Full Length Research Paper

Postharvest responses of cut *Dendrobium* orchids to exogenous ethylene

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The time to senescence of flowers in *Dendrobium* hybrids is different and one of the major reasons for this variation in vase life can be related to differences in the level of ethylene sensitivity. This study was conducted to determine the sensitivity of six cut *Dendrobium* hybrids to exogenous ethylene. The cut orchid inflorescences were treated with or without 10 µL/L ethylene gas for 24 h, and then placed in bottles containing a standard vase solution. Water loss, vase life, anthocyanin contents and ethylene production were determined after the treatments. The results indicated that different hybrids showed distinct variances in ethylene sensitivities and degrees of deterioration. *Dendrobium* 'Darren Glory' and *D.* 'Jaquelyne Concert' × *D.* 'Rinappa' exhibited the utmost hyponasty, weight loss and degradation of anthocyanin content in sepals and petals. Thus, these two hybrids were categorized as the very sensitive group. On the other hand, *D.* 'Sonia Red', *D.* 'Shavin White', *D.* 'Sonia White' and *D.* 'Sonia Bom' showed lower sensitivity and hence were categorized in the less sensitive group. The results of this experiment and similar experiments to determine the resistant and susceptible cultivars to ethylene injury can be helpful in improving the postharvest life of cut orchid flowers.

Key words: Ethylene sensitivity, vase life, anthocyanin content, ACC synthase, ACC oxidase.

INTRODUCTION

Orchids, belonging to the Orchidaceae family, are found throughout the world except the Polar Regions. Orchidaceae is one of the largest families which has about 800 genera with 25,000 species (Sheehan and Sheehan, 1994). *Dendrobium* is the second largest genus in the family after *Bulbophyllum (*Puchooa, 2004). It has unique characteristics among the cut orchids because of varieties of colour, larger number of florets in the inflorescence and recurrent flowering (Fadelah et al., 2001).

Ethylene has a major role in the senescence of most cut flowers. Moreover, increasing ethylene gas concentration inside the flower packages during shipment can cause premature senescence (Uthaichay et al., 2007). Ethylene plays some roles in petal senescence and ovary development (Ketsa and Rugkong, 1999; O'Neill et al.,

1993; Porat et al., 1994). The response of plants to the hormones is influenced by the biosynthesis and sensitivity to the hormones. The age of the flowers also play a role in ethylene sensitivity (Trewavas, 1981; Whitehead et al., 1984). The probable mechanism of ethylene action on flower senescence was suggested as when the ethylene molecule binds to membrane-based binding site, a second ligand is released and interacts with the 5′ promoter regions of genes related to ethylene-regulated senescence. With the transcription of the genes (mRNA), the enzymes productions in poly ribosomes are encoded (Borochov et al., 1997; Reid and Wu, 1992).

Ethylene is produced through the conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) and ACC to ethylene by ACC oxidase (ACO) (Srivastava, 2002). Carnation (Woltering and Van Doorn, 1988), petunia (Serek et al., 1995), alstroemeria (Wagstaff et al., 2005) and *Dendrobium* 'Pompadour' (Ketsa and Rugkong, 1999) are ethylene-sensitive-flowers. The major pigments

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in flowers are anthocyanins which are derivatives from flavonoids. Anthocyanins with other co-pigments cause differential of colors (Harborne, 1994). Storage can gradually reduce the anthocyanin content both in the control and 1-MCP treated patumma flowers (Chutichudet et al., 2011).

Cut *Dendrobium* hybrids are susceptible to damage by ethylene which can eventually reduce their longevity. Whereas *Dendrobiums* are sensitive to ethylene damage (Woltering, 1987), the level of the sensitivity seems to vary depending on the hybrids. Hence, it is essential to identify the degree of ethylene sensitivity in *Dendrobium* hybrids and the prospective impact of the exposure to ethylene on the length of their vase life. Thus, the objective of this study was to compare and then group these six orchids based on the ethylene sensitivity.

MATERIALS AND METHODS

Inflorescences of *Dendrobium* 'Darren Glory'(DG), *D.* 'Sonia Red' (SR), *D.* 'Sonia White' (SW), *D.* 'Sonia Bom' (SB), *D.* 'Shavin White' (SHW) and *D.* 'Jacqueline Concert' × *D.* 'Rinappa' (JCR) were purchased from a commercial farm in Bukit Changgang, Banting, Selangor, Malaysia. Inflorescences with 60 to 70% open florets were harvested between 8 to 9 a.m. and then immediately transported to the Postharvest Laboratory, Department of Crop Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, within one hour.

Ethylene treatment

Inflorescences of the six cultivars (each with 34 inflorescences) were divided into two groups and each group (17 inflorescences) was placed inside a plexiglass chamber (51 × 46.5 × 33.5 cm^3). After being completely 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). To ensure that the ethylene gas was homogenized inside the chamber, a small fan was placed on the base of the chambers for circulation. After 24 h, the chambers were opened and each inflorescence was trimmed to 12 cm from the first open floret before being put into a cylindrical PE bag (thickness 10 µM) containing 60 ml vase solution [distilled water + 250 mg/L 8-hydroxyquinoline citrate (8-HQC) + 150 mg/L citric acid + 4% sucrose, pH = 3.5]. Each plastic bag was then held in a 300 ml glass bottle with cotton wool placed around the stem for the purpose of holding it straight. The inflorescences were kept in the laboratory. The means temperature, relative humidity and light intensity were 25°C, 78% and 6.57 µmol, respectively.

Loss of fresh weight

The weight of each inflorescence together with the P.E. bag, bottle and vase solution was recorded daily for five days without replenishing the vase solution. The differences between the daily measurements were calculated as percentage loss of fresh weight per day.

Vase life

Yellowing and epinasty of florets and buds, number of opened florets and buds drop were recorded visually. Vase life was regarded as ceased when > 30% of any one of these symptoms appeared.

Ethylene production

Two inflorescences of each treatment were incubated inside a 5.2 L container for 4 h and 1 ml sample from the headspace was taken using a syringe and injected into a gas chromatograph (Clarus 500 Gas Chromatograph Perkin Elmer, USA) fitted with a flame ionization detector and 30 m capillary column. The temperature of column was 70°C, while the temperature of injector and detector were 200°C. The mean of ethylene production was calculated as nl/kg/h.

Anthocyanin content

Anthocyanin content of the florets was determined using the method of Nakamae and Nakamura (1983). Ten discs (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, placed in a 25 ml vial containing 10 ml methanol and 0.1% HCl (v/v) and then stored at 25°C in darkness for 24 h. Absorbances of these solutions were measured at 530 nm. Measurements were taken on day 1, 3 and 5.

Statistical analysis

In all the experiments, inflorescences were arranged in a completely randomized design (CRD). The experiment on determination of vase life and weight loss was replicated five times, while that for anthocyanin content and ethylene production experiment was replicated three times. Data were analyzed using analysis of variance (ANOVA) and the treatment means were compared using t-test, least significant difference (LSD) at p ≤ 0.05. When there were significant interactions between factors, their effects on parameters measured were determined using regression analysis by SAS soft ware version 9.1.

RESULTS

Effect of exogenous ethylene on fresh weight loss and vase life

The studied cut orchid hybrids had different weight losses through day 1, 3 and 5 in vase solution, with *D.* Darren Glory showing significantly highest weight loss compared to other hybrids (Table 1). In addition, the main effect of ethylene on weight loss of the orchids was significant (Table 1), although comparison of weight losses between exposed and non-exposed orchids to ethylene showed that only *D.* 'Shavin White' had significantly higher weight loss in flowers exposed to ethylene (Figure 1). Moreover, the interaction between ethylene treatment and days in vase solution was significant (p ≤ 0.05). Flowers exposed to ethylene had constant weight loss until third day in vase solution (Figure 2). After 3 days in vase solution, flowers exposed to ethylene showed a quadratic increase in weight loss and by 5 days in vase solution, the flowers had 26% weight loss. However, there was no significant

Table 1. Effects of ethylene gas on weight loss, anthocyanin content and ethylene production in six cut *Dendrobium* hybrids *D.* 'Sonia White'(SW), *D.* 'Darren Glory'(DG), *D.* 'Jaquelyne Concert' x *D.* 'Rinappa'(JCR), *D.* 'Sonia Bom' (SB), *D.* 'Sonia Red'(SR) and *D.* 'Shavin White' (SHW) during five days in vase solution.

Factor		Weight loss (%)	Anthocyanin (A^o)	Ethylene (nL/kg/h)	Vase life (day)
Hybrids	SW	0.68[c]	0.018[e]	3.5[a]	7.5[b]
	DG	0.92[a]	0.252[d]	3.32[ab]	7.1[b]
	JCR	0.69[c]	1.129[a]	2.27[c]	11.1[a]
	SB	0.69[c]	0.537[c]	2.82[b]	7.8[b]
	SR	0.63[c]	0.851[b]	2.99[ab]	10.1[a]
	SHW	0.80[b]	0.014[e]	2.86[b]	8.1[b]
Ethylene	With	0.76[a]	0.450[a]	3.01[a]	5.47[b]
	Without	0.70[b]	0.406[b]	2.91[a]	11.77[a]
Day	1	0.71[b]	0.447[a]	2.94[b]	n.a
	3	0.70[b]	0.397[a]	3.46[a]	n.a
	5	0.79[a]	0.440[a]	2.48[c]	n.a
Hybrids × Ethylene		n.s	n.s	n.s	**
Hybrids × Day		n.s	*	**	n.a
Ethylene × Day		*	*	n.s	n.a
Hybrids × Ethylene × Day		n.s	n.s	n.s	n.a

** = 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 letter were significantly different at $P = 0.05$ using least significant difference (LSD). n.a = Not applicable, vase life was terminated when 30% of florets and buds had wilted.
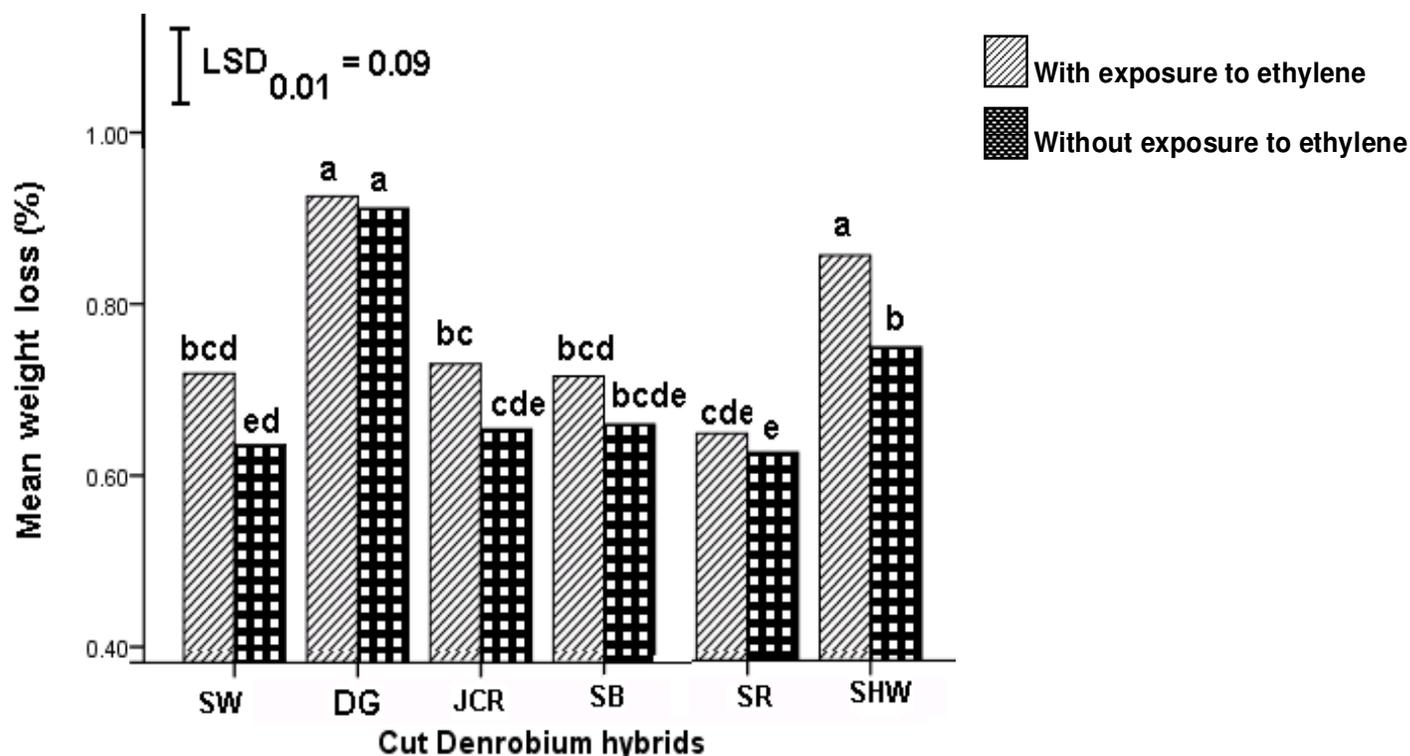

Figure 1. Mean weight loss of six cut Dendrobium hybrids; D. 'Sonia White' (SW), 'Darren Glory' (DG), 'Acqueline Concert × Rinappa' (JCR), Sonia Bom' (SB), 'Sonia Red' (SR) and 'Shavin White' (SHW) with and without exposure to ethylene through day 1, 3 and 5 in vase solution. Means with different letter(s) are highly significant by least significant difference at $p < 0.01$.
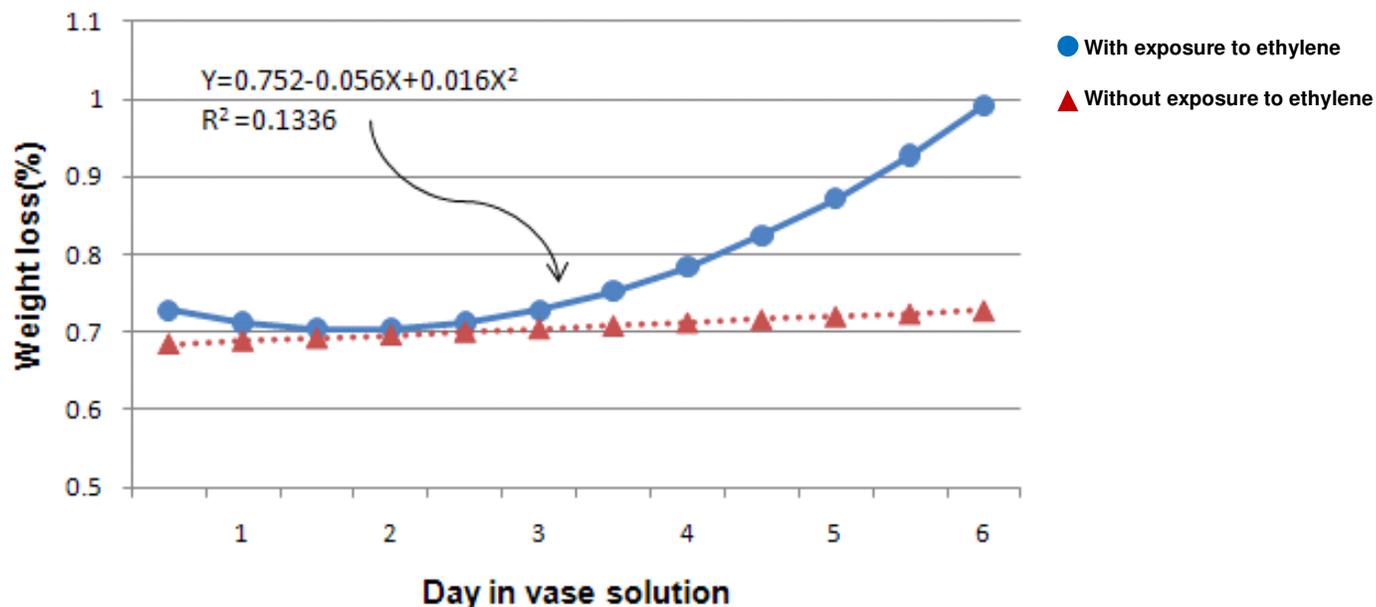
Figure 2. Relationship between 24 h exposure to 10 µL/L ethylene and days in vase solution on weight loss. Broken lines indicate non-significance.
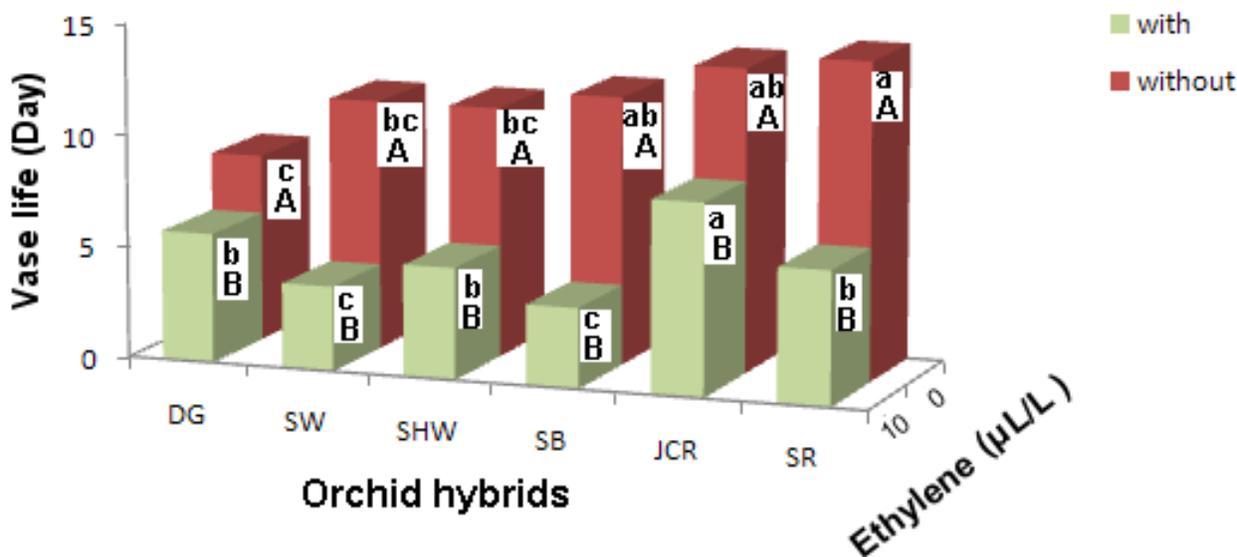

Figure 3. Interaction between hybrids; Dendrobium 'Darren Glory' (DG), D. 'Sonia Red' (SR), D. 'Sonia White' (SW), D. 'Sonia Bom' (SB), D. 'Shavin White' (SHW) and D. 'Jacqueline Concert' × D. 'Rinappa' (JCR) and ethylene treatment (0 and 10 µL/L) on vase life. Means within the row (0 and 10 µL/L) and column (hybrids) followed by different small and capital letter (s) respectively, were significantly different at p ≤ 0.01 using LSD.

relationship between weight loss and days in vase solution for non - ethylene exposed flowers.

Vase life of the cut orchid hybrids significantly decreased with exposure to ethylene and they wilted in less than one week (Table 1). The interaction between hybrids and ethylene treatments was highly significant for vase life (Figure 3). On non-ethylene exposed flowers, D. 'Darren

Glory' and D. 'Sonia Red' had the shortest and longest vase life, respectively. However, with exposure to ethylene, D. 'Jaquelyne Concert' × D. 'Rinappa' had highest vase life of 8.6 days, while D. 'Sonia White' and D. 'Sonia Bom' had the lowest vase life of 3.6 days. Drooping or epinasty of florets and bud drop were observed in all ethylene treatments with varied severities.
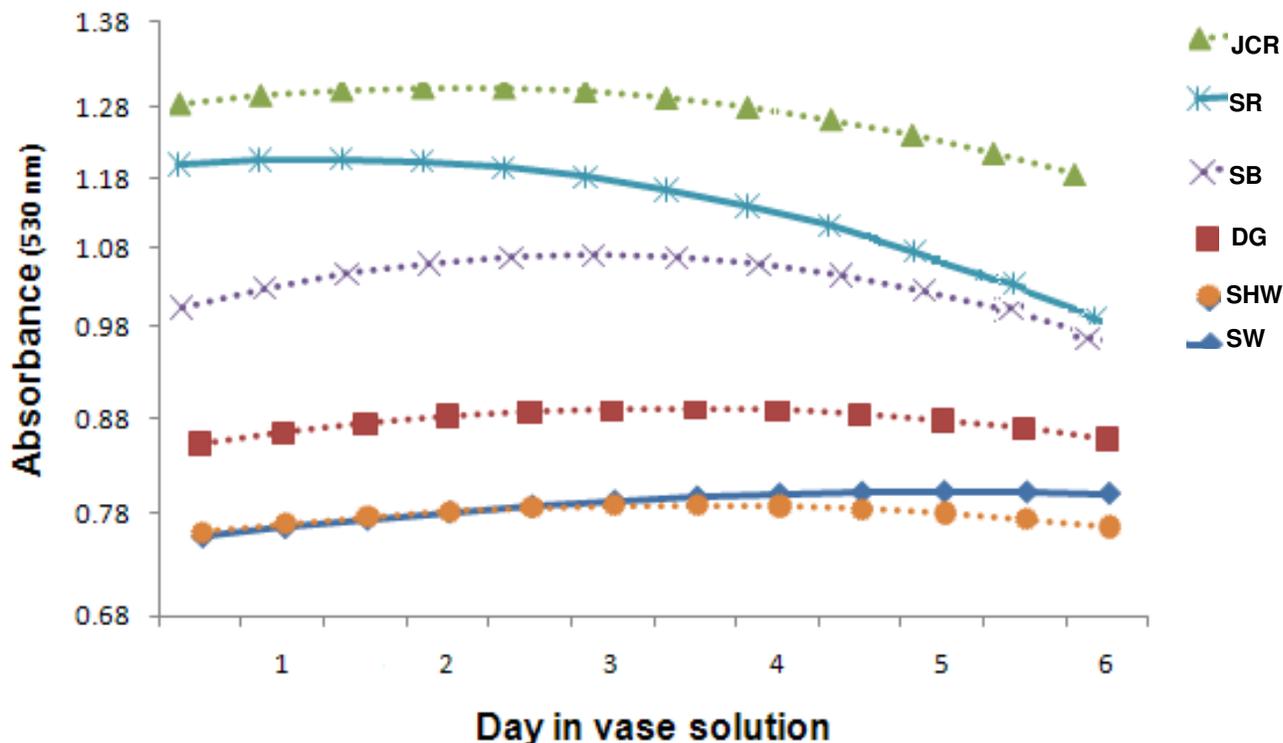
Figure 4. Relationship between anthocyanin content and days in vase solution in six cut Dendrobium hybrids; Dendrobium 'Darren Glory' (DG), D. 'Sonia Red' (SR), D. 'Sonia White' (SW), D. 'Sonia Bom' (SB), D. 'Shavin White' (SHW) and D. 'Jacqueline Concert' × D. 'Rinappa' (JCR). Broken lines indicate non-significance. $Y_{(SW)} = 0.7018 + 0.0098X - 0.00096 X^2$, $R^2_{(SW)} = 0.521$ and $Y_{(SR)} = 1.193 + 0.029X - 0.0104X^2$, $R^2_{(SR)} = 0.353$.

Effect of exogenous ethylene on anthocyanin contents

Table 1 shows that anthocyanin contents in petal and sepal of the cut orchids were highly significantly different and the highest amount of anthocyanin was found in *D.* Jacqueline Concert × *D.* Rinappa, while the lowest was found in *D.* Sonia White and *D.* Shavin White. Interaction between hybrids and days in vase solution on anthocyanin content was significant ($p \leq 0.05$) (Table 1). Furthermore, there were quadratic relationships between anthocyanin content and days in vase solution for *D.* 'Sonia Red'. Anthocyanin content of D. 'Sonia Red' in vase solution from day 3 till day 5 reduced by 14.91% (Figure 4). While for *D.* 'Sonia White', there was an ascending trend in anthocyanin content throughout 5 days in vase solution and anthocyanin content also increased to 2.22%. Meanwhile, there was no significant relationship between hybrids and days in vase solution on anthocyanin content for other hybrids.

Effect of exogenous ethylene on the endogenous ethylene production in six cut orchid hybrids

The ethylene production in the each studied orchid

hybrids was significantly different. The rates of ethylene production between day 1, 3 and 5 in vase solution were different, and the peak of ethylene production was on day 3. However, there were no significant differences between ethylene exposed and non-ethylene exposed hybrids for average ethylene production at day 1, 3 and 5 in vase solution (Figure 5). Meanwhile, interaction between hybrids and days in vase solution on ethylene production was highly significant ($p < 0.01$) (Table 1). Figure 6 demonstrated that ethylene production of *D.* Darren Glory at day 1, 3 and 5 in vase solution changed quadratically as at day 3 in vase solution; ethylene production increased by 25% compared to day 1, but after 3 days in vase solution on day 6, there was no ethylene production (Figure 6). In addition, there were no significant relationship between other hybrids and days in vase solution for ethylene production.

DISCUSSION

Treatment with exogenous ethylene (10 µL/L) for 24 h accelerated the early senescence of perianth in the cut orchids due to loss of water as indicated by percent weight loss that increased within five days in vase solution compared to control (non-exposed to ethylene).
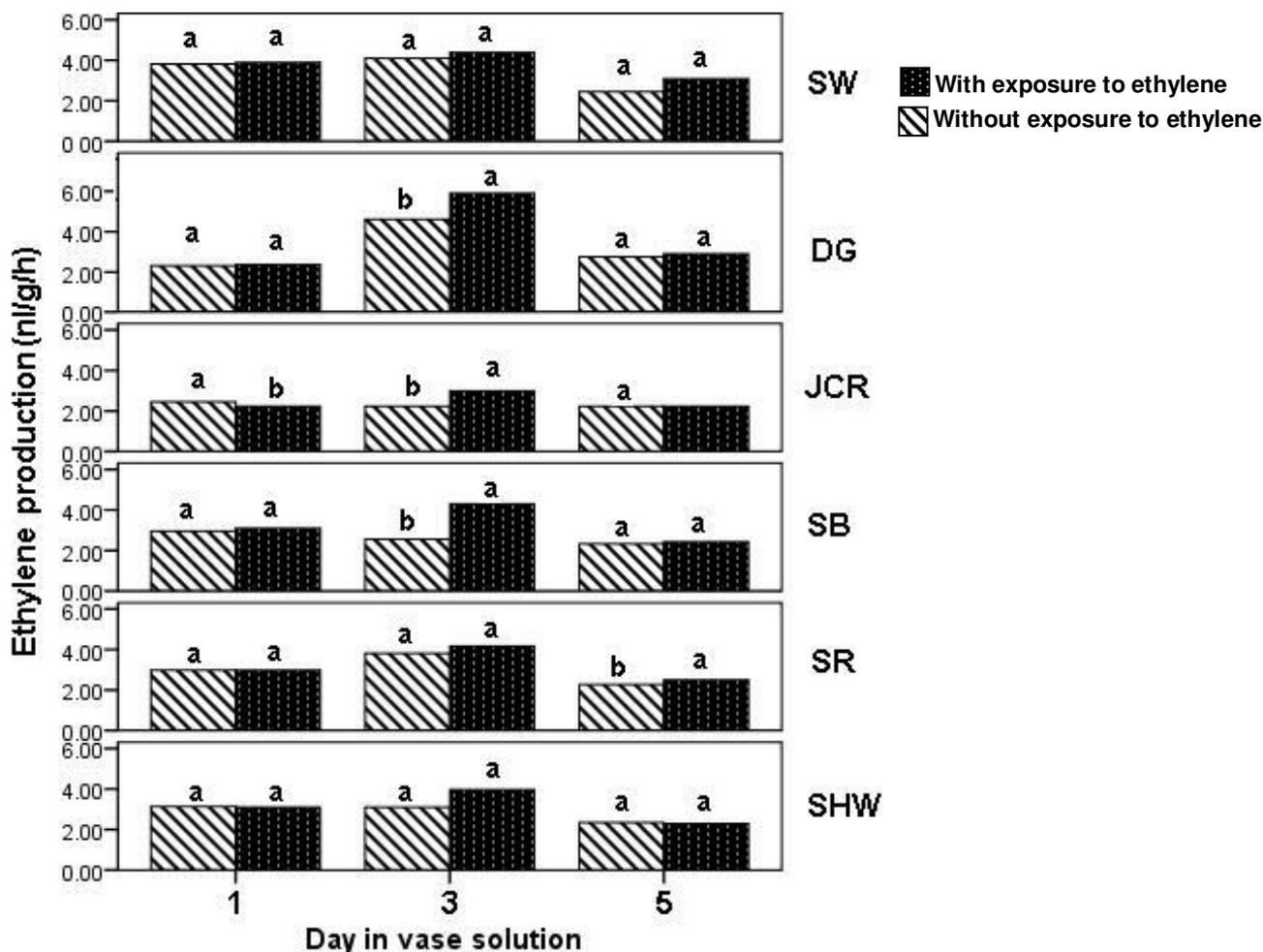
Figure 5. Comparison of the ethylene production of six cut Dendrobium hybrids, Dendrobium 'Darren Glory' (DG), D. 'Sonia Red' (SR), D. 'Sonia White' (SW), D. 'Sonia Bom' (SB), D. 'Shavin White' (SHW) and D. 'Jacqueline Concert' × D. 'Rinappa' (JCR), with and without exposure to ethylene through day 1, 3 and 5 in vase solution. Means with different letter(s) are highly significant by least difference at $p \leq 0.05$.

Interaction between ethylene treatment and days in vase solution on weight loss revealed that with exposure to ethylene, there was rapid weight loss of flowers compared to non ethylene exposed flowers after 3 days in vase solution (Figure 2). This rapid weight loss could be due to pre-activated programming of cell death (PCD) in the perianth. Thus, when PCD is stimulated to occur than normal, the rate of respiration would be increased and also the evaporation and transpiration in the perianth would continue without replacement of water, thereby resulting in water loss and wilting. This therefore suggests that early senescence and wilting is correlated to level of ethylene sensitivity. The results of ethylene sensitivity are in agreement 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). The weight losses in the cut

orchids such as *D.* 'Darren Glory' and *D.* 'Shavin White' were higher than the others (Figure 1) and this could be related to the level of ethylene sensitivity. Furthermore, exogenous ethylene reduced the vase life of orchid hybrids between 31 to 70% in *D.* 'Darren Glory' and *D.* 'Sonia Bom', respectively. In addition, interaction between hybrids and ethylene treatments on vase life was significant, indicating different sensitivities to ethylene. However, the reduction of vase life percentage could not be considered as a sufficient criterion to indicate the ethylene sensitivity because a hybrid like *D.* 'Darren Glory' had only 30% reduction vase life but it had the shortest vase life and highest ethylene production.

Yellowing, drooping, epinasty and venation of florets were major observable signs of senescence (Lerslerwong and Ketsa, 2008). Anthocyanin content was reduced in these cut *Dendrobium* flowers exposed to ethylene. This deterioration and discoloration could be due to an
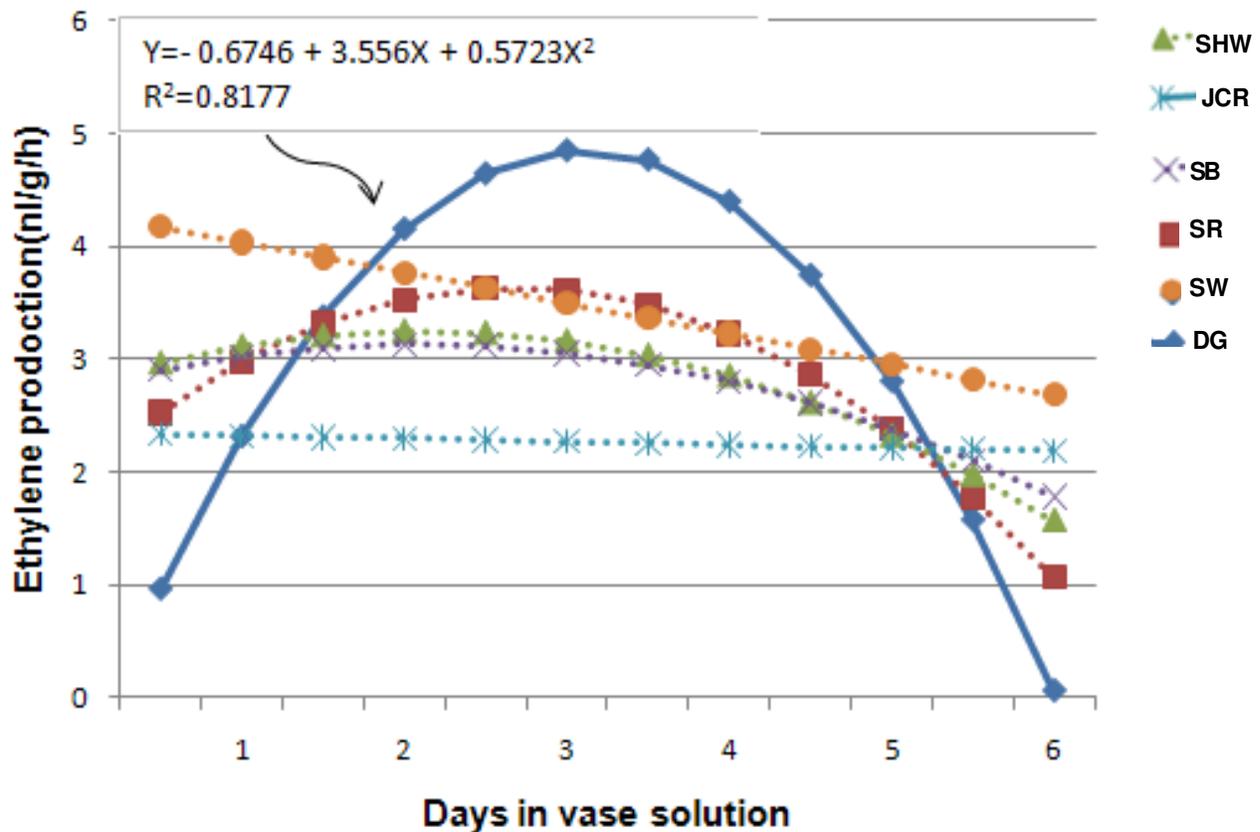
Figure 6. Relationship between ethylene production and days in vase solution in six cut Dendrobium hybrids, Dendrobium 'Darren Glory' (DG), D. 'Sonia Red' (SR), D. 'Sonia White' (SW), D. 'Sonia Bom' (SB), D. 'Shavin White' (SHW) and D. 'Jacqueline Concert' × D. 'Rinappa' (JCR). Broken lines were not significant.

increase of vacuole's pH or enzyme effects such as polyphenol oxidase and peroxidase on senescing flowers as reported by Zhang et al. (2003, 2001). Except for *D.* 'Sonia White', the results of this experiment confirmed the findings of Rebecca et al. (2008) who found that anthocyanin content decreased in senescing cut orchid flowers. However, for *D.* 'Sonia White', anthocyanin content showed an increase trend because when the florets start to senesce, their colour became darker due to browning process (Figure 4). This suggests that the browning of perianth was induced by a loss of water through the sepal and petal via transpiration. The resulting negative water status in the cells causes the diffusion of anthocyanins and other flavonoids from the vacuoles to the functional cytoplasma. Through enzymatic and/or air oxidation, these substances become brown especially in white hybrids and they are precipitated, causing the browning phenomenon (Ferreira, 1983). Thus, absorbance at 530 nm for these flowers increased through 5 days in vase solution. The interaction between hybrids and days in vase solution on anthocyanin content suggested that anthocyanin degradation depended on the hybrids and their sensitivity to ethylene. In addition, anthocyanin content of flowers is

correlated to the stage of flowers growth; when it reaches to maximum level, its biosynthesis would be maximum too (Janna et al., 2005). Furthermore, during wilting, decomposition of anthocyanin would be increased with exposure to ethylene.

The results of exposure to ethylene on ethylene production in *D.* 'Darren Glory', *D.* 'Jaquelyne Concert' × *D.* 'Rinappa' and *D.* 'Sonia Bom' showed that their ethylene production on day 3 was higher than non-exposed to ethylene (Figure 5). These findings confirm the results of Ketsa and Rugkong (1999) that exogenous ethylene resulted in autocatalytic increase in endogenous ethylene of the cut *Dendrobium* 'Pompadour' flowers. However, there were no significant differences on day 3 in other orchid hybrids either exposed or non-exposed to ethylene. This could be due to level of ethylene sensitivity. Interaction between hybrids and ethylene production on day 1, 3 and 5 showed that the trend of ethylene production depending of hybrids is different. Furthermore, with exposure to ethylene occurred a burst in ethylene production and early senescence, especially in sensitive cut orchids. More also, ethylene production had a negative correlation with vase life and positive correlation with weight loss and anthocyanin degradation

with and without exogenous ethylene and this was similar with the results of Bartoli et al. (1997) for chrysanthemum flowers.

Finally, responses among the cut *Dendrobium* hybrids to exogenous ethylene were different. Thus, based on sensitivity to ethylene, the hybrids could be divided into two groups, ethylene sensitive and less ethylene sensitive. The hybrids in the sensitive group are: *D.* 'Darren Glory' and *D.* 'Jacqueline Concert' × *D.* 'Rinappa' because these hybrids were more prone to floret epinasty in addition to bud drop, floret wilting and reduced vase life, while the other hybrids could be considered less ethylene sensitive.

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