm. A standard curve of sugar (Appendix D) was drawn by using Excel soft ware.



Figure 3.3 Spectrophotometer (HITACHI, U-1500, USA) used for total sugar determination.

Step 3 Determination of total sugar content

At first, samples of florets and flower-stems were diluted 10 times with 80 % ethanol in different ratios to reduce the chances of error in sampling amount. The ratios of 2:98 to 8:92 for florets samples and 4:96 to 12:88 for the samples of flower-stems were maintained. For example, to get the 2:98 ratio of florets sample, 20 μ l clear solution of florets sample was combined with 980 μ l of 80 % ethanol with the help of pipette. Then 3 samples of 100 μ l were taken from diluted solution and put in test tubes. Further, 100 μ l of 5 % phenol and 1 ml of 98 % H_2SO_4 were added in all samples and mixed well with vortex (VORTEX-GENIE 2, G-560E, USA). After standing for 20 min, the absorbance values of these samples were measured by spectrophotometer (HITACHI, U-1500, USA) setting at wave length of 490 nm.

Step 4 Calculation for total sugar content

Finally, total sugar content within florets and flower-stems of 4 different harvesting stages were calculated by using the following formula obtained from the standard curve of D-fructose.

$$X = y - 0.0685 / 0.0244, \quad R^2 = 0.9266.$$

Here, x = amount of total sugar (µg), y = mean absorbance of sample

After this, the amount of total sugar was multiplied by the dilution factor which was different for florets and flower-stems and also for different days of determination. For example, on day-0 for floret samples, 2 gm floret tissues and 18 ml of 80 % ethanol were taken during sample preparation. It meant there was 2 gm in total volume 20 ml (18+2) which represented the dilution factor, 2/20 or 1/10. Similarly, 2 µl clear solution of florets was combined with 98 µl of 80 % ethanol that represented the dilution factor, 2/100 or 1/50. Thus, there was 1/500 (1/10 x 1/50) total dilution factor. Based on this total dilution factor, the value of total sugar (µg) obtained from formula was multiplied by 500. Lastly, the value of total sugar content was found as µg/g FW which was converted into mg/g FW by divided it by 1000.

3.1.5 Bud-opening

The number of buds and florets in each inflorescence of all 4 harvesting stages was recorded at the beginning of the experiment. Then, the number of opened buds in each inflorescence was recorded daily. At the end of the experiment, the percentage of additional bud-opening was calculated for all harvesting stages as following:

$$\text{Bud-opening (\%)} = \frac{\text{Additional number of buds opened at the end of vase-life}}{\text{The number of unopened buds on day-0}} \times 100$$

3.1.6 Determination of vase-life

To determine the vase-life, the symptoms of wilting and abscission (dropping) of florets and buds (Figure 3.4) were recorded in each inflorescence every day. The end of vase-life of each individual inflorescence was determined when 50 % of opened florets and/or buds wilted or abscised.

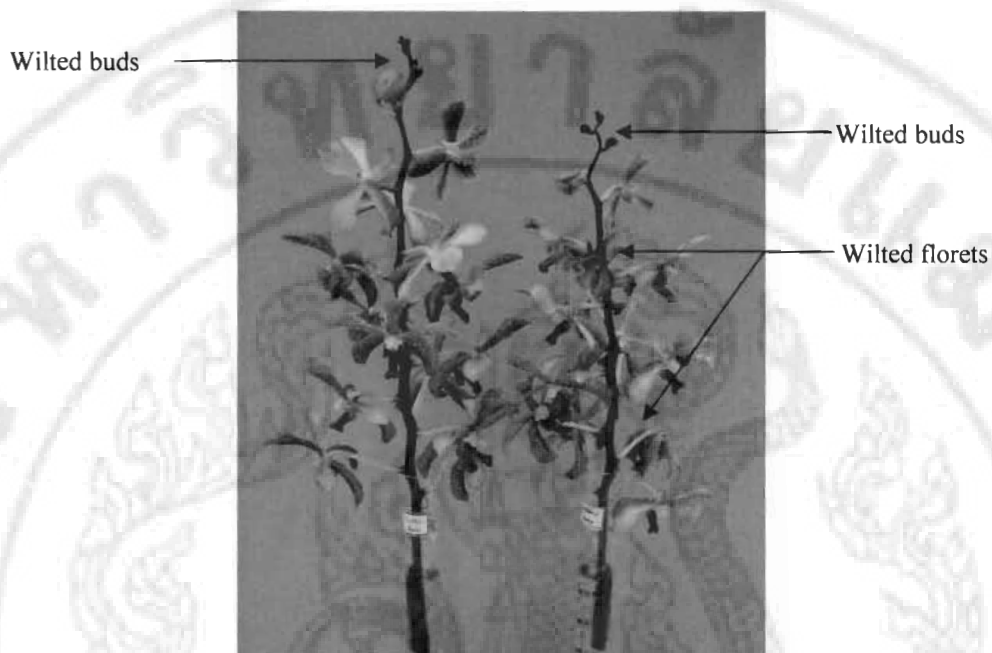


Figure 3.4 Symptoms of wilted florets and buds.

3.1.7 Experimental design and data analysis

Completely Randomized Design (CRD) was employed in this experiment. Four harvesting stages of *Mokara* Madame Panne cut orchid were used as treatments with 10 replications. Analysis of variance (ANOVA) was used as a tool to determine significant differences among treatments. And means were separated by Duncan's Multiple Range Test (DMRT) at Probability (p) = 0.05 level.

3.2 Experiment – 2

To identify the effects of various preservative solutions on vase-life and bud-opening of *Mokara* Madame Panne cut orchid, experiment-2 was further divided into two sections. In section-1 (preliminary part), different preservatives were evaluated individually while they were tested in various combinations in section-2 (main part).

3.2.1 Section-1 (preliminary part)

Plant material

Based on the result of the first experiment, it was decided to use only harvesting stage-3 bearing 7-8 opened florets (Figure 3.5) for section-1 of experiment-2. The same as in experiment-1 the required inflorescences / cut flowers were bought in Maejo Orchid Farm, Maejo, Chiang Mai. For uniformity, the length of flower-stems was 13 cm from the lowermost opened floret. Then, individual inflorescences were held in glass bottles containing 250 ml preservative solutions and 1 ml paraffin oil.



Figure 3.5 Harvesting stage-3 (7-8 opened florets) of *Mokara* Madame Panne cut orchid used in experiment-2.

Preservative solutions

According to need, preservative solutions (holding solutions with preservatives) were prepared by using RO water one day before onset of the experiment. The following preservatives and their concentrations were applied singly in holding solutions as treatments:

T_1 = 2 % sucrose

T_6 = 225 ppm 8-HQS (T_6)

T_2 = 4 % sucrose

T_7 = 50 ppm aluminium sulfate [$Al_2(SO_4)_3$]

T_3 = 150 ppm citric acid

T_8 = 100 ppm $Al_2(SO_4)_3$

T_4 = 300 ppm citric acid

T_9 = Control (only R O water)

T_5 = 150 ppm 8-hydroxyquinoline sulfate (8-HQS)

Bud-opening as well as experimental design and data analysis

The same as mentioned in experiment-1.

Determination of vase-life

The same process of vase-life related data collection as described in experiment-1 was also followed in this section. However, the vase-life of each individual inflorescence was considered ended when symptoms of wilting and abscission were noticed in 50 % of total florets and buds.

3.2.1 Section-2 (main part)

Plant material

The same as mentioned in section-1 of experiment-2. During this experiment the mean temperature was 28°C ($\pm 2^{\circ}\text{C}$) and the mean relative humidity was 66 % ($\pm 6\%$) with 12 hr /day natural light.

Preservative solutions

Five different combinations of preservative solutions were prepared based on the literature review and the result of the section-1 (Table 4.5) as followed:

$T_1 = 150 \text{ ppm } 8\text{-HQS} + 2\% \text{ sucrose or HQS+Sugar}$

$T_2 = 50 \text{ ppm } \text{Al}_2(\text{SO}_4)_3 + 2\% \text{ sucrose or AS+Sugar}$

$T_3 = 150 \text{ ppm } 8\text{-HQS} + 50 \text{ ppm } \text{Al}_2(\text{SO}_4)_3 \text{ or HQS+AS}$

$T_4 = 150 \text{ ppm } 8\text{-HQS} + 50 \text{ ppm } \text{Al}_2(\text{SO}_4)_3 + 2\% \text{ sucrose or HQS+AS+Sugar}$

$T_5 = \text{RO water as control}$

More over, the method of preservative solutions preparation was the same as in section-1. The initial pH values of these preservative solutions were recorded as following:

HQS + Sugar (pH-5.7)

AS + Sugar (pH-4.1)

HQS+ AS (pH-4.1)

HQS + AS + Sugar (pH-4.3)

RO water / control (pH-6.9)

Solution (water + preservatives) uptake and solution loss measurement

Each container was filled with 250 ml preservative solution. And paraffin oil was poured on the top of the preservative solution to prevent evaporation loss. Other all methods applied for solution uptake and solution loss were the same as described in experiment-1 under water uptake and water loss measurement.

Total sugar determination

Same as in experiment-1, the total sugar content within florets and flower-stems of *Mokara Madame Panne* cut orchid held in different preservative solutions was also obtained in experiment-2 from four steps.

Step 1 Sample preparation

Most of the methods were the same as in experiment-1. However, samples were prepared from 5 treatments for 6 times at 3-day intervals (on days 0, 3, 6, 9, 12 and 15) in this experiment. For each sampling of floret, florets and buds of 3 selected inflorescences situated in the middle 10 cm part leaving 16 cm from cut end were taken.

Step 2 Making standard curve

The same process as described in experiment-1. The standard curve drawn is shown in Appendix E.

Step 3 Determination of total sugar content

The same method as mentioned in experiment-1

Step 4 Calculation for total sugar content

The same as described in experiment-1. Difference only in formula and R^2 obtained from standard curve of D-fructose.

$$X = y - 0.0587 / 0.0128, \quad R^2 = 0.8756$$

Here, x = amount of total sugar (μg), y = mean absorbance of sample.

Change in floret color

Different values (L^* , a^* and b^*) of color change were evaluated in the florets of *Mokara Madame Panne* cut orchid held in five different preservative solutions on days 0, 3, 6, 9, 12 and 15. For this purpose, 4th floret from the base of peduncle was selected. And data were collected only from dorsal sepal / tepal with the help of colorimeter (MINOLTA Co. Ltd.) (Figure 3.6).



Figure 3.6 Colorimeter (MINOLTA) applied for measuring color change in the floret of *Mokara* Madame Panne cut orchid.

Bud-opening as well as experimental design and data analysis

The same as mentioned in experiment-1.

Determination of vase-life

The same process as mentioned in section-1 of experiment-2.

Chapter 4

Results

4.1 Experiment – 1

4.1.1 Respiration rate and ethylene production

The highest respiration rate (385 mg CO₂/kg/hr) was recorded from the inflorescences *Mokara* Madame Panne at harvesting stage-1 on day-0, though all harvesting stages showed the high respiration rates (Figure 4.1 A and Table 4.1). The respiration rates decreased greatly during the experiment and no significant differences were detected among the harvesting stages (Figure 4.1 A and Table 4.1).

Inflorescences of harvesting stage-1 (more buds stage) also produced more ethylene than those of the other stages on day-0 (Figure 4.1 B and Table 4.1). On day-7, only harvesting stage-1 and harvesting stage-2 of *Mokara* Madame Panne cut orchid / inflorescences had high ethylene production rates. However there was no significant different in ethylene production among the harvesting stages after day-7 (Figure 4.1 B and Table 4.1). Data of respiration and ethylene production for harvesting stage-3 missed on day-0 due to experimental error.

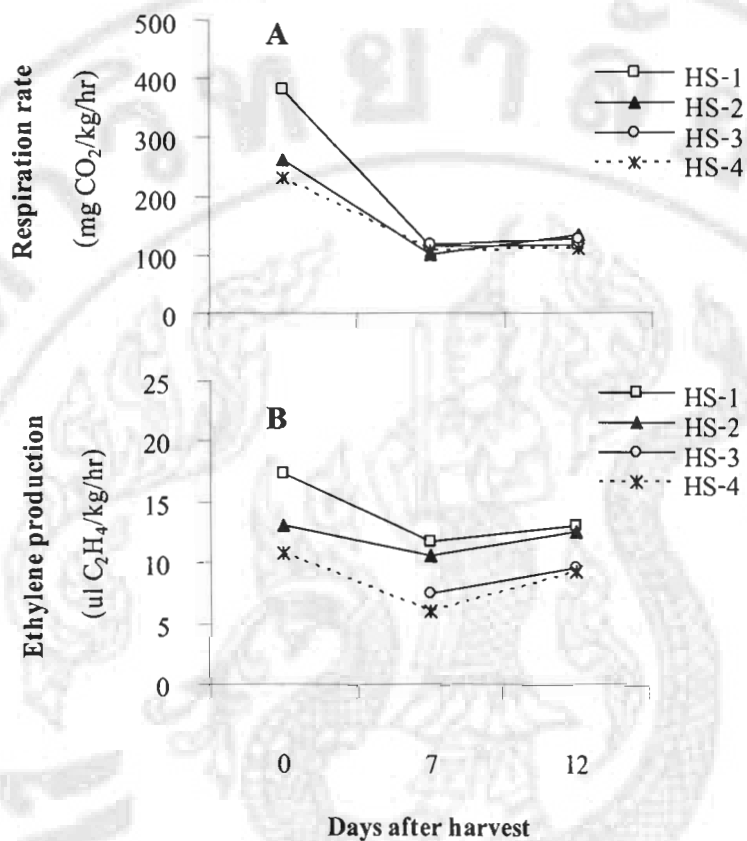


Figure 4.1 Rate of respiration (A) and ethylene production (B) in different harvesting stages, 3-4 opened florets (HS-1), 5-6 opened florets (HS-2), 7-8 opened florets (HS-3) and 9-10 opened florets (HS-4) of *Mokara Madame Panne* cut orchid held in reverse osmosis water.

Table 4.1 Rate of respiration and ethylene production in different harvesting stages, 3-4 opened florets (HS-1), 5-6 opened florets (HS-2), 7-8 opened florets (HS-3) and 9-10 opened florets (HS-4) of *Mokara Madame Panne* cut orchid held in reverse osmosis water.

Treatments	Day-0	Day-7	Day-12
Respiration rate (mg CO ₂ /kg/hr)			
HS-1	385.0 a	115.6	118.8
HS-2	264.7 b	102.7	137.3
HS-3	-	119.5	130.9
HS-4	233.7 b	109.7	111.8
F-test	**	NS	NS
Ethylene production (μl C ₂ H ₄ /kg/hr)			
HS-1	17.40 a	11.81 a	13.24
HS-2	13.20 b	10.71 a	12.68
HS-3	-	7.64 b	9.65
HS-4	10.80 b	6.15 b	9.34
F-test	**	**	NS

** = highly significantly different at $p < 0.01$ and NS = not significantly different ($p > 0.05$)
Means within a column followed by different letter were significantly different at $p = 0.05$ by DMRT.

4.1.2 Water uptake and water loss

The maximum water uptake (4.5-5.2 ml/inflorescence/day) and water loss (2.14-3.34 g/inflorescence/day) were found from the inflorescences of all harvesting stages of *Mokara Madame Panne* on day-1 (Figure 4.2 A, B and Table 4.2). Then, both water uptake and water loss decreased sharply on day-2. After that, there was found continuous decrease with slight fluctuations; however the water loss was still higher in harvesting stage-1 initially (Figure 4.2 A, B and Table 4.2). Similarly on the first day, inflorescences of all harvesting stages had higher water balance (1.8-2.4 g/inflorescence/day) (Figure 4.2 C and Table 4.2). Whereas the other days, water balance decreased gradually and not significantly different among the harvesting stages in most of the holding period (Figure 4.2 C and Table 4.2).

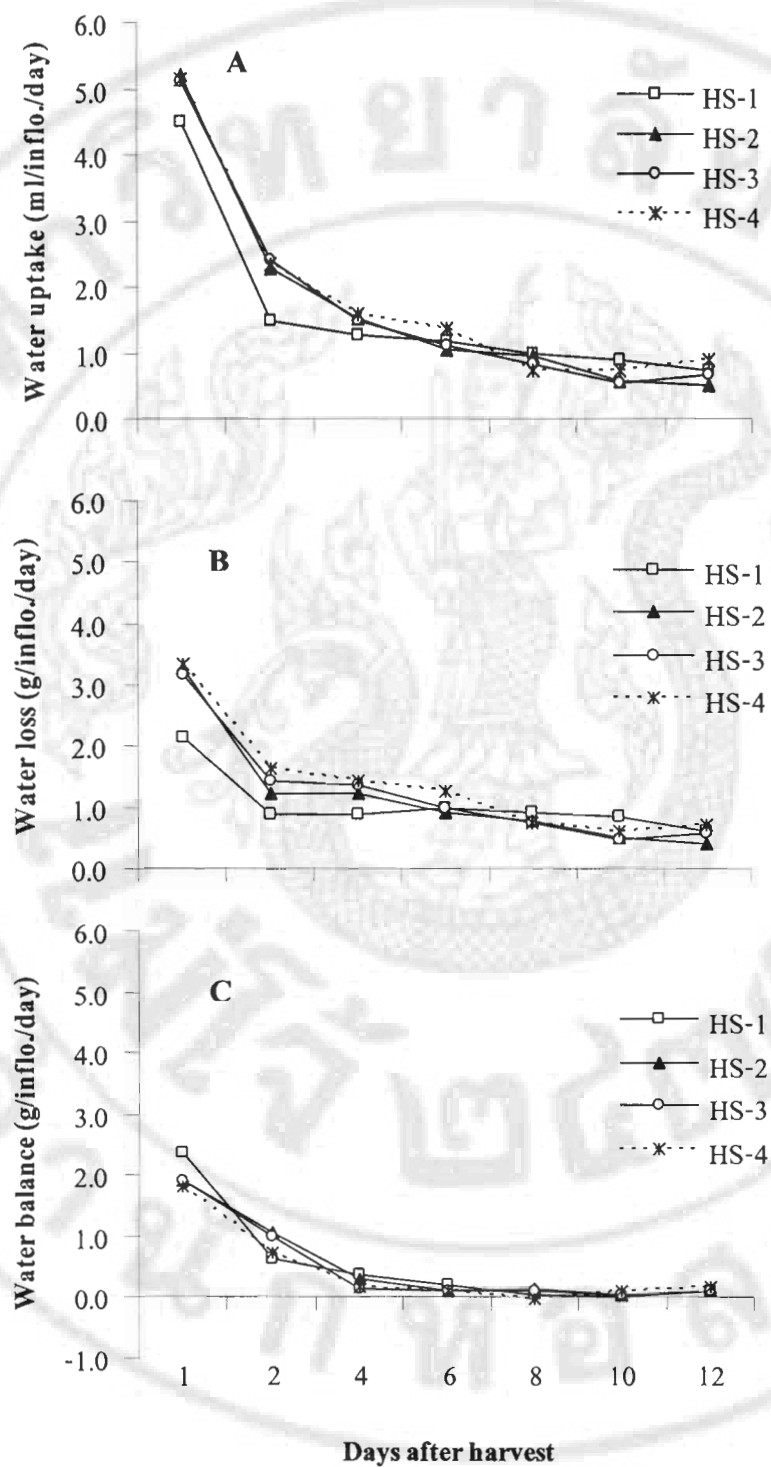


Figure 4.2 Water uptake (A), water loss (B) and water balance (C) in different harvesting stages, 3-4 opened florets (HS-1), 5-6 opened florets (HS-2), 7-8 opened florets (HS-3) and 9-10 opened florets (HS-4) of *Mokara Madame Panne* cut orchid held in reverse osmosis water.

Table 4.2 Water uptake, water loss and water balance in different harvesting stages, 3-4 opened florets (HS-1), 5-6 opened florets (HS-2), 7-8 opened florets (HS-3) and 9-10 opened florets (HS-4) of *Mokara Madame Panne* cut orchid held in reverse osmosis water.

Treatments	Days after harvest						
	1	2	4	6	8	10	12
Water uptake (ml/inflo./day)							
HS-1	4.50	1.50 b	1.28	1.18	0.98	0.88 a	0.72 ab
HS-2	5.20	2.30 a	1.52	1.04	0.94	0.56 b	0.50 b
HS-3	5.10	2.40 a	1.50	1.10	0.84	0.54 b	0.68 ab
HS-4	5.14	2.36 a	1.60	1.36	0.72	0.74 ab	0.90 a
F-test	NS	**	NS	NS	NS	*	*
Water loss (g/inflo./day)							
HS-1	2.14 b	0.88 c	0.90 b	0.98	0.92	0.86 a	0.62 ab
HS-2	3.28 a	1.24 b	1.22 ab	0.92	0.80	0.50 b	0.40 b
HS-3	3.18 a	1.42 ab	1.36 a	1.00	0.74	0.48 b	0.58 ab
HS-4	3.34 a	1.62 a	1.42 a	1.26	0.74	0.63 ab	0.73 a
F-test	**	**	*	NS	NS	*	*
Water balance (g/inflo./day)							
HS-1	2.36	0.62 c	0.38	0.20 a	0.06	0.02	0.10 b
HS-2	1.92	1.06 a	0.30	0.12 b	0.14	0.06	0.10 b
HS-3	1.92	0.98 ab	0.14	0.10 b	0.10	0.06	0.10 b
HS-4	1.80	0.74 bc	0.18	0.10 b	-0.02	0.11	0.17 a
F-test	NS	*	NS	*	NS	NS	*

NS = not significantly different ($p > 0.05$), * = significantly different at $p \leq 0.05$ and

** = highly significantly different at $p < 0.01$

Means within a column followed by different letter were significantly different at $p = 0.05$ by DMRT.

4.1.3 Total sugar content

The total sugar content in the florets of harvesting stage-1 of *Mokara Madame Panne* cut orchid on day-0 was much higher (1.35 mg/g FW) than those of the other harvesting stages (Figure 4.3 A and Table 4.3). On day-5, it decreased greatly (0.8 mg/g FW). Over all, the florets of harvesting stage-1 and harvesting stage-2 had higher levels of total sugar than those of the other harvesting stages in most of the period of vase-life (Figure 4.3 A and Table 4.3).

At the same time, the total sugar content in the flower-stems of harvesting stage-1 was significantly different from those of the other harvesting stages over the study period (Figure 4.3 B and Table 4.3). The differences in the total sugar content among the stages decreased according to time; however it was still higher in harvesting stage-1 (Figure 4.3 B and Table 4.3).

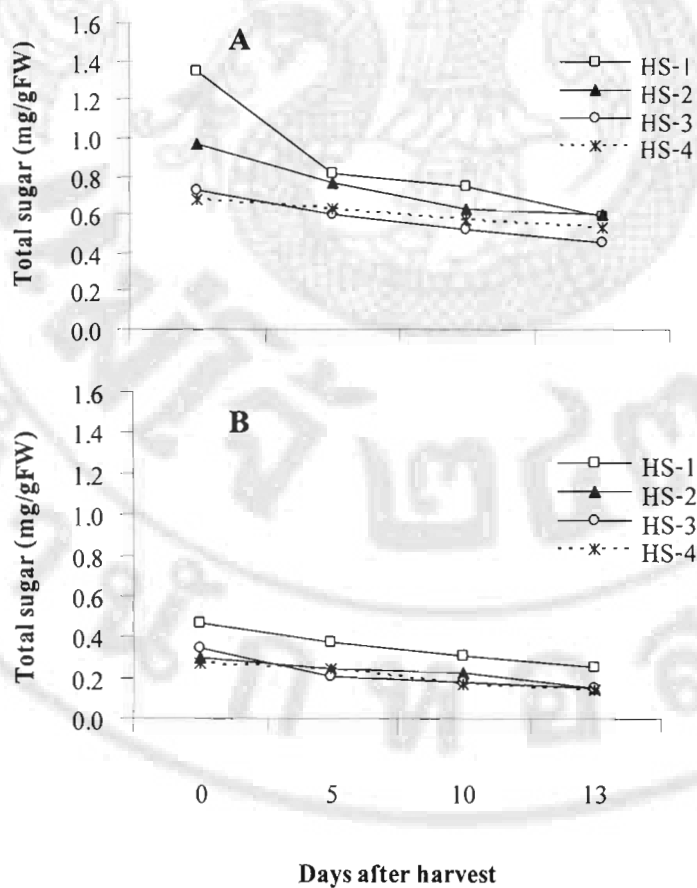


Figure 4.3 Total sugar content in florets (A) and flower-stems (B) of different harvesting stages, 3-4 opened florets (HS-1), 5-6 opened florets (HS-2), 7-8 opened florets (HS-3) and 9-10 opened florets (HS-4) of *Mokara Madame Panne* cut orchid held in reverse osmosis water.

Table 4.3 Total sugar content in florets and flower-stems of different harvesting stages, 3-4 opened florets (HS-1), 5-6 opened florets (HS-2), 7-8 opened florets (HS-3) and 9-10 opened florets (HS-4) of *Mokara* Madame Panne cut orchid held in reverse osmosis water.

Treatments	Days after harvest			
	0	5	10	13
Total sugar in florets (mg/g FW)				
HS-1	1.35 a	0.81 a	0.75 a	0.59 a
HS-2	0.97 b	0.77 a	0.63 b	0.60 a
HS-3	0.73 c	0.60 b	0.52 c	0.46 c
HS-4	0.68 c	0.63 b	0.57 bc	0.53 b
F-test	**	*	**	**
Total sugar in flower-stems (mg/g FW)				
HS-1	0.47 a	0.37 a	0.31 a	0.25 a
HS-2	0.30 b	0.24 b	0.22 ab	0.15 b
HS-3	0.35 b	0.21 b	0.18 b	0.15 b
HS-4	0.27 b	0.24 b	0.17 b	0.14 b
F-test	**	**	**	*

** = highly significantly different at $p < 0.01$ and * = significantly different at $p \leq 0.05$

Means within a column followed by different letter were significantly different at $p = 0.05$ by DMRT.

4.1.4 Bud-opening and vase-life

There were not found significant differences in the bud-opening (actually additional bud-opening) and vase-life among 4 harvesting stages of *Mokara* Madame Panne cut orchid (Table 4.4). However, harvesting stage-3 showed a little higher bud-opening and vase-life both than the other harvesting stages (Table 4.4).

Table 4.4 Bud-opening and vase-life of different harvesting stages, 3-4 opened florets (HS-1), 5-6 opened florets (HS-2), 7-8 opened florets (HS-3) and 9-10 opened florets (HS-4) of *Mokara* Madame Panne cut orchid held in reverse osmosis water.

Treatments	Bud-opening (on day-12)	Vase-life
	(%)	(days)
HS-1	48.8	11.8
HS-2	57.2	11.6
HS-3	59.5	13.4
HS-4	55.6	11.2
F-test	NS	NS

NS = not significant different ($p > 0.05$)

4.2 Experiment – 2

4.2.1 Section-1 (preliminary part)

Bud-opening and vase-life

No preservative solutions tested in this section had significant effect on bud-opening of *Mokara* Madame Panne cut orchid (Table 4.5). However, 150 ppm HQS, 50 ppm $\text{Al}_2(\text{SO}_4)_3$ and 4 % sucrose showed high bud-opening in comparison to control.

Similarly, none of the preservative solutions significantly extended the vase-life of *Mokara* Madame Panne cut orchid compare to the control (Table 4.5).

Table 4.5 Bud-opening and vase-life of *Mokara* Madame Panne cut orchid held in different individual preservative solutions.

Treatments	Bud-opening (on day-10) (%)	Vase-life (Days)
2 % sucrose	70.6	11.1 bc
4 % sucrose	73.5	9.7 c
150 ppm citric acid	63.5	11.1 bc
300 ppm citric acid	66.6	10.6 bc
150 ppm 8-HQS	77.8	13.6 a
225 ppm 8-HQS	71.0	12.0 ab
50 ppm $\text{Al}_2(\text{SO}_4)_3$	76.8	13.3 a
100 ppm $\text{Al}_2(\text{SO}_4)_3$	68.0	13.2 a
Control	65.0	12.7 a
F-test	NS	*

NS = not significant different ($p > 0.05$) and * = significantly different at $p \leq 0.05$

Means within a column followed by different letter were significantly different at $p = 0.05$ by DMRT.

4.2.2 Section-2 (main part)

Solution (water + preservatives) uptake and solution loss

Inflorescences of *Mokara* Madame Panne held in all 5 preservative solutions had very high amount of solution (water + preservatives) uptake (3.6-6.4 ml/inflorescence/day) on day-1 compared to the other days. However, the inflorescences with AS+Sugar had the lowest amount of solution uptake among the preservative solutions (Figure 4.4 A and Table 4.6). On days 4, 6 and 8, the preservative solution containing HQS+AS supported significantly higher amount of solution uptake than the control. In the most of the remaining days, inflorescences held in HQS+AS had also high amount of solution uptake, though not significantly different from the control (Figure 4.4 A and Table 4.6).

The amount of solution (water + preservatives) loss in the inflorescences of *Mokara* Madame Panne held in HQS+AS had significantly higher than those of the control on days 6, 8, 12, 16 and 18 (Figure 4.4 B and Table 4.7). Same as the solution uptake, the maximum solution balance (3.9-4.3 g/inflo./day) was occurred in the all inflorescences on day-1 except in the inflorescences held in AS+Sugar. However, the solution balance among the treatments was not significantly different during the most of the holding period (Figure 4.4 C and Table 4.8).

Additionally, there was observed unique drooping symptoms different from normal wilting of florets (Figure 4.5) in the inflorescences held in sugar (2 %) containing preservative solutions.

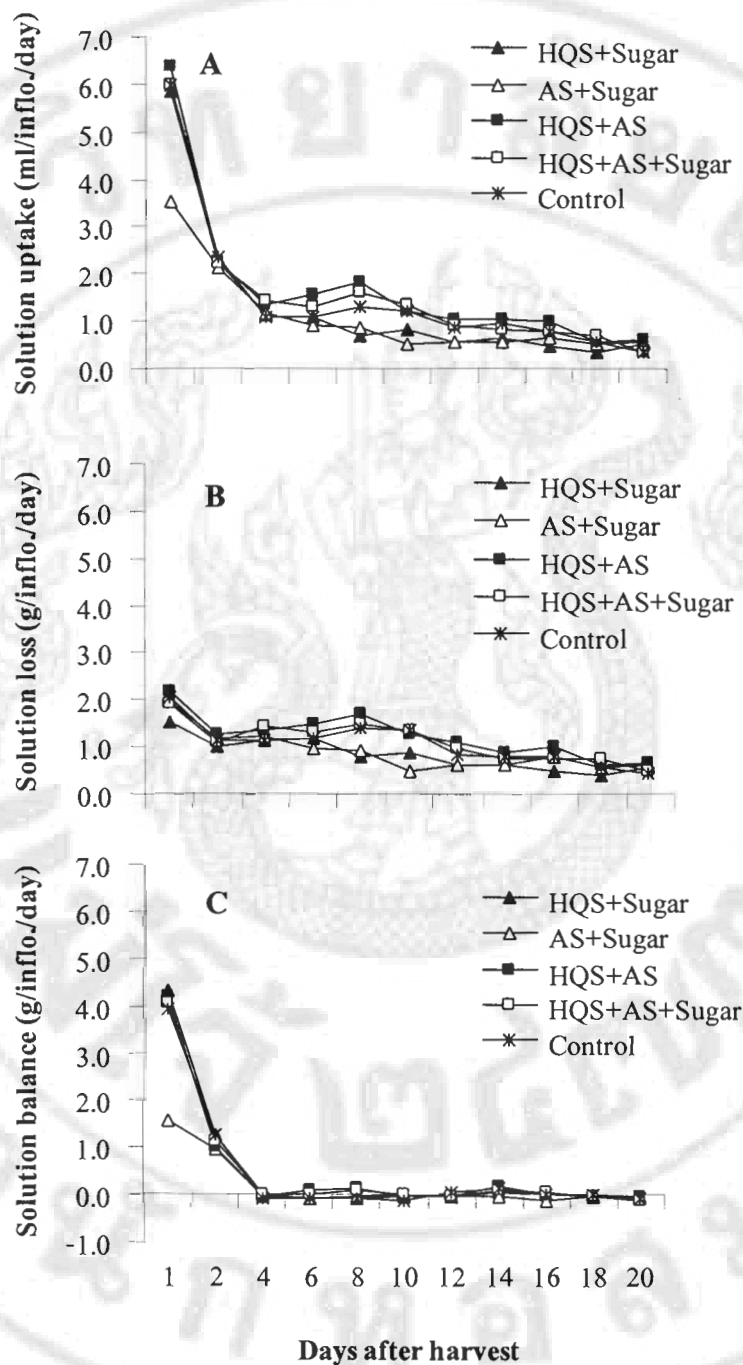


Figure 4.4 Solution (water + preservatives) uptake (A), solution loss (B) and solution balance (C) in the inflorescences of *Mokara Madame Panne* orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

Table 4.6 Solution (water + preservatives) uptake in the inflorescences of *Mokara* Madame Panne orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and RO water (control).

Treatments	Solution uptake (ml/inflo./day)										
	Days after harvest										
	1	2	4	6	8	10	12	14	16	18	20
HQS+Sugar	5.88 a	2.27	1.12 b	1.09 bc	0.68 c	0.84 b	0.58 b	0.67 c	0.46 c	0.36 b	0.51 ab
AS+Sugar	3.55 b	2.14	1.19 ab	0.90 c	0.86 c	0.51 c	0.57 b	0.59 c	0.64 bc	0.54 a	0.57 a
HQS+AS	6.40 a	2.26	1.34 ab	1.58 a	1.85 a	1.24 a	1.06 a	1.03 a	1.00 a	0.59 a	0.62 a
HQS+AS+Sugar	6.00 a	2.25	1.46 a	1.32 b	1.60 ab	1.36 a	0.91 a	0.84 ab	0.80 ab	0.70 a	0.36 b
Control	5.98 a	2.38	1.08 b	1.10 bc	1.33 b	1.24 a	0.89 a	0.95 a	0.78 ab	0.58 a	0.36 b
F-test	**	NS	*	**	**	**	**	**	**	**	**

** = highly significantly different at $p < 0.01$, * = significantly different at $p \leq 0.05$ and NS = not significantly different ($p > 0.05$)

Means within a column followed by the different letter were significantly different at $p = 0.05$ by DMRT.

Table 4.7 Solution (water + preservatives) loss in the inflorescences of *Mokara* Madame Panne orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and RO water (control).

Treatments	Solution loss (g/inflo./day)											
	Days after harvest											
	1	2	4	6	8	10	12	14	16	18	20	
HQS+Sugar	1.54 b	1.00 b	1.12 b	1.16 b	0.77 c	0.87 b	0.60 c	0.62 b	0.46 c	0.40 b	0.57 ab	
AS+Sugar	1.97 a	1.16 ab	1.12 b	1.09 b	0.91 c	0.50 c	0.60 c	0.62 b	0.77 b	0.58 a	0.62 ab	
HQS+AS	2.18 a	1.25 a	1.37 ab	1.48 a	1.70 a	1.27 a	1.10 a	0.86 a	0.99 a	0.63 a	0.65 a	
HQS+AS+Sugar	1.92 a	1.11 ab	1.44 a	1.32 ab	1.49 ab	1.36 a	0.95 ab	0.74 ab	0.76 b	0.74 a	0.48 ab	
Control	2.04 a	1.12 ab	1.15 ab	1.18 b	1.41 b	1.35 a	0.84 b	0.80 a	0.78 b	0.59 a	0.44 b	
F-test	**	*	*	**	**	**	**	**	**	**	*	

** = highly significantly different at $p < 0.01$ and * = significantly different at $p \leq 0.05$

Means within a column followed by the different letter were significantly different at $p = 0.05$ level by DMRT.

Table 4.8 Solution (water + preservatives) balance in the inflorescences of *Mokara Madame Panne* orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and RO water (control).

Treatments	Solution balance (g/inflo./day)											
	Days after harvest											
	1	2	4	6	8	10	12	14	16	18	20	
HQS+Sugar	4.34 a	1.27	0.00	-0.07 b	-0.09 c	-0.03 bc	-0.02	0.05 ab	0.00 a	-0.04	-0.06	
AS+Sugar	1.58 b	0.98	-0.02	-0.07 b	-0.05bc	0.01 a	-0.03	-0.03 b	-0.13 b	-0.04	-0.05	
HQS+AS	4.22 a	1.01	-0.03	0.10 a	0.15 a	-0.03 bc	-0.04	0.17 a	0.01 a	-0.04	-0.03	
HQS+AS+Sugar	4.08 a	1.14	0.02	0.00 ab	0.11 ab	0.00 a	-0.04	0.10 ab	0.04 a	-0.04	-0.12	
Control	3.94 a	1.26	-0.07	-0.08 b	-0.08 c	-0.11 c	0.05	0.15 a	0.00 a	-0.01	-0.08	
F-test	**	NS	NS	*	*	*	NS	**	**	NS	NS	

** = highly significantly different at $p < 0.01$, * = significantly different at $p \leq 0.05$ and NS = not significantly different ($p > 0.05$)
Means within a column followed by the different letter were significantly different at $p = 0.05$ level by DMRT.



Figure 4.5 Symptom of drooping florets held in sugar containing preservative solutions.

Total sugar content

Initially, the total sugar content increases both in the florets and flower-stems of *Mokara Madame Panne* cut orchid held in the preservative solutions contained sucrose after harvest (Figure 4.6 A, B and Table 4.9). From day 6, when the total sugar content decreased in florets, the content increased in flower-stems and vice versa in the treatments containing sucrose (Figure 4.6 A, B and Table 4.9). On the other hand, the treatments without sucrose showed gradual decrease of the total sugar both in florets and flower-stems over the study period (Figure 4.6 A, B and Table 4.9).

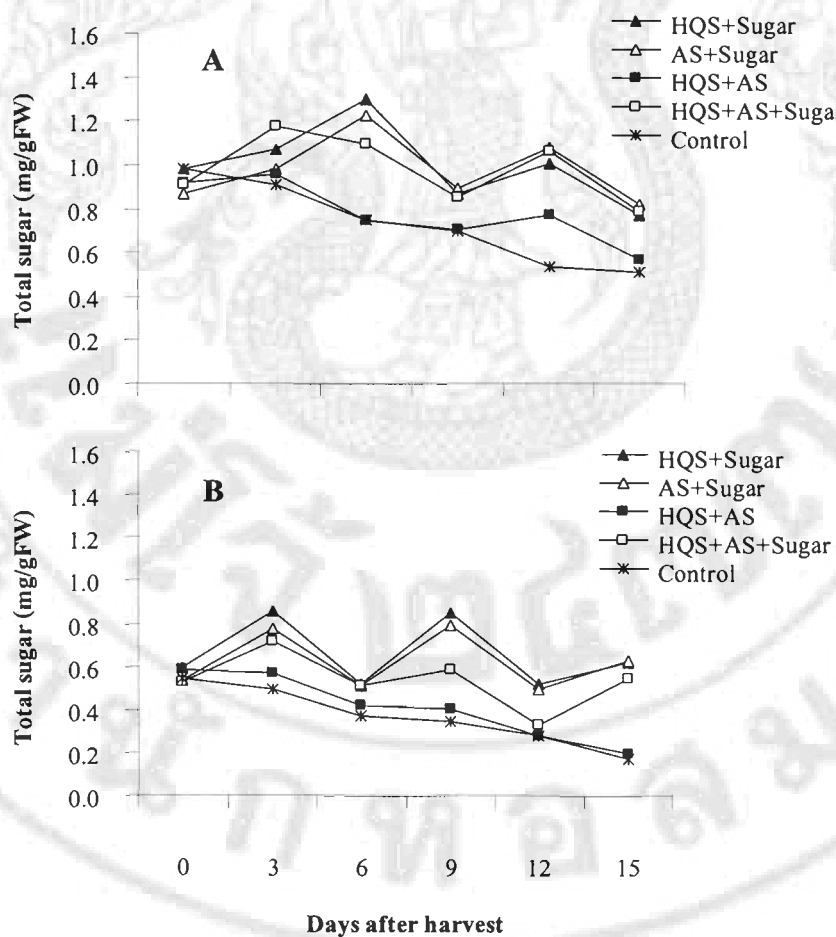


Figure 4.6 Total sugar content in florets (A) and flower-stems (B) of *Mokara Madame Panne* cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

Table 4.9 Total sugar content in florets and flower-stems of *Mokara Madame Panne* cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

Treatments	Days after harvest					
	0	3	6	9	12	15
Total sugar in florets (mg/g FW)						
HQS+Sugar	0.98	1.07 ab	1.30 a	0.87	1.01 a	0.77 a
AS+Sugar	0.87	0.98 bc	1.23 a	0.89	1.08 a	0.82 a
HQS+AS	0.92	0.96 bc	0.75 b	0.71	0.77 b	0.56 b
HQS+AS+Sugar	0.91	1.18 a	1.10 a	0.85	1.06 a	0.79 a
Control	0.98	0.91 c	0.74 b	0.70	0.54 b	0.51 b
F-test	NS	**	**	NS	*	**
Total sugar in flower-stems (mg/g FW)						
HQS+Sugar	0.60	0.86 a	0.52	0.85 a	0.52 a	0.62 a
AS+Sugar	0.54	0.78 a	0.51	0.80 a	0.50 a	0.63 a
HQS+AS	0.59	0.57 b	0.42	0.41 c	0.28 b	0.20 b
HQS+AS+Sugar	0.54	0.72 a	0.51	0.59 b	0.33 b	0.55 a
Control	0.56	0.50 b	0.37	0.35 c	0.28 b	0.17 b
F-test	NS	**	NS	**	**	**

NS = not significantly different ($p > 0.05$), ** = highly significantly different at $p < 0.01$ and * = significantly different at $p \leq 0.05$

Means within a column followed by the different letter were significantly different at $p = 0.05$ level by DMRT.

Change in floret color

There was not significant change in the floret (petals + sepals) color of *Mokara* Madame Panne cut orchid held in various preservative solutions during the experiment (Figure 4.7, 4.8, 4.9 and Table 4.10, 4.11, 4.12). Only on day-15, the a^* value of floret containing preservative solution HQS+AS was significantly higher than that of the floret containing only RO water (control) (Figure 4.8, and Table 4.11).

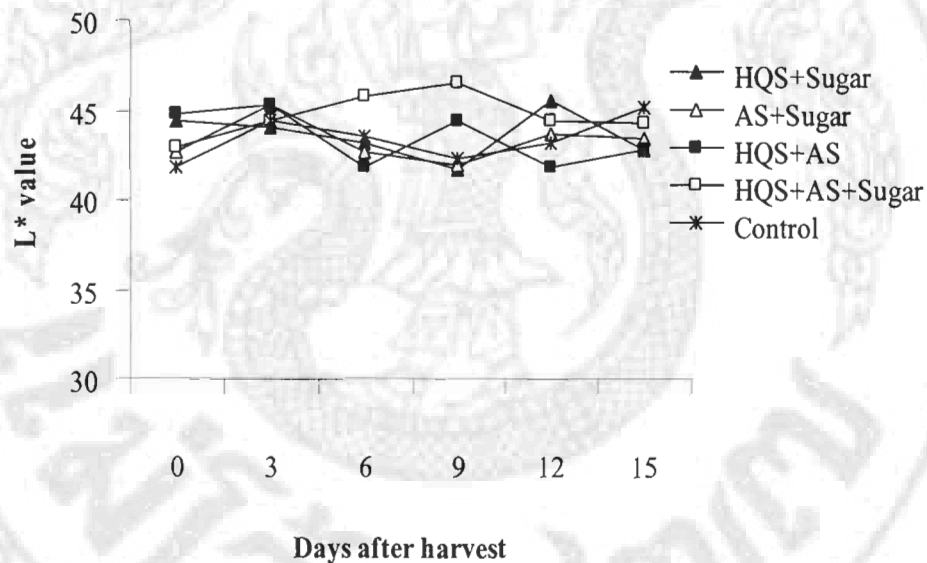


Figure 4.7 L^* value of the floret of *Mokara* Madame Panne cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

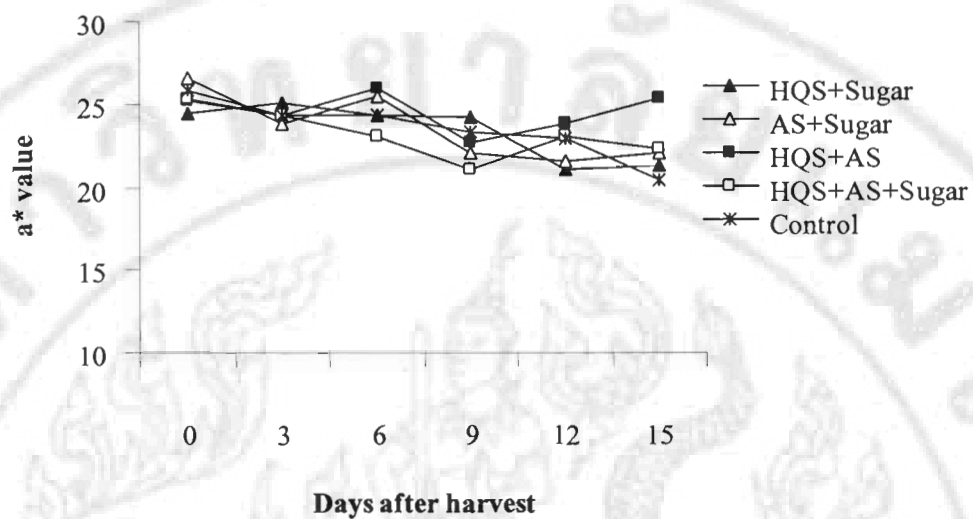


Figure 4.8 a^* value of the floret of *Mokara Madame Panne* cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

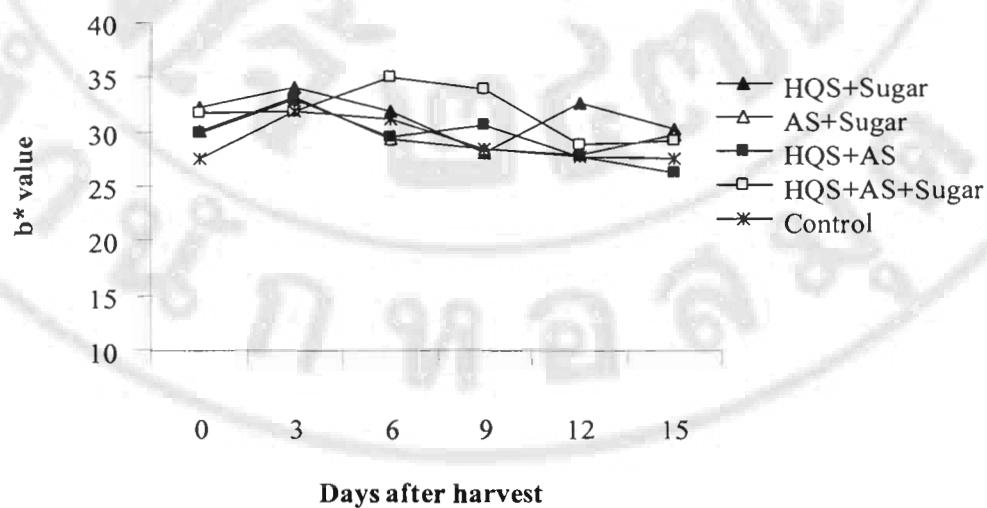


Figure 4.9 b^* value of the floret of *Mokara Madame Panne* cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

Table 4.10 L* value of the floret of *Mokara* Madame Panne cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

Treatments	L* value of floret					
	Days after harvest					
	0	3	6	9	12	15
HQS+Sugar	44.40	44.03	43.27	41.70	45.60	42.90
AS+Sugar	42.67	45.30	42.67	42.00	43.70	43.47
HQS+AS	44.83	45.27	41.90	44.50	41.90	42.87
HQS+AS+Sugar	43.00	44.40	45.83	46.50	44.47	44.27
Control	41.83	44.50	43.53	42.40	43.23	45.20
F-test	NS	NS	NS	NS	NS	NS

Table 4.11 a* value of the floret of *Mokara* Madame Panne cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

Treatments	a* value of floret					
	Days after harvest					
	0	3	6	9	12	15
HQS+Sugar	+24.47	+25.13	+24.40	+24.27	+21.03	+21.37 ab
AS+Sugar	+26.60	+23.80	+25.53	+22.07	+21.53	+22.07 ab
HQS+AS	+25.17	+24.30	+25.93	+22.67	+23.83	+25.30 a
HQS+AS+Sugar	+25.33	+24.37	+23.07	+21.03	+23.07	+22.33 ab
Control	+25.90	+24.37	+24.30	+23.37	+23.00	+20.43 b
F-test	NS	NS	NS	NS	NS	*

NS = not significantly different ($p > 0.05$) and * = significantly different at $p \leq 0.05$

Means within a column followed by the different letter were significantly different at $p = 0.05$ level by DMRT.

Table 4.12 b* value of the floret of *Mokara Madame Panne* cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

Treatments	b* value of floret					
	Days after harvest					
	0	3	6	9	12	15
HQS+Sugar	+32.27	+34.13	+32.03	+28.17	+32.77	+30.30
AS+Sugar	+30.17	+33.20	+29.47	+28.43	+27.97	+29.67
HQS+AS	+29.90	+33.13	+29.60	+30.70	+27.73	+26.33
HQS+AS+Sugar	+31.73	+32.00	+34.97	+34.03	+28.80	+29.17
Control	+27.60	+32.00	+31.20	+28.43	+27.73	+27.60
F-test	NS	NS	NS	NS	NS	NS

NS = not significantly different ($p > 0.05$)

Bud-opening and vase-life

None of the preservative solutions (HQS+Sugar, AS+Sugar, HQS+AS and HQS+AS+Sugar) significantly increased the bud-opening of *Mokara Madame Panne* cut orchid compared to the control treatment (Table 4.13 and Figure 4.10).

However, these preservative solutions had an effect on the vase-life of *Mokara Madame Panne* cut orchid. The preservative solution containing HQS+AS could extend the vase-life up to 7 days, (26 days) that was significantly higher than that of the control (19 days)(Table 4.13 and Figure 4.11).

Table 4.13 Bud-opening and vase-life of *Mokara* Madame Panne cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

Treatments	Bud-opening (on day-18)	Vase-life
	(%)	(days)
HQS+Sugar	66.5	18.7 b
AS+Sugar	64.4	18.3 b
HQS+AS	70.2	25.7 a
HQS+AS+Sugar	68.4	22.5 ab
Control	70.0	19.3 b
F-test	NS	*

NS = not significantly different ($p > 0.05$) and * = significantly different at $p \leq 0.05$

Means within a column followed by the different letter were significantly different at $p = 0.05$ level by DMRT.

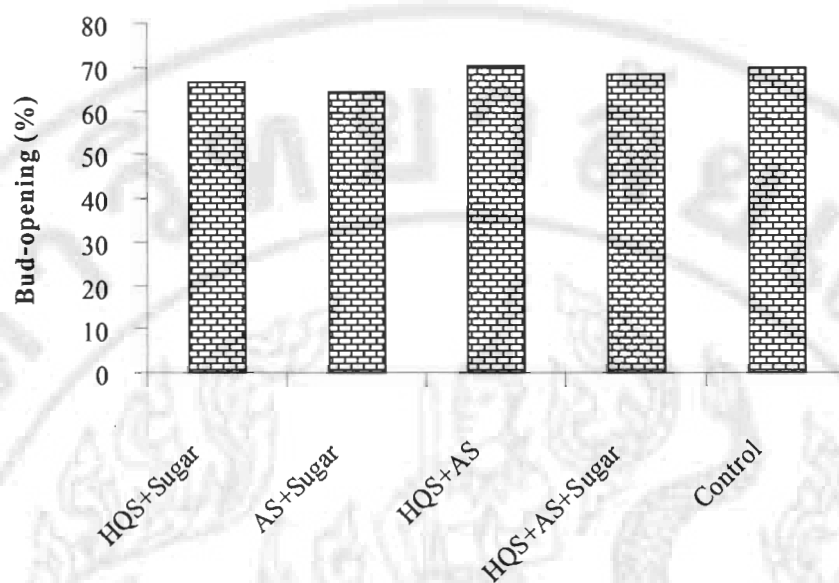


Figure 4.10 Bud-opening of *Mokara Madame Panne* cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

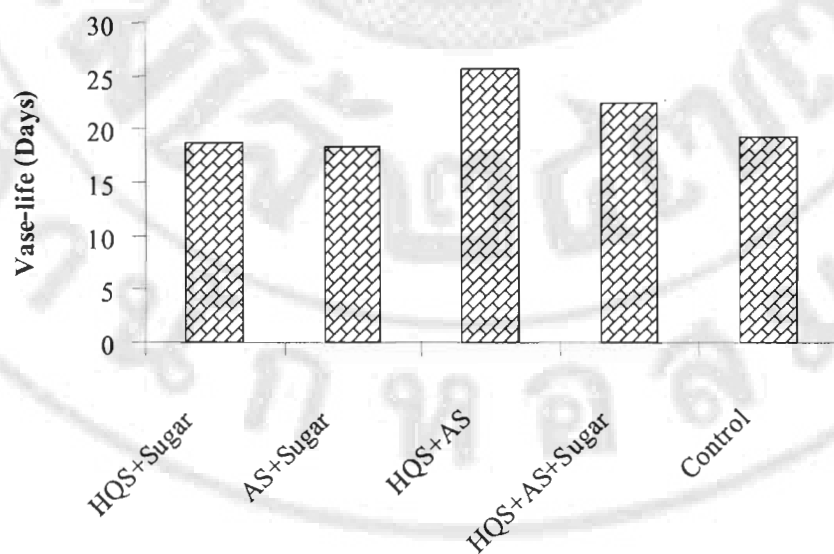


Figure 4.11 Vase-life of *Mokara Madame Panne* cut orchid held in different preservative solutions, HQS+Sugar, AS+Sugar, HQS+AS, HQS+AS+Sugar and control.

Chapter 5

Discussion

5.1 Experiment – 1

5.1.1 Respiration rate and ethylene production

Inflorescences of *Mokara* Madame Panne harvested at harvesting stage-1 (HS-1) showed the highest respiration rate only on day-0. It might be due to more number of buds as the respiration rates of *Dendrobium* inflorescences bearing only buds were higher than those of the inflorescences bearing only opened florets (Ketsa et al., 2001). It indicated a more active metabolism and sink activity of buds than opened florets. As day advanced, the respiration rates decreased in the inflorescences of all harvesting stages, possibly due to decreasing number of buds and also decreasing amount of stored food reserves. In fact, there was decreasing trend of total sugar both in florets and flower-stems of *Mokara* Madame Panne cut orchid (Figure 4.3 and Table 4.3).

Ethylene was also produced more by the younger harvesting stages (HS-1 and HS-2) than the other harvesting stages during the study period. This result is supported by the finding of Ketsa and Thampitakorn (1995) as mentioned in *Dendrobium* orchid.

5.1.2 Water uptake and water loss

All 4 harvesting stages of *Mokara* Madame Panne cut orchid exhibited much higher water uptake, on day-1 of the experiment which was similar to the results obtained for *Dendrobium* Pompadour (Ketsa et al., 2001) and tuberose cv. Double (Bhaskar et al., 1999). It is possible because inflorescences might need more water to overcome water stress caused by separating from mother plants. In contrast, the levels of water uptake and water loss decreased greatly on day-2, probably due to already maintained rehydration by the inflorescences of *Mokara* Madame Panne on day-1.

Over all, there were decreasing trends of water uptake, water loss and water balance in the inflorescences of all harvesting stages during the study period. It was associated with the increasing senescence and decreasing vase-life.

5.1.3 Total sugar content

Although total sugar content in the florets of harvesting stage-1 of *Mokara Madame Panne* cut orchid was higher than that of the other stages on day-0, it decreased sharply on day-5. The similar result was reported in the buds of *Aranda* orchid (Hew et al., 1989 cited by Hew and Yong, 2004). It was possible that a large amount of sugars might be utilized for maintaining high respiration rate and for bud-opening during the initial period. At latter on day-10 and day-13, the levels of total sugar within the florets of all harvesting stages decreased slowly, because the rates of respiration also decreased during that period due to increased senescence.

Similarly, the total sugar content in flower-stems of all harvesting stages decreased according to increased time of holding. It means sugars stored in flower-stems might be utilized by buds and florets during respiration for their survival as suggested in diploid and tetraploid *Dendrobium* (Ketsa et al., 2001).

5.1.4 Bud-opening and Vase-life

There were no significant differences in the bud-opening and vase-life among the harvesting stages of *Mokara Madame Panne* cut orchid. This result seems logical because all harvesting stages succeeded to maintain the various postharvest physiological changes during the holding period. Thus, in this study, only 3-4 opened florets (26 %) stage of *Mokara Madame Panne* inflorescences could be successfully utilized as cut flower. In case of *Dendrobium* orchid, inflorescences with 9-10 open florets (minimum 37.5 %) were found appropriate in bud-opening (Ketsa and Wongs-Aree, 1995).

However, while considering aesthetic value (attractive in looking, Figure 3.1), the mature harvesting stages with 50 % or more opened florets might be more appropriate than younger harvesting stages with < 50 % opened florets to be supplied for local markets. In fact, there is practice to be harvested and sold inflorescences of *Mokara Madame Panne* orchid bearing 50 % or more opened florets (personal communication with growers and traders).

5.2 Experiment – 2

5.2.1 Section-1

Bud-opening and Vase-life

No any preservative solutions could improve the bud-opening and extend the vase-life of *Mokara* Madame Panne cut orchid compared to the control. It indicated ineffectiveness of various preservatives tested here when applied singly, as reported in different cultivars of *Dendrobium* (Ketsa and Kosonmethakul, 2001).

5.2.2 Section-2

Solution (water + preservatives) uptake and solution loss

The combination of HQS+AS (initial pH 4.1, Page 30) significantly improved the solution uptake in the inflorescences of *Mokara* Madame Pane during initial period of holding. It might be due to acidifying solution as reported in cut tuberose (Halevy and Mayak, 1981 cited by Bhaskar et al., 1999).

The solution loss was also significantly higher in the inflorescences held in HQS+AS than that of the inflorescences placed in control in most of the study period. Since water loss by normal transpiration is also essential for continuous water uptake and for extending the vase-life of cut flowers (Bhaskar et al., 1999), preservative solution containing HQS+AS was found useful for maintaining water uptake and for extending the vase-life of *Mokara* Madame Pane cut orchid in this experiment.

On the other hand, there was observed symptom of drooping florets (Figure 4.5) in the inflorescences held in sugar (2 %) containing preservative solutions that was different from normal wilting symptom. It indicated that 2 % sucrose in holding solution was high for *Mokara* Madame Panne cut orchid which might not support for sufficient solution uptake (due to negative solute potential in holding solution) and drooping florets were observed. In fact, there was lower solution uptake in the inflorescences held in sugar containing preservative solutions (combined with single germicide) than in those inflorescences held in RO water (control) in most of the days of holding after one week of harvest (Figure 4.4 A and Table 4.6).

Total sugar content

The total sugar content in florets and flower-stems of *Mokara* Madame Panne cut orchid was higher when held in the preservative solutions containing sugar than those held in control or non-sugar containing solution. It was the similar result as reported in cut 'Sonia' rose flowers (Ichimura et al., 2003). There was always high content of total sugar in florets compared to the flower-stems especially in sugar containing treatments. It indicated that florets of *Mokara* Madame Panne cut orchid can uptake more sugar than flower-stems from holding solution. It might be possible because florets can provide more area for transpiration and normal transpiration supports water or solution uptake as mentioned in cut tuberose (Bhaskar et al., 1999).

The total sugar from florets and flower-stems of *Mokara* Madame Panne cut orchid was lost alternatively after 6 day of harvest in the solution containing sugar. It meant loss of total sugar from florets and flower-stems was not in the same rate and trend.

The preservative solution with sucrose could not extend bud-opening and vase-life of *Mokara* Madame Panne cut orchid compared to the control in this experiment. It suggested that the available content of total sugar within florets and flower-stems of *Mokara* Madame Panne cut orchid might be sufficient to maintain the physiological changes after harvest as in cut *Narcissus tazetta* var. *chinensis* (Ichimura and Goto, 2002).

Change in floret color

No significant change of color was detected from the florets of *Mokara* Madame Panne cut orchid held in either sucrose containing solutions or not. It meant exogenous sucrose and the other preservatives, 8-HQS and $\text{Al}_2(\text{SO}_4)_3$ had no effect on the floret color of *Mokara* Madame Panne cut orchid after harvest.

The preservative solutions containing 8-HQS showed little blackening on the lower most parts of flower-stems after two weeks of holding. It indicated the toxic nature of 8-HQS. This might be one reason that all effective holding solutions applying for wet-pack of orchid cut flowers are made of 8-HQS, AgNO_3 and sugars (Ketsa and Boonrote, 1990 cited by Ketsa and Kosonmethakul, 2001).

Bud-opening and Vase-life

There was no significant difference in the bud-opening of *Mokara* Madame Panne cut orchid among the preservative solutions including control. It indicated ineffectiveness of different preservatives applied here to improve the bud-opening of *Mokara* Madame Panne cut orchid. In contrast, preservative solution containing HQS+AS+glucose greatly increased the bud-opening of *Dendrobium* orchid (Ketsa and Kosonmethakul, 2001). It could be due to the different genetic make up of the two different orchid flowers.

The preservative solution containing HQS+AS provided maximum vase life (26 days) to *Mokara* Madame Panne cut orchid. It happened possibly because of the acidification of the preservative solution (initial pH 4.1) by the inclusion of $Al_2(SO_4)_3$, same as in cut tuberose (Bhaskar et al., 1999). It also might be due to suppression of micro-organism by the synergetic effect of these two preservatives as reported in *Dendrobium* orchid (Ketsa and Kosonmethakul, 2001). The combination of these preservatives enhanced the water uptake in the inflorescences of *Mokara* Madame Panne orchid. However, preservative solution containing $Al_2(SO_4)_3$, AS+Sugar could not extend the vase-life. This finding supported the combined effect of two germicides, 8-HQS and $Al_2(SO_4)_3$, as mentioned in *Dendrobium* orchid (Ketsa and Kosonmethakul, 2001).

The results showed that there was no further benefit from additional sugar in the preservative solution. It might be because of the sufficient availability of sugars in *Mokara* Madame Panne cut orchid to maintain their vase-life and other postharvest qualities as mentioned in other cut flowers. Sucrose pulsing (with 5 and 10% for 20 hr) could not extend the vase-life of cut *Narcissus tazetta* var. *chinensis* (Ichimora and Goto, 2002) and 2-3 % sucrose also did not provide any useful effects in cut tuberose (Jowkar and Salehi, 2005), probably due to high levels of sugars already presence in various floral organs and increase in microbes in holding solution, respectively.

In this experiment, the vase-life of *Mokara* Madame Panne cut orchid held in RO water (control) was 19 days. This finding agrees with Yam and Thame (1999) who reported that most commercial cultivars of *Mokara* last for at least two weeks after harvest.

Chapter 6

Conclusion and Recommendations

6.1 Conclusion

Four harvesting stages did not affect the vase-life and bud-opening of *Mokara* Madame Panne cut orchid. Thus, all harvesting stages of *Mokara* Madame Panne cut orchid tested here had potential to be used as cut flowers. However, mature harvesting stages (with 50 % or more opened florets) might be better than younger harvesting stages (with < 50 % opened florets) for local markets due to their attractiveness in looking from beginning.

The preservative solution containing 150 ppm 8-HQS + 50 ppm $\text{Al}_2(\text{SO}_4)_3$ provided the longest vase-life (26 days) for *Mokara* Madame Panne cut orchid. The combination of two preservatives, 8-HQS and $\text{Al}_2(\text{SO}_4)_3$ extended 7 days vase-life compared to the control. And there is no necessary to include sucrose or glucose in holding solution at least for this cultivar of *Mokara* orchid.

6.2 Recommendations

The following recommendations are suggested on the basis of results and experiences of conducted experiments:

1. It will be important to apply consumer acceptability test in order to prove whether mature harvesting stages (with 50 % or more opened florets) of *Mokara* Madame Panne cut orchid is suitable or not for local markets in further research.
2. It is needed to confirm whether younger harvesting stages (with < 50 % opened florets) of *Mokara* Madame Panne cut orchid are good or not for distant markets and export in future study.
3. More number of maturity stages of *Mokara* Madame Panne cut orchid can also be evaluated to know their effects on postharvest life and qualities.
4. Effects of different harvesting stages of other *Mokara* hybrids/cultivars on their vase-life and bud-opening should be studied.
5. Sucrose at the lower rates (0.5 and 1 %) can be tested to find out whether it is effective or not for *Mokara* cut orchid.
6. Storability and simulation of shipping for *Mokara* cut orchid should be considered in future study.
7. Various species of bacteria in the vase solutions and flower-stems of *Mokara* cut orchid should be identified which are responsible for stem plugging.
8. It will be more informative if the content of different sugars such as sucrose, glucose and fructose is determined in various floral parts to know their roles in postharvest physiology of *Mokara* cut orchid.

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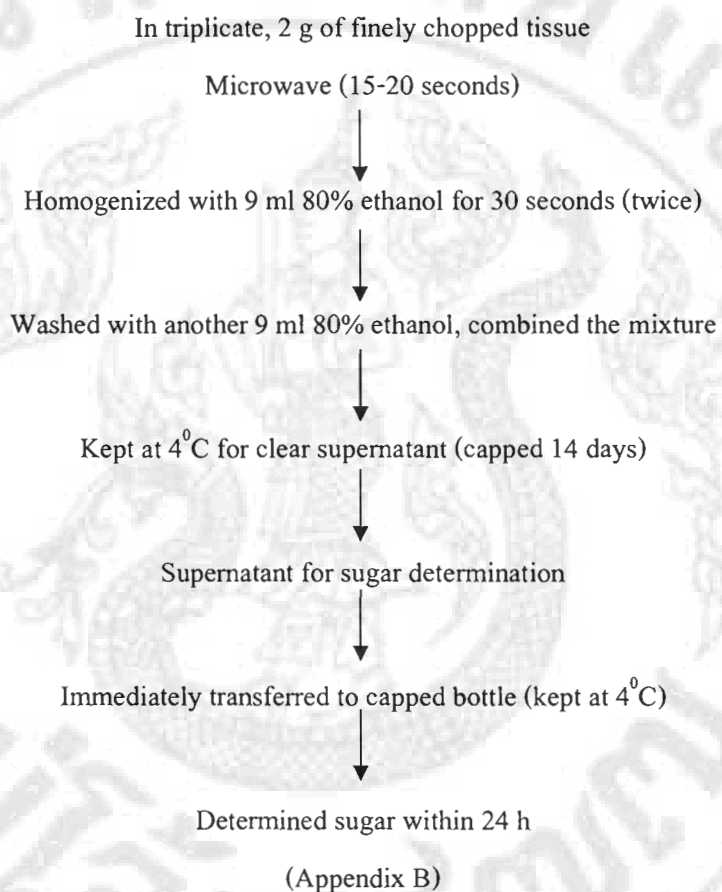
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Appendices

Appendix A. Protocol used to extract sugar from fresh tissues (Dubois et al., 1956)



Appendix B. Determination of total sugar by colorimetric method (Dubois et al., 1956)

Sample of tissue, volume = 100 μ l

(Example, 25 μ l tissue extract + 75 μ l 80% ethanol, mix well)



Add 100 μ l 5% phenol, and 1 ml of H_2SO_4

(Mixed well by vortex)



Stand 20 min at room temperature, read at A_{490} nm

(Use formula obtained from 'standard curve' using glucose, unit = mg sugar gFW^{-1})

Appendix C. Protocol used to prepare stock solution of reducing sugar (D-fructose)

Pure sugar (fructose) = 1mg/ml (0.1 %)

(1mg sugar + 1 ml distilled water)

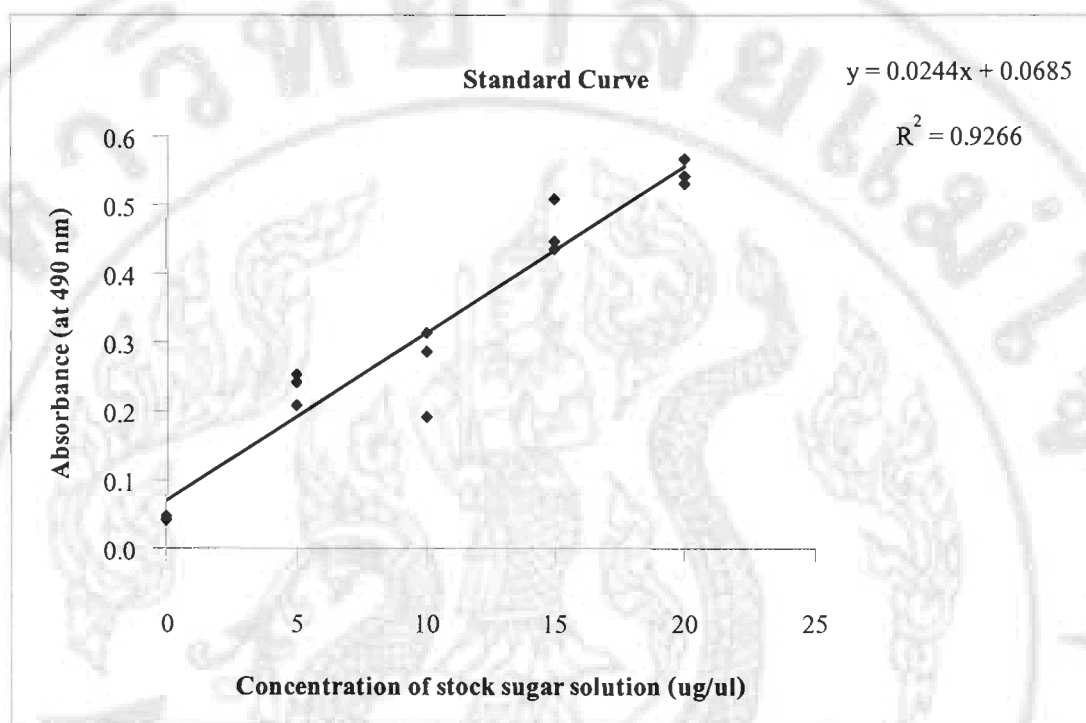
Or

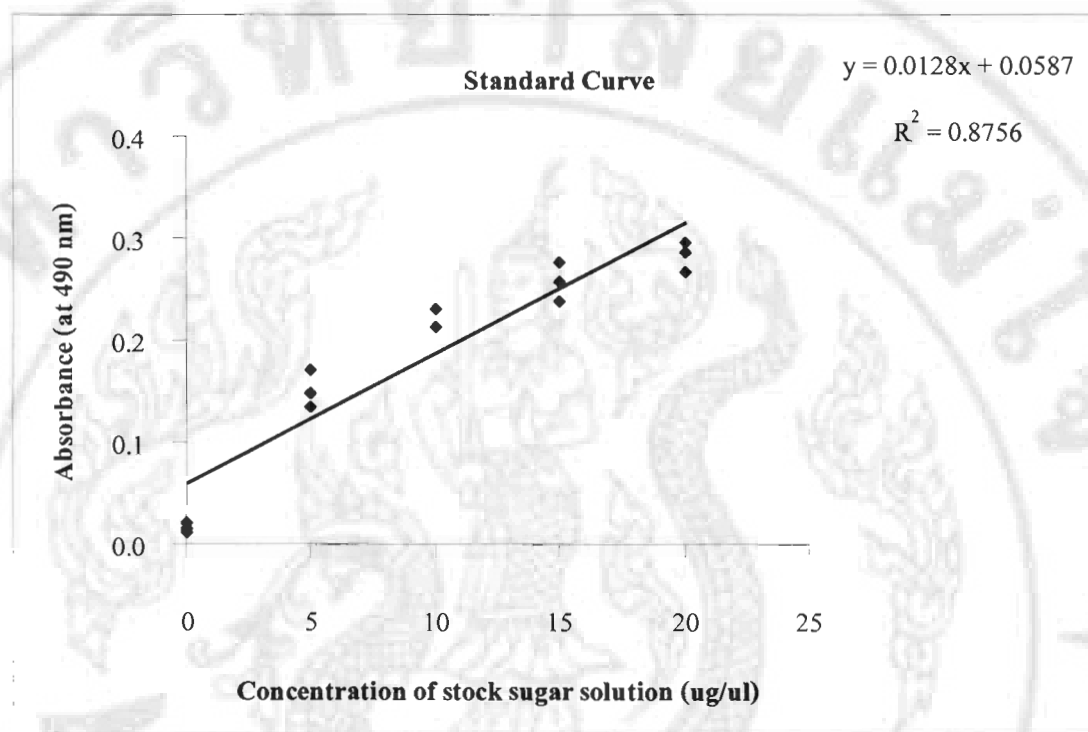
50 mg sugar + 50 ml distilled water mix well

Make samples of 1 ml

(Capped plastic bottles)

Store in refrigerator

Appendix D. Standard curve for total sugar determination during experiment-1

Appendix E. Standard curve for total sugar determination during experiment-2

Appendix F

Full research paper presented (oral presentation) in 7th National Horticultural Congress
held on 27-29 May, 2008 at Naresuan University; Phitsanulok (Thailand)

Postharvest Physiological Changes in Different Maturity Stages of 'Mokara Madame Panne' Cut Orchid

Khagendra Prasad Sharma¹, Theeranuch Jaroenkit², Sakesan Usahatanonta³ and Yongyut Khamsee⁴

Abstract

Some postharvest physiological changes were studied among the four different maturity stages of 'Mokara Madame Panne' cut orchid to investigate their effects on vase-life, held in reverse osmotic water. Four maturity stages included: opening of 3-4 florets, 5-6 florets, 7-8 florets and 9-10 florets as stage 1, stage 2, stage 3, and stage 4 respectively. Respiration rate on day-0 of experiment was highest in maturity stage 1; however, it decreased and was not different on day-7 and 12 among the stages. Similarly, the rate of ethylene production was also found higher in maturity stage 1 on day-0; and then, all stages showed the similar trends of low ethylene production. The amount of total sugar within florets of *Mokara* inflorescences was much more in the maturity stage 1 on day-0. After that, it decreased in all maturity stages. However, it was still higher in stages 1 and 2 in most of the days of holding. In the same way, the amount of total sugar within flower-stems (peduncles) of maturity stage 1 was always higher during the whole period of study; whereas the other stages had the same trends of decreasing sugar. In most of the days of vase life, there were similar decreasing tendencies of water uptake, water loss and water balance with slight fluctuations among the various flower stages. As a result, vase-life (11-13 days) of all maturity stages of *Mokara* cut orchid was not observed significantly different, although stage 3 and stage 4 exhibited maximum flower-bud opening in most of the holding period. In conclusion, different maturity stages of *Mokara* inflorescences in our study had no effect on vase life. Because even 3-4 florets (26 %) opening stage that is maturity stage 1 was found successful in maintaining the various physiological changes and vase life same as the other stages.

Introduction

Out of the over 800 genera of orchid, genus '*Mokara*', a multigeneric vandaceous hybrid, is also considered very successful. It is an artificial genus that was created in Singapore by involving *Arachnis*, *Ascocentrum* and *Vanda* orchids (Yew-Hwa, 1995; Yam and Thame, 1999). Variety '*Mokara Madame Panne*' bears red spotted yellowish florets and very popular in Thailand. Its major flowering period is May to October and in general, its inflorescences are harvested at ½ (half) or 2/3 (two third) blooming stages (personal communication with growers). However, it has not been scientifically studied which stage is the best for its maximum vase-life. Ketsa and Wongs-Aree (1995) reported that the number of open florets in each individual inflorescence of *Dendrobium* orchid must be adequate before harvest because they will ensure the maximum water supply even in the presence of preservatives in order to maximize bud opening with less defects (twisting and half opening).

Ethylene production and respiration are the major physiological processes that are responsible for deterioration and short life of harvested commodities. It is generally accepted that ethylene production increases during flower senescence and ethylene can accelerate flower senescence (Reid, 1989; cited by Ketsa and Thampitakorn, 1995). Due to respiration, there is continuous loss of stored food reserves (especially sugars) in the horticultural commodities and thus it hastens their senescence. So, this study was carried out to investigate the postharvest physiological changes and their effects on vase-life of different maturity stages of '*Mokara Madame Panne*' cut orchid.

Keywords: *Mokara* orchid, Maturity stages, Postharvest physiological changes

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Materials and Methods

Four maturity stages of '*Mokara Madame Panne*' orchid flower were used as treatments:

- (1) 3-4 florets open (maturity stage 1 or stage 1), (2) 5-6 florets open (maturity stage 2 or stage 2),
- (3) 7-8 florets open (maturity stage 3 or stage 3), and (4) 9-10 florets open (maturity stage 4 or stage 4).

Experiment was in completely randomized design (CRD). Individual inflorescences (10 for each treatment) were placed in 50 ml glass cylinders containing 40 ml reverse osmotic (RO) water. Data collection included respiration and ethylene production, water uptake and water loss, total sugar in florets and flower-stems, flower-bud opening, and wilting and abscission of florets and flower-buds. The vase-life of each individual inflorescence was considered terminated when symptoms of wilting and abscission were noticed in 50 % opened florets or flower-buds or in both. All data were analyzed statistically by using analysis of variance procedure and mean comparisons were assessed by Duncan's Multiple Range Test (DMRT). There were 30°C ($\pm 2^\circ\text{C}$) mean temperature, 65 % relative humidity and natural light (12 hr day) during this experiment.

Results

Respiration and ethylene production rates

Respiration rates of all maturity stages of *Mokara* inflorescences were recorded very high (234-385 mg CO₂ / kg / hr) on day 0, though highest rate was found in stage 1 (Fig. 1 A). There after, we observed decreased rates and no significant differences among the stages during their vase-life (Fig. 1 A). Maturity stage 1 (more buds stage) also produced more ethylene than the other stages on day 0 (Fig. 1 B). As day advanced, there were seen similar low ethylene production rates among the stages of *Mokara* flower (Fig. 1 B). Data of respiration and ethylene production for stage 3 on day 0 missed due to experimental error.

Water uptake and water loss

There were much more water uptake (4.5-5.2 ml/inflorescence/day) and water loss (2.14-3.34 gm/inflorescence/day) both, in all maturity stages of *Mokara* inflorescences on first day of experiment which decreased sharply on day second (Fig. 2 A, B). After that, there was found gradual decrease with slight fluctuations. Similarly on day first, there was more water balance (1.8-2.4 gm/inflorescence/day) in comparison to the other days that decreased according to increased time of holding (Fig. 2 C).

Amount of total sugar

The amount of total sugar in the florets of maturity stage 1 of *Mokara* on day 0 was much higher (1.35 mg/g FW) than those of the other stages (Fig. 3 A). But on day 5, it decreased greatly (0.8 mg/g FW) and on day 10 and 13, the levels of total sugar in florets of all stages decreased slowly (Fig. 3 A).

At the same time, the amount of total sugar in the flower-stems of maturity stage 1 was highly significantly different from the amount of other stages on day 0 and 5 (Fig. 3 B). The differences in the amount of total sugar among the stages decreased according to time, however it was still higher in maturity stage 1 (Fig. 3 B).

Flower-buds opening and vase-life

Maturity stage 3 and maturity stage 4 exhibited the maximum flower-bud opening in most of the days of holding. However, no any harvesting stages of *Mokara* cut orchid showed significant difference in vase-life (Table 1). At the latter period of vase life, maturity stage 2 also found successful in exhibiting the higher (> 70 %) bud opening (Table 1).

Discussion

Flower-bud opening in maturity stages 3 and 4 of *Mokara* inflorescences was much higher in most of the study period which was coincident with 'the more mature the harvesting stage, the greater in percentage of buds opening is' as in *Leucocoryne* also (Elgar et al, 2003). However, all four maturity stages of '*Mokara Madame Panne*' cut orchid did not show the significant differences in their vase life. This result seems logical, because there were not found significant differences in water uptake and water loss, respiration and ethylene production as well as in total sugar of florets and flower-stems (peduncles) among the stages in most of days of holding. Although, on day-0 of experiment, maturity stage 1 had the highest rates in respiration and ethylene production. These may be due to more number of flower-buds in that stage. Ketsa and Thampitakorn (1995) reported that flower-buds of *Dendrobium* orchid produced more ethylene than opened florets. The amount of total sugar in florets of maturity stage 1 was also very high in comparison to the other stages on day-0 which was sharply decreased on day-5. It may be possible, because a large amount of sugars may be utilized for increased rate of respiration and bud opening during initial period. In this study, only 3-4 florets (26 %) open stage of *Mokara* inflorescences was also observed successful to be utilized as cut flower. Previously, in case of *Dendrobium* orchid, Ketsa and Wongs-Aree (1995) mentioned that inflorescences of 6 varieties with 9-10 open florets (minimum 37.5 %) and 13-15 flower buds were found appropriate in bud opening and vase-life.

Summary

No significant differences were detected in various physiological changes and vase life among 4 maturity stages of *Mokara* cut orchid. However, stage 3 and stage 4 showed the maximum bud opening in most of the days of holding. At the latter period, maturity stages 2 and 1 also exhibited the high rates of bud opening more than 70 % and 60 %, respectively. Thus, all four harvesting stages of '*Mokara Madame Panne*' tested here, are seemed potential to be used as cut flowers. More florets open stages (stage 3 and stage 4) can be appropriate for local markets and less florets open stages (stage 1 and stage 2) may be more suitable for distant markets due to less chances of damage during handling.

Acknowledgement

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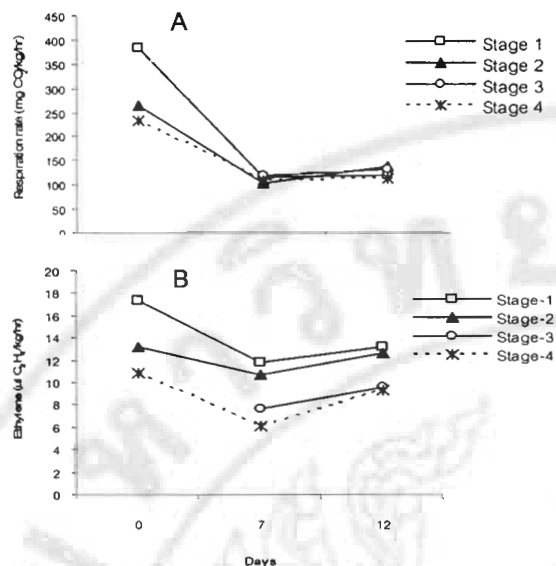


Fig 1 Rates of respiration (A) and ethylene production (B) in different maturity stages of 'Mokara Madame Panne' cut orchid held in reverse osmotic water.

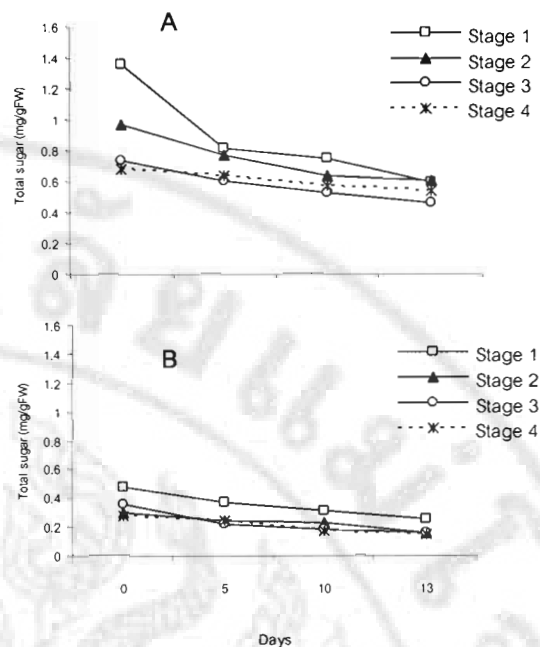


Fig 3 Amount of total sugar in florets (A) and flower-stems (B) of different maturity stages of 'Mokara Madame Panne' cut orchid held in reverse osmotic water.

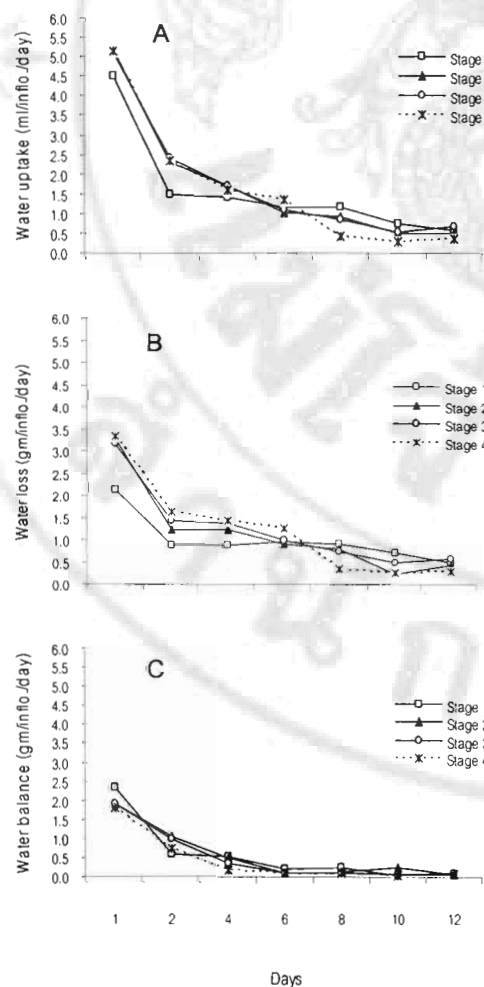


Fig 2 Water uptake (A), water loss (B) and water balance (C) in different maturity stages of 'Mokara Madame Panne' cut orchid held in reverse osmotic water.

Table 1 Bud opening and vase-life of 'Mokara Madame Panne' cut orchid held in reverse osmotic water.

Treatment	Bud-opening (%)			Vase-life (days)
	Day 0	Day 6	Day 12	
Stage 1	26.0 d	56.5 c	62.1 b	11.8 a
Stage 2	38.2 c	66.5 b	73.3 a	11.6 a
Stage 3	50.3 b	78.0 a	79.4 a	13.4 a
Stage 4	58.1 a	78.5 a	81.0 a	11.2 a

Values sharing the same letter with in a column indicate that they were not significantly different at 0.05 level by DMRT for bud-opening and vase-life respectively.



Appendix G

Curriculum Vitae

Appendix G Curriculum Vitae

Name: Khagendra Prasad Sharma

Date of Birth: 11th October, 1967

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1987-1992	B.Sc. (Ag. and A.H.) CSA University of Agriculture and Technology, Kanpur, India
2006-2008	M.S. Horticulture Maejo University, Chiang Mai, Thailand

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1992-1994	Asst. Horticulture Development Officer District Agriculture Development Office, Jumla Under Department of Agriculture, Nepal
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Publication: Paper presented in 7th National Horticultural Congress held on 27-29 May, 2008 at Naresuan University; Phitsanulok (Thailand)