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Postharvest responses of six cut *Mokara* spp. hybrids to exogenous ethylene

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Abstract

Ethylene-sensitive cut flowers despite having good shape and colour, display short vase life and usually after shipment, their ethylene injury would be very high, which is a negative point for exportation. The objectives of the study were first, evaluation of level of ethylene sensitivity and second, categorizing of six cut *Mokara* hybrids based on ethylene sensitivity: *Mokara* 'Chak Kuan Blue', *M.* 'Chao Praya Classic', *M.* 'Calypso Jumbo', *M.* 'Boy Blue', *M.* 'Red' and *M.* 'Chitty Gold'. The inflorescences were treated with 10 µL/L ethylene gas for 24 hours before placing them in bottles containing standard solution [distilled water + 250 mg/L 8-hydroxyquinoline citrate (8-HQC) + 150 mg/L citric acid + 4% sucrose, pH=3.5]. Water loss, vase life, anthocyanin contents and ethylene production were determined after treatments. Expressions of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and oxidase genes (ACO), before and after exposure to ethylene in the lips of third florets were also determined using semi-quantitative RT-PCR method. Ethylene caused tremendous reduction of vase life with an average of 59%, indicating ethylene sensitivity in studied *Mokara* cut hybrids. This was displayed by florets and buds wilted and dropped in all tested hybrids. Weight loss and anthocyanin degradation increased when the flowers were exposed to ethylene 11.5 and 16%, respectively. Both genes were expressed in fully open florets, but after exposure to ethylene, the levels of expressions were higher in all hybrids. However, different hybrids showed distinct variances in ethylene sensitivities and degrees of deterioration. *M.* 'Calypso Jumbo' and *M.* 'Red' exhibited the utmost anthocyanin degradation in sepals and petals and declined in the length of vase life. Thus, these two hybrids were categorized as the very sensitive group. *M.* 'Chak Kuan Blue', *M.* 'Chao Praya Classic', *M.* 'Boy Blue' and *M.* 'Chitty Gold' categorized as less sensitive group.

Keywords: ethylene sensitivity, vase life, anthocyanin content, ACC synthase, ACC oxidase.
Abbreviations: ACC_1-aminocyclopropane-1-carboxylic acid; ACO_1-aminocyclopropane-1-carboxylic acid oxidase; ACS_1-aminocyclopropane-1-carboxylic acid synthase; GC_gas chromatograph; 8-HQC_8-hydroxyquinoline citrate; MCKB_*Mokara* 'Chak Kuan Blue'; MCPC_*Mokara* 'Chao Praya Classic'; MCJ_*Mokara* 'Calypso Jumbo'; MBB_*Mokara* 'Boy Blue'; MR_ *Mokara* 'Red'; MCG_*Mokara* 'Chitty Gold.

Introduction

One of the largest families in the plant kingdom is Orchidaceae with about 750 different genera, at least 25,000 native species and more than 30,000 cultivated hybrids (Hew and Yong, 1997). *Mokara* is the generic name of one hybrid that has been produced in Singapore in 1969 (Lee et al., 1996). It was generated from the hybridization of three genera, including: Arachnis, Ascocentrum and Vanda (Lee et al., 1996). *Mokara* hybrids normally seem very similar to Aranda. They are currently very popular cut flowers. Until 1996, about one hundred *Mokara* hybrids had been registered. The majority of *Mokara* hybrids are diploid or triploid. Diploid Mokaras are generally produced from crossing diploid Arachnis with diploid Ascocendas (Yew-Hwa, 1995). Plants of diploid Mokaras are generally small and not very strong, and the diameter of their flowers is only 4.5 to 5.5 cm. Triploid Mokaras are generated from crossing between certain clones of diploid Aranda hybrids as female parents and diploid Ascocendas. Triploid Mokaras are much more strong and plentiful than their diploid equivalents, producing bigger flowers of nice shape with diameter of 8 to 9 cm (Lee et al., 1996).

The basic pigments of flower are anthocyanins, which are derived from flavonoids. The anthocyanins create various colours with the Co-pigments (Harborne, 1994). They are concentrated in epidermal and sub-epidermal cells of flowers, fruits and foliar and their colour depend on micro-environmental condition like metal and flavonoid concentration and pH condition (Mazza and Miniati, 1993). The anthocyanin content gradually decreased both in control and 1-MCP treated Patumma flowers throughout the storage period (Chutichudet et al, 2011). Arditti et al. (1979) reported that exposure of Cymbidium flowers with 10 µL/L ethylene for up to 78 hours encourages anthocyanin formation in both gynostemia (columns) and labella (lips). Anthocyanin content was reduced in Arabidopsis by exogenous ethylene. Negative regulation of anthocyanin accumulation in arabidopsis by ethylene might be a mechanism, whereby the proper balance between carbon assimilation and anthocyanin

accumulation is maintained in target tissues, via the suppression of light- and sugar-induced anthocyanin pigmentation (Das et al., 2011).

Endogenous ethylene is produced through conversion of S-adenosylmethionine (AdoMet) to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) and ACC is oxided to ethylene by ACC oxidase (ACO) (Srivastava, 2002). The increasing of ethylene production after exposure to ethylene in Dendrobium 'Khao Sanan' is correlated to the expression of Den-ETR1, Den- ACS and Den-ACO genes and it will be ended due to premature senescence (Lerslerwong and Ketsa, 2008).

Ethylene sensitivity in cut orchids is high, even at exposure to very low level of ethylene (Goh et al., 1985). In the majority of Dendrobium cultivars, the wilting of flowers and buds occurs, because of highly sensitivity to ethylene and increasing of ethylene gas inside the packages containing flowers, which during shipment can promote premature senescence (Uthaichay et al., 2007). Ethylene sensitivity is normally manifested as flower, bud drops or abscission (Kuroda et al., 2004). Sensitivity to ethylene might be raised when the expression of 1-aminocyclopropane-1-carboxylic acid oxidase gene (ACO) is increased (Wagstaff et al., 2005). In many of the Dendrobium cultivars, the flowers and buds wilting occurs due to high sensitivity to ethylene and the increase of ethylene gas inside the packages which contain flowers during the shipment. The shipment can promote the premature senescence. The sensitivity is depicted as flowers or buds abscission. The sensitivity might rise when the expression of 1- aminocyclopropane-1-carboxylic acid oxidase gene (ACO) is increased (Uthaichay et al., 2007). It is also possible that ACC is oxided to ethylene by the oxidase bringing about the sensitivity to *Mokara*. However, the postharvest cut Mokara's health depends on senescence and abscission and on the degree of ethylene sensitivity to the buds and florets.

Ethylene sensitivity varies with cultivars and hybrids for most flowers. Extensive variation were found in postharvest quality and longevity of cut roses cultivars (Macnish et al., 2010), *Dendrobium* hybrids (Almasi et al., 2012), within different species and crosses of *Chamelaucium* Desf. (Macnish et al. 2004) and potted carnation cultivars (Onozaki et al., 2009) with exogenous ethylene application. These variations were often reflected by their ethylene sensitivity levels.

Postharvest quality of cut orchids depends on abscission and senescence, as well as on the degree of ethylene sensitivity of florets and buds, which varies widely within and among *Mokara* hybrids. Therefore, the objective of this study was to determine the ethylene sensitivity level of six cut *Mokara* hybrids.

Results

Effect of ethylene on weight loss and vase life

Table 1. showed that weight loss of hybrids were significantly different across the exposure to ethylene and day in vase solution. Weight losses of MCG were lower compared to MCPC, MCKB and MCJ. However, there were no significant differences between the former hybrid with MCJ and MBB.

There was a significant interaction between exposure to ethylene and day in vase solution (Table 1). When the flowers were exposed to ethylene, the weight loss increased until day 3 after which, it began to decrease (Fig 1). This

Table 1. Effects of ethylene exposure (with and without exposure), different hybrids [*Mokara* hybrids; *M.* 'Chak Kuan Blue' (MCKB), *M.* 'Chao Praya Classic' (MCPC), *M.* 'Calypso Jumbo' (MCJ), *M.* 'Boy Blue' (MBB), *M.* 'Red' (MR)and *M.* 'Chitty Gold'(MCG)] and days after exposure (day 1, 3 and 5) on weight loss.

Factor		Weight Loss (%)
Hybrids (H)	MCPC	0.19 ± 0.01 a
	MCKB	0.18 ± 0.02 a
	MCJ	0.16 ± 0.02 ab
	MBB	0.15 ± 0.01 bc
	MR	0.14 ± 0.01 bc
	MCG	0.13 ± 0.01 c
Ethylene (E)		
	With	0.18 ± 0.01 a
	Without	0.14 ± 0.00 b
Day in vase solution (D)		
	1	0.16 ± 0.01 b
	2	0.15± 0.01 b
	3	0.23 ± 0.02 a
	4	0.14± 0.01 b
	5	0.11 ± 0.01 c
Interactions		
H x E		n.s
H x D		n.s
E x D		**
H x E x D		n.s

**= Highly significant at $p < 0.01$ and n.s = not significant ($p > 0.05$). Means within a column followed by a different letter were significantly different at $p = 0.05$ using least significant difference (LSD).

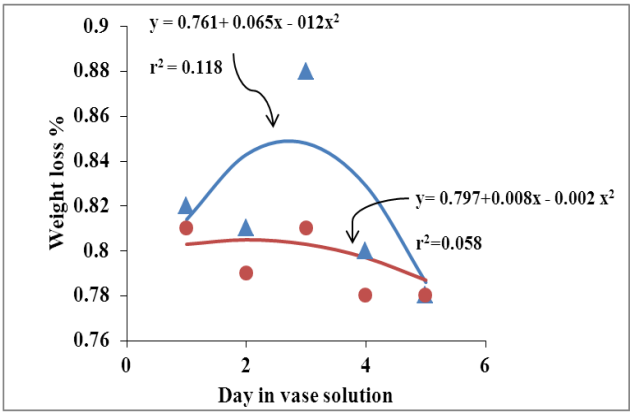

Fig 1. Effect of day in vase solution and exposure to ethylene (with ▲ and without ●) on percentage of weight loss.

may be due to the senescence process of the flowers which started to occur from third day onwards. In the control flowers, weight losses were more gradual.

The responses of flowers exposed to ethylene on their vase lives varied among hybrids (Table 2). Hybrids MCG and MBB had significantly longer vase lives, even though after exposure to ethylene compared to other hybrids (Fig 2). This showed that these two hybrids were less sensitive to ethylene. When not exposed to ethylene, vase lives of hybrids MCKB, MCG and MCJ were significantly higher than MCPC. However, MCPC was not considerably different from MR and MBB. The latter two were also not meaningfully different from MCKB, MCG and MCJ.

Effect of ethylene on anthocyanin content

Anthocyanin contents between *Mokara* hybrids were significantly different (Table 3). The highest anthocyanin content was found in MCJ and the lowest in MCPC and MCG. The differences between the former hybrid and the latter two were more than six-folds. The results showed that anthocyanin contents were more sensitive to ethylene compared to weight loss and vase life. Anthocyanin contents were also significantly higher in flowers that were not exposed to ethylene compared with the flowers that were exposed to. The anthocyanin content on the average was 16% higher. However, the number of days the flowers were in vase solutions did not affect the anthocyanin contents.

Effect of exogenous ethylene on endogenous ethylene production

Ethylene production varied significantly between hybrids (Table 3). The highest production was found in MCKB and MCG. However, MCKB was not significantly different when compared with other hybrids.

There was a significant interaction between exposures to ethylene with days in vase solution (Fig 3). When the flowers were exposed to ethylene, the production was about the same for the first three days, after which they began to decrease abruptly. However, with control flowers, the reduction was gradual. This suggested that ethylene exposure maintained the endogenous production for the first three days. Then, the production decreased which could coincide with premature senescence of the flowers. Normally, ethylene production is expected to be lower in senesced flowers.

Expression of ACC Synthase and ACC Oxidase genes in response to ethylene

Fig 4. showed the effect of ethylene treatment on expression of ACC synthase and ACC oxidase genes. These results were expressions of both genes on the day of exposure to ethylene and one day later. The two enzymes were expressed in all the six hybrids. But for all of them, the levels of expression were higher one day after exposure compared with expressions on the day of treatment. All hybrids produced the same band at the same location in response to Den-ACO and Den-ACS primers. It can be inferred that the genes related to these enzymes in the cut orchids are active in fully open florets and were more expressed when exposed to ethylene.

Discussion

Exposure to exogenous ethylene (10 µL/L for 24h) enhanced the early senescence in the cut orchids and increased their weight loss. This could be due to premature senescence of flower perianths. The results affirmed the findings of Ketsa et al. (2001) on diploid inflorescence of *Dendrobium* 'Caesar' and Chutichudet et al. (2011) on patumma. They reported that exogenous ethylene induced weight loss in the cut *Dendrobium* and patumma. The findings also revealed that weight loss is correlated to the number of florets and buds (Tan, 1995). This is because as the senescence progressed, the number of dropping florets and buds increased and; thus, the weight of the inflorescence decreased accordingly.

Rapid weight loss could be due to pre-activated programming of cell death (PCD) in the perianth. With exposure to ethylene, premature senescence will be induced

Table 2. Effects of ethylene on hybrids [*Mokara* hybrids; *M.* 'Chak Kuan Blue' (MCKB), *M.* 'Chao Praya Classic' (MCPC), *M.* 'Calypso Jumbo' (MCJ), *M.* 'Boy Blue' (MBB), *M.* 'Red' (MR)and *M.* 'Chitty Gold'(MCG)] and days after exposure (day 1, 3 and 5) on vase life.

Factors		Vase life (day)
Hybrids (H)	MCG	7.6 ± 1.24 a
	MCKB	7.0 ± 1.50 ab
	MBB	6.7 ± 0.68 ab
	MCJ	6.6 ± 1.35 ab
	MR	5.7 ± 0.91 bc
	MCPC	4.4 ± 0.54 c
Ethylene (E)	With	3.6 ± 0.25 b
	Without	9.1 ± 0.49 a
Interaction	H x E	*

*=significant at $p < 0.05$. Means within a column followed by a different letters were significantly different at $p = 0.05$ using LSD.

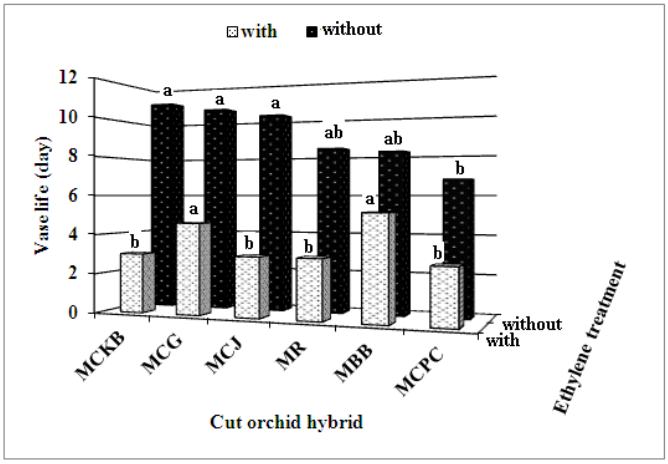

Fig 2. Effects of hybrids [(*Mokara* 'Chak Kuan Blue' (MCKB), *M.* 'Chao Praya Classic' (MCPC), *M.* 'Calypso Jumbo' (MCJ), *M.* 'Boy Blue' (MBB), *M.* 'Red' (MR) and *M.* 'Chitty Gold'(MCG)] and ethylene exposure on vase life. Means comparison were between hybrids for exposed and non-exposed to ethylene. Different letters showed significant differences by LSD at $p \leq 0.01$.

and as a result PCD will be activated (Trobacher, 2009). Thus, when PCD is stimulated, the rate of respiration would be increased followed by more evaporation and transpiration in the perianth and eventually more weight loss. This weight loss would continue without compensation through proper water uptake. This trend will ultimately result in water loss and wilting.

Vase lives were differently affected between hybrids when they were exposed to ethylene. This suggested that different hybrids responded in different ways to ethylene, or in other words, they have different levels of sensitivities to various concentrations of ethylene. Similar results were reported by Ebrahimzadeh et al. (2011) on carnation cultivars. Hybrids which are more ethylene sensitivite display more ethylene responsiveness (such as more anthocyanin degradation, water loss and reduced vase life) and in contrast hybrids which are less sensitivite exibit low ethylene sensitivity. It has also been reported that the sensitivity to ethylene is associated with vase life in orchid flowers which were significantly varied among cultivars (Sun et al., 2009).

Rebecca et al. (2008) found that when cut *Dendrobium* 'Sonia' and 'Savin White' were aged, discolouration and

pigment leakage became visible. The same had been the case with the study on *Brunfelsia calycina*, where the pigment in the flowers changed from dark purple to white at certain stages of development (Vaknin et al. 2005).

The exogenous ethylene induced the endogenous ethylene in cut orchids significantly higher than the control. This showed that exogenous ethylene can cause the autocatalytic ethylene production in the orchid flowers and expression induction of ACC synthase and oxidase genes. Consequently, it will cause tepal senescence. These results were comparable to the results of Nabigo et al. (2010) on roses, Scariot et al. (2009) on cut *Ranunculus asiaticus*. Ebrahimzadeh et al. (2011) reported that ethylene production was stimulated by exogenous ethylene but at different rates among the various carnations cultivars.

The expression of ACS and ACO genes with exposure to ethylene were up-regulated in all cut *Mokara* orchids, which is in agreement with results of Wagstaff et al. (2005) on Alstroemeria and Lerslerwong and Ketsa, (2008) on *Dendrobium* flowers. In addition, Borochov and Woodson (1989) reported that ethylene exposure induces senescence of flower petals and declined vase life with the activation of 1-amino-cyclopropane-1-carboxylic acid (ACC) synthase and/or ACC oxidase.

The results of ethylene sensitivity were consistent with studies carried out on *Dendrobium* 'Khao Sanan' (Lerslerwong and Ketsa, 2008), mini Phalaenopsis (Sun et al., 2009), Oncidium 'Gower Ramsey' (Huang and Paull, 2009) and cut roses (Chamani et al., 2005). All of them found that exogenous ethylene induced premature senescence of these flowers.

Materials and methods

Plant materials

Inflorescences of *Mokara* 'Calypso Jumbo' (MCJ), M. 'Chao Praya Classic' (MCPC), M. 'Chitty Gold' (MCG), M. 'Boy Blue' (MBB), M. 'Red' (MR) and M. 'Chak Kuan Blue' (MCKB) were purchased from a nursery located in Bukit Changgan, Selangor, Malaysia. After harvest, the flowers (within one hour) were taken to the laboratory in the Department of Crop Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Ethylene treatment

The Inflorescences of six hybrids, each with 34 inflorescences were divided into two groups of 17 inflorescences (5, 6, 3 and 3 inflorescences used for vase life, ethylene production, anthocyanin content and gene expression evaluation, respectively). They were placed inside a plexiglass chamber (51 × 46.5 × 33.5 cm). After being sealed, 10 µL/L ethylene gas balanced with nitrogen was injected into the chamber. The concentration of ethylene gas was checked by a gas chromatograph (GC) (Srivastava, 2002). A fan was placed at the base of the chamber for circulation to ensure that the gas was homogenized inside the chamber. In the control chamber, no ethylene was injected. The inflorescences were inside the dark chambers with mean temperature and relative humidity of 25±2°C and 80 ± 2%, respectively for 24 hours. After 24 hours, the chambers were aerated and inflorescences trimmed up to 12 cm from the first open floret before being put into a cylindrical polyethylene (PE) bags having a thickness of 10 µm. Each bag contained 60 ml vase solution [distilled water + 250 mgL^{-1} 8-hydroxyquinoline citrate (8-HQC) + 150 mgL^{-1} citric acid + 4% sucrose, pH=3.5]. The plastic bag was then held in a 300 ml glass bottle with cotton wool placed around the stem in order to hold it straight. The inflorescences were placed in the laboratory with the temperature, humidity and light intensity (25±2 °C, 78% ±2 and 6.57 µmol/m^2/s, respectively). Parameters determined were weight loss, vase life, anthocyanin contents, expression of ACS and ACO and ethylene production.

Table 3. Effects of ethylene, hybrids [*Mokara* hybrids; *M.* 'Chak Kuan Blue' (MCKB), *M.* 'Chao Praya Classic' (MCPC), *M.* 'Calypso Jumbo' (MCJ), *M.* 'Boy Blue'(MBB), *M.* 'Red' (MR)and *M.* 'Chitty Gold' (MCG)] and days in vase solution exposure (day 1, 3 and 5) on anthocyanin content and ethylene production.

Factor		Anthocyanin (A^o)	Ethylene production (nl/kg/hr)
Hybrids (H)			
	MCKB	0.28 ± 0.01 c	2.56 ± 0.12 ab
	MCPC	0.05 ± 0.00 d	2.40 ± 0.09 b
	MCJ	0.43 ± 0.02 a	2.43 ± 0.07 b
	MBB	0.33 ± 0.01 b	2.37 ± 0.05 b
	MR	0.35 ± 0.02 b	2.45 ± 0.09 b
	MCG	0.07 ± 0.01 d	2.67 ± 0.10 a
Ethylene (E)			
	With	0.21 ± 0.02 b	2.58 ± 0.05 a
	Without	0.25 ± 0.02 a	2.38 ± 0.04 b
Day in vase solution (D)			
	1	0.24 ± 0.03 a	2.58 ± 0.07 a
	3	0.23 ± 0.03 a	2.58 ± 0.07 a
	5	0.23 ± 0.02 a	2.27 ± 0.02 b
Interactions			
H x E		n.s	n.s
H x D		n.s	n.s
E x D		n.s	*
H x E x D		n.s	n.s

**= Highly significant at $p < 0.01$, *=significant at $p < 0.05$ and n.s = not significant ($p > 0.05$). Means within a column followed by a different letters were significantly different at $p = 0.05$ using LSD.

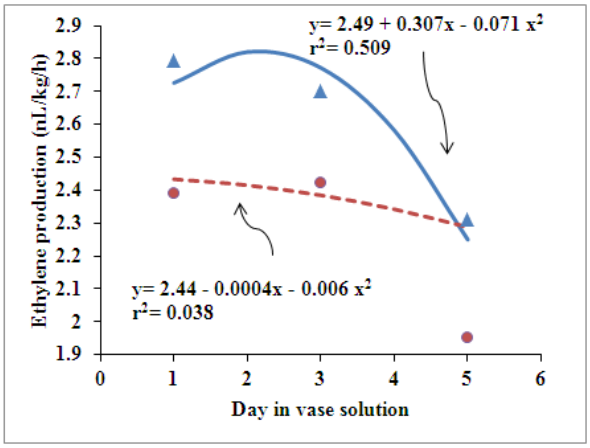

Fig 3. Relationship between ethylene treatment [0 ( ) and 10 ( ) µl/l] and days in vase solution on endogenous ethylene production.

Fresh weight changes and weight loss of cut Mokara hybrids

The weight of each inflorescence, together with the P.E. bag,

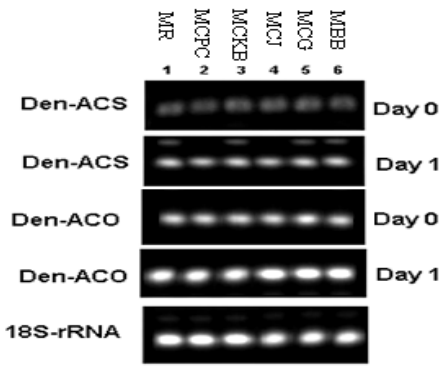

Fig 4. Expression of Den-ACS and Den-ACO genes in *Mokara* hybrids; *M.* 'Chak Kuan Blue' (MCKB), *M.* 'Chao Praya Classic' (MCPC), *M.* 'Calypso Jumbo' (MCJ), *M.* 'Boy Blue' (MBB), *M.* 'Red' (MR) and *M.* 'Chitty Gold'(MCG) using semi-quantative RT-PCR analysis was performed in lip. Total RNA was extracted from the lip of third open floret on days before (0) and after (1) ethylene exposure. Both genes were expressed in fully open florets in all cut *Mokara* hybrids but the bands of both genes after exposure to ethylene were markedly brighter.

bottle and vase solution was recorded daily for five days without replenishing the vase solution. The measurement differences were calculated daily as a percentage of loss of weight due to dropped of fresh buds and florets per day.

Vase life

The process of epinasty or yellowing of the buds and florets was recorded visually. It was assumed that the vase life was ceased when any of the yellowing symptoms is greater than 30 percent appeared (Almasi et al., 2012).

The production of ethylene

Two inflorescences of each treatment were incubated inside a 5.2 L container for four hours. A 1 ml sample was taken from the head space using a syringe and injected into a gas chromatograph (Perkin Elmer Clarus 500 Gas Chromatograph, USA) fitted with flame ionization detector and 30m capillary column. The column temperature was 70 ^{o}C; however, the injector and detector temperatures were 200^{o}C. The mean of ethylene production was calculated as nl/kg/h (Friedman et al., 2005).

Anthocyanin content

Anthocyanin content of the florets was determined using the method of Nakamae and Nakamura (1983). Ten discs of (0.53 cm in diameter) from petals and sepals of first floret were bored using a cork borer. The discs were washed in distilled water in a 25ml vial which contained 10 ml 0.1% methanol (v/v). It was then stored at 25^{o}C in darkness for 24 hours. The purpose of this process was to measure the absorbency of the solutions at 530 nm (Ketsa and Rugkong, 1999). The measurements were taken on the first, third and fifth days of the experiment.

RNA extraction

The third floret from each inflorescence was excised before and after the treatments (days 0 and 1). They were stored at -70 ^{o}C. Total RNA was extracted from 90 mg frozen lip of each floret using Qiagen RNeasy plant mini kit based on manufacturer's protocol. The RNA's quality was checked by the use of a NanoDrop Spectrophotometer to ensure that the nucleic acid concentration was between 40-75 ng/ L.

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Qiagen one-step RT-PCR Kit was used to carry out the expression of ACC synthase and ACC oxidase genes by semi-quantitative RT-PCR method. In this kit, reactions of reverse transcription (as cDNA) and PCR amplification takes place in one tube. A pair of primers of ACC synthase gene (Forward 5´-CAACCTGGTTCAGAACAG-CAAC-3´and Reverse 5´-GGGAAGCTGGATTGTTCTGC-3´) and a pair of primers of ACC oxidase (Forward 5´-ATGGAGCTTCT-TGAGGGTTC-3´and Reverse 5´-TCAAGCAGTAGGAA-TCGGCTG-3´) were used for semi-quantitative RT-PCR (Lerslerwong and Ketsa, 2008).

The PCR machine (Bio RAD, My cycler^{TM} Thermal cycler) condition was set as follows: 30 min 50^{o}C for reverse transcription, 15 min 95^{o}C initial PCR activation and 3 steps cycling (30x) 1: 30 sec 94^{o}C denaturation 2: 30 sec 50^{o}C annealing 3: 60 sec 72^{o}C extension. The products of RT-PCR were run on the agarose gel as 500 mg agarose powder was mixed with 50ml Tris/Borate/EDTA (TBE) buffer in 150 ml chronicle flask. The mixture was then placed in the microwave for 2 min at 100 ^{0}C. It was then left under a fume chamber for cooling, but before it achieved a complete solid state. A 3 µL ethidium bromide was added to the gel and then it was poured into the gel tray. The comb was taken out after 30 minutes when the wells were ready for loading. The two dyes mixed with 10 µL RT-PCR product was loaded into the well. After all the samples were loaded into the wells, the electrophoresis power supply (Wealtec, Elite 300) was programmed as: 70 V, 400 A and 70 min. The bands of DNA were viewed and photographed by the use of Gel Documentation System (Alpha Innotech, Chemimager TM5500).

Statistical analysis

The experiment was conducted using a Completely Random Design (CRD) with a six (hybrids) by two (0 and 10 ethylene exposure) factorial array of treatments. The study on the determination of vase life and weight loss was replicated five times, whereas the experiment for anthocyanin content and ethylene production was replicated three times. The analysis of data was done using the variance analysis (ANOVA). Treatment means were compared by least significant difference (LSD) at $p \leq 0.05$. Due to the significant interaction between the factors which highlighted the effect of ethylene on the buds and florets and the vase life, their effects on the measured parameters were determined using regression analysis by SAS software version 9.1.

Conclusion

The results showed that the sensitivity of cut *Mokara* to exogenous ethylene exposure varied among hybrids. Different hybrids responded diversely to the parameters evaluated. Overall, the hybrids can be categorized into two groups, which were sensitive to ethylene, *M.* 'Red' (MR) and *M.* 'Calypso Jumbo' (MCJ) and the other group being less sensitive, *M.* 'Chitty Gold' (MCG),), *M.* 'Chak Kuan Blue' (MCKB), *M.* 'Chao Praya Classic' (MCPC) and *M.* 'Boy

Blue' (MBB). This grouping is beneficial for further work on breeding or postharvest.

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References

Almasi P, Mahmud TMM, Ahmad SH, Kadir J, Mirshekari A (2012) Postharvest responses of cut *Dendrobium* orchids to exogenous ethylene. Afr J Biotechnol 11: (16) 3895-3902.

Borochov A, Woodson WR (1989) Physiology and biochemistry of flower petal senescence. Hort Rev 11: 15-43.

Chamani ABE, Khalighi BA, Joyce ADC, Donald E, Irving A, Zamani BZA, Mostofi BY, Kafi BM (2005) Ethylene and anti-ethylene treatment effects on cut 'First Red'rose. Appl Horti 7: 3-7.

Chutichudet P, Chutichudet B, Boontiang K (2011) Influence of 1-MCP fumigation on flowering, Weight loss, Water uptake, longevity, anthocyanin content and colour of patumma *(Cucurma alismatifolia) CV*, Chiang Mai Pink. Int J Agric Res 5: 1-10.

Cullina W (2004) Understanding Orchids: An Uncomplicated Guide to Growing the World's Most Exotic Plants. Houghton Mifflin Harcourt, pp. 55-63.

Das PK, Geul B, Choi SB, Yoo SD, Park YI (2011) Photosynthesis-dependent anthocyanin pigmentation in Arabidopsis. Plant Signal Behav 6: 23-25.

Ebrahimzadeh A, Jimenez-Becker S, Manzano-Medina S, Jamilena-Quesada M, Lao-Arenas MT (2011) Evaluation of ethylene production by ten Mediterranean carnation cultivars and their response to ethylene exposure. Span J Agric Res 9: 524-530.

Friedman H, Hagiladi A, Resnick N, Barak A, Umiel N (2001) Ethylene-insensitive related phenotypes exist naturally in a genetically variable population of *Dianthus barbatus*. Theor Appl Genet 103: 282–287.

Goh CJ, Halevya AH, Engelb R, Kofranek AM (1985) Ethylene evolution and sensitivity in cut orchid flowers. Sci Hort 26: 57-67.

Harborne JB (1994) Flavonoids: Advances in Research Since 1986. Chapman and Hall Press, London, UK, pp. 299–343.

Hew CS, Yong JWH (1997) The physiology of tropical orchids in relation to the industry. World Scientific Publishing Co, pp. 25-36.

Huang CC, Paull RE (2009) The responses of Oncidium cut flowers to ethylene and 1-MCP. Tai J Agric Res 58: 1-6.

Ketsa S, Rugkong A (1999) Senescence of *Dendrobium* 'Pompadour' flowers following pollination. J Hortic Sci Biotech 74: 608-613.

Ketsa S, Rugkong A (2000) Ethylene production, senescence and ethylene sensitivity of *Dendrobium* 'Pompadour' flowers following pollination. J Hortic Sci Biotech 75: 149-153.

Ketsa S, Uthairatanakij A, Prayurawong A (2001) Senescence of diploid and tetraploid cut inflorescences of *Dendrobium*. Sci Hort 91: 133-141.

Kuroda S, Hirose Y, Shiraishi M, Davies E, Abe S (2004) Co-expression of an ethylene receptor gene, ERS1, and ethylene signaling regulator gene, CTR1, in Delphinium during abscission of florets. Plant Physiol Bioch 42: 745-751.

Lee YH, Wong SM, Tan WK, Goh CJ (1996) Breeding vandaceous orchids for commercial cut-flowers in Singapore: an overview. Euphytica 89: 235-241.

Lerslerwong L, Ketsa S (2008) Autocatalytic Ethylene Production by *Dendrobium* Flowers during Senescence Induced by Exogenous Ethylene. Thai J Agr Sci 41: 91-99.

Macnish A1, Irving DE, Joyce DC, Vithanage V, Wearing AH, Lisle AT (2004) Variation in ethylene-induced postharvest flower abscission responses among *Chamelaucium* Desf. (*Myrtaceae*) genotypes. Sci Hort 102: 415-432.

Macnish AJ, Leonard RT, Borda AM, Nell TA (2010) Genotypic Variation in the Postharvest Performance and Ethylene Sensitivity of Cut Rose Flowers. HortScience 45: 790-796.

Mazza G, Miniati E (1993) Color stabilization and intensification. In: Mazza G, Miniati E (eds) Anthocyanins in Fruits, Vegetables, and Grains. CRC Press, Boca Raton, pp. 1–20.

Nabigol A, Naderi R, Mostofi Y, Khalighi A, Bujar M (2009) Soluble carbohydrates content and ethylene production in cut rose cultivars. Hort Environ Biotech 50: 122-126.

Nakamae H, Nakamura N (1983) Effects of metabolic inhibitors on anthocyanin accumulation in petals of Rosa hybrida Hort. cv. Ehigasa. Plant Cell Physiol 24: 995-1005.

Rebecca PS, Zuliana R, Wijenthiran K, Boyce AN, Chandran S (2008) A Comparative Study on Selected Antioxidants During Pollination Induced Senescence on *Dendrobium* Sonia and *Dendrobium* Savin White. Asian J Plant Sci 7: 724-729.

Reid MS, Wu MJ (1992) Ethylene and flower senescence. Plant Growth Regul 11: 37–43.

Scariot V, Larcher F, Caser M, Costa E, Beruto M, Devecchi M (2009) Flower Longevity in Ten Cultivars of Cut Ranunculus asiaticus L. as Affected by Ethylene and Ethylene Inhibitors. Europ J Hort Sci 74: 137–142.

Seidenfaden G (1994) The Orchids of Peninsular Malaysia and Singapore. Amer Orchid Society, pp. 4-17.

Srivastava ML (2002) Plant Growth and Development: Hormones and environments. Academic Press. USA, pp. 233-251.

Sun Y, Christensen B, Liu F, Wang H, Muller R (2009) Effects of ethylene and 1-MCP (1-methylcyclopropene) on bud and flower drop in mini Phalaenopsis cultivars. Plant Growth Regul 59: 83-91.

Tan H (1995) A Guide to the Orchids of Singapore. Singapore Science Centre, pp. 69-73.

Trobacher CP (2009) Ethylene and programmed cell death in plants. Botany 87: 757-769.

Uthaichay N, Ketsa S, van Doorn WG (2007) 1-MCP pretreatment prevents bud and flower abscission in *Dendrobium* orchids. Postharvest Biol Tec 43: 374-380.

Vaknin H, Bar-Akiva A, Ovadia R, Nissim-Levi A, Forer I, Weiss D, Oren-Shamir M (2005) Active anthocyanin degradation in *Brunfelsia calycina* (yesterday-today-tomorrow) flowers. Planta 222: 19–26.

Wagstaff C, Chanasut U, Harren FJM, Laarhove LJ, Thomas B, Rogers HJ, Stead AD (2005) Ethylene and flower longevity in alstroemeria: relationship between tepal senescence, abscission, and ethylene biosynthesis. J Exp Bot 56: 1007-1016.

Yew-Hwa L (1995) Intergeneric hybridization in vandaceous orchid breeding Acta Hort 420: 129-131.