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Effect of 6-Benzylaminopurine on flowering of a *Dendrobium* orchid

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Abstract

Dendrobium orchids are popularly used as cut flowers because they come in a wide range of vibrant colours besides being able to produce high number of flowers per inflorescence. There is a significant interest in developing methods to promote early flowering in commercial *Dendrobium* orchids. In this study, the potential effect of benzylaminopurine (BAP) on inducing inflorescence production of a *Dendrobium* hybrid (*Dendrobium* Angel White) was investigated. *D*. Angel White plantlets were subjected to spray containing different BAP concentrations. The results indicated that the application of BAP increased the percentage of inflorescence production, induced earlier flowering, and contributed to the differences in inflorescence length and the number of leaves and flowers produced. However, the application of BAP did not significantly influence the size of the flowers. This study showed that BAP is a potential plant growth regulator that can speed up the flowering process of *D*.Angel White.

Keywords: 6-Benzylaminopurine, cytokinin, *Dendrobium*, flowering, foliar spray, inflorescence.
Abbreviations: BAP- 6-Benzylaminopurine, DAW- *Dendrobium* Angel White.

Introduction

Orchids are an important group of ornamental plants comprising several thousand species and hybrids. Orchids attract almost every kind of individual including professional breeders, amateurs and normal collectors because of their naturally beautiful and uniquely shaped flowers that come in a wide spectrum of vibrant colours. In the year 2005 alone, orchids held 8% share of the worldwide floriculture trade (Martin and Madassery, 2006). Potted *Dendrobium* orchids are produced in a large scale in many countries including China, Taiwan, Thailand, The Phillipines and United States, Japan and Germany. *Dendrobium* is the second-largest orchid genus consisting of more than 1,000 natural species (Puchooa, 2004). These hybrids are in the foremost position in floriculture trade especially in ornamental cut flower industry because of the large variety of beautiful flower sprays (Puchooa, 2004) , its capability in blooming continuously and a prolonged post-harvest life relative to other orchid hybrids (Kuehnle, 2006). However, under normal conditions *Dendrobium* hybrids have a long juvenile period requiring at least two to five years to reach maturity and flowering stage (Hee et al., 2007). Therefore, there is a need to develop a method to speed up the flowering process of *Dendrobium* in order to be competitive in the ever-growing orchid industry. Orchid flower initiation is usually associated with light intensity, (Kataoka et al., 2004), temperature and photoperiod (Vaz et al., 2004) or hormonal changes (Campos and Kerbauy, 2004). Plant growth regulators (PGRs) such as gibberellins, auxins, cytokinins, and abscisic acid have been successfully used in the orchid cut flower industry for many purposes including for flower initiation and development. Cytokinins are considered as a critical physiological signal in triggering the process of flowering (Bonhomme et al., 2000). The level of cytokinins has been reported to increase in the apical meristem during floral transition and flower development in *Arabidopsis thaliana* L. (Corbesier et al., 2003). Nguyen et al. (2006) reported that cytokinin treatment increased the percentage of flowering and helped in developing normal floral buds in roses. The effects of supplying exogenous cytokinin in inducing *in vitro* flowering were also observed in *D*. Sonia 17 (Tee et al., 2008), *D.* Madame Thong-In (Sim et al., 2007), *D.* Chao Praya Smile (Hee et al., 2007), *Cymbidium niveo-marginatum* Mak (Kostenyuk et al., 1999) and *Phalaenopsis* Pink Leopard 'Petra' (Duan and Yazawa, 1995). (BAP) was used to induce *in vitro* flowering of *D.* Chao Praya Smile in just six months from germination (Hee et al., 2007). A mixture of BAP and coconut water was used to induce *in vitro* flowering of *D.* Madame Thong-In in just five months. (Sim et al., 2007). *Cymbidium niveo-marginatum* Mak on the other hand was induced to flower *in vitro* 90 days after the treatment using a more complicated formula which includes BAP treatment, decreased nitrogen concentration, increased phosphate concentration, and root removal (Kostenyuk et al., 1999). The objective of this study was to clarify the effect of BAP in the form of foliar spray on inducing early flowering in a local commercial *Dendrobium* hybrid known as *D.* Angel White.

Results and discussion

BAP treatment and inflorescence production

Based on Fig.1, percentage of inflorescence production for plants with control treatment was 20% compared to (85%) when the plants were sprayed with 200 mg/L of BAP followed by 250 mg/L (75%) and 300 mg/L (45%). This

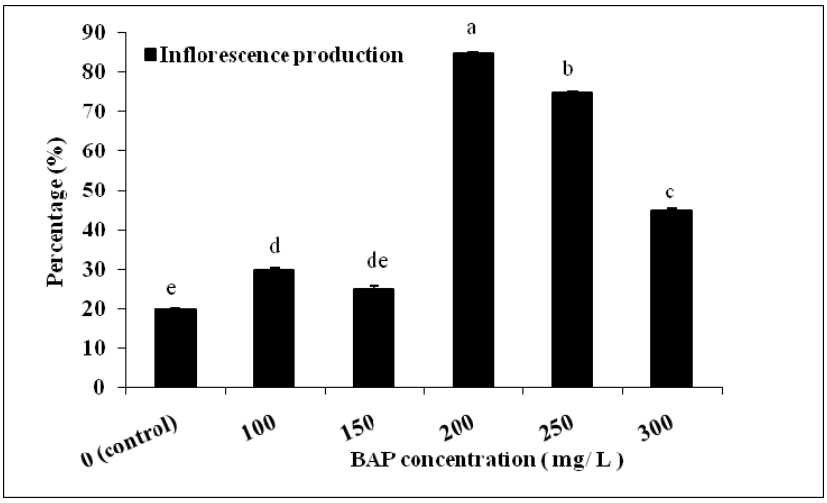

Fig 1. The effect of different BAP concentrations on the percentage of inflorescence production in *D*. Angel White. Bar indicates the standard error of mean. The different letters indicate that the values are significantly different (p≤o.o5).

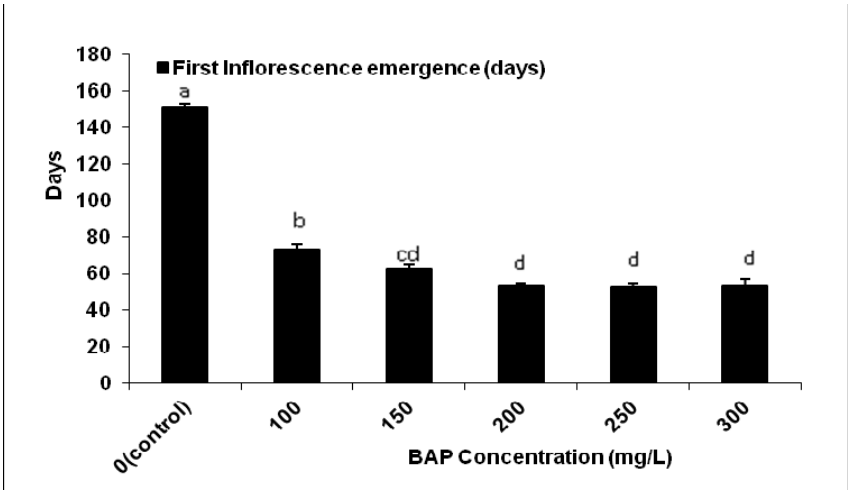

Fig 2. The effect of different BAP concentrations on the days taken for the first inflorescence emergence *D*. Angel White. Bar indicates the standard error of mean. The different letters indicate that the values are significantly different (p≤o.o5).

confirms that the applications of exogenous BAP promote the production of inflorescences in *D.* Angel White although the inflorescence production was not significantly different in plants treated with 100 and 150 mg/L BAP. These results are in agreement with those obtained by Blanchard and Runkle, (2008) who reported that the application of BAP was responsible for inflorescence initiation in *Phalaenopsis* and *Doritaenopsis* orchids. BAP has also promoted flowering in plants other than orchids. Ishimori et al. (2009) induced *in vitro* flowering of *Lilium rubellum* by combining BAP with the right temperature.

BAP treatment and number of days required for inflorescence production

Based on Fig.2, first inflorescence stalk was formed on day 53 in the plants exposed to 200, 250 or 300 mg/L of BAP followed by nine days later for plants sprayed with 150 mg/L of BAP. It took an average of 74 days for plants sprayed with 100 mg/L BAP to produce inflorescence stalk. Control plants, however, produced the first inflorescence stalk only on day 151 which was 98 days later than the plants treated with 200, 250 and 300 mg/L of BAP. None of the plants in this study produced more than one inflorescence stalk. The success of BAP in regulating inflorescence initiation in orchids has been previously reported by Blanchard and Runkle, (2008) who indicated that BAP could at least partially regulate the inflorescence initiation in *Doritaenopsis* and *Phalaenopsis* orchids. The flower promoting effect of BAP on orchids also occurred in *in vitro* plants (Duan and Yazawa, 1995; Sim et al., 2007; Tee et al., 2008).

BAP treatment and floral bloom

Effect of BAP was also examined on the time required for the first floral bloom (as shown in Fig. 3) from the day the treatment was initiated. Similar trend was observed in the number of days needed for the first flower to bloom in which higher BAP concentrations significantly reduced the number of days when compared with control plants. Plants treated with 300 mg/L BAP were the earliest to have its flowers to bloom (59.2 days), followed by 200 mg/L of BAP (60.1

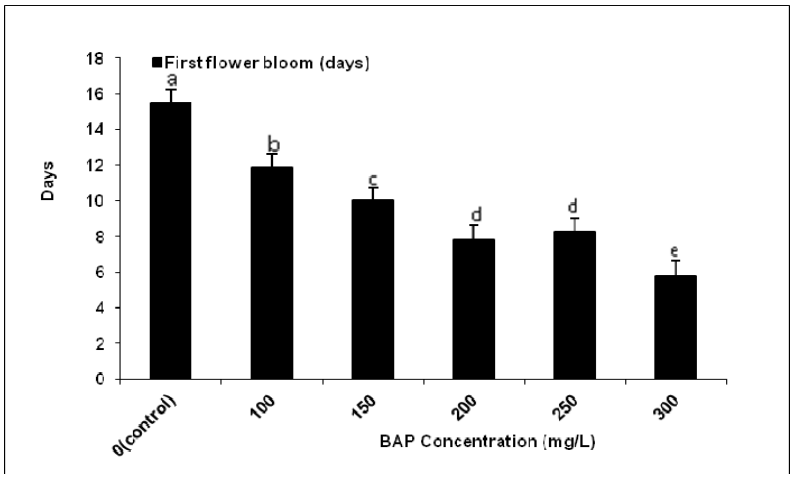

Fig 3. The effect of different BAP concentrations on the days taken for the first floral bloom in *D*. Angel White. Bar indicates the standard error of mean. The different letters indicate that the values are significantly different (p≤o.o5).

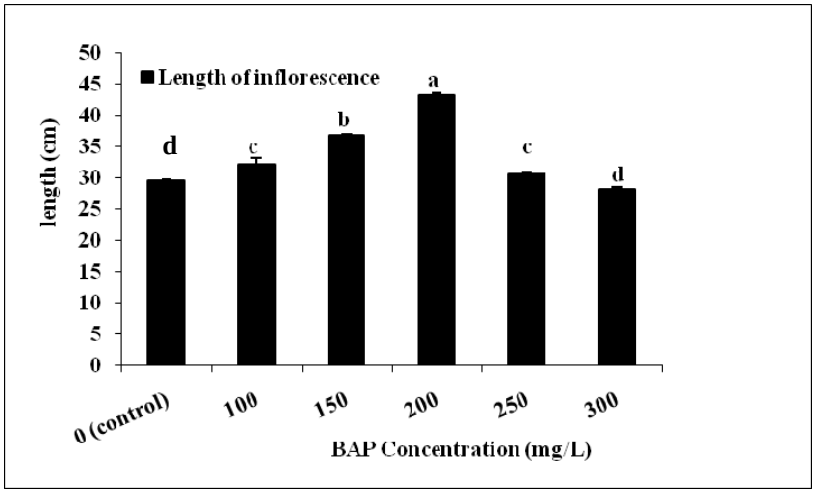

Fig 4. The effect of different BAP concentrations on the inflorescence length (cm) of *D*. Angel White. Bar indicates the standard error of mean. The different letters indicate that the values are significantly different (p≤o.o5).

days), 250 mg/L (61.8 days), 150 mg/L (70.4 days) and 100 mg/L (85 days). Control plants took the longest number of days for the first flower to bloom (166.5 days). In this study, exposure to all the concentration of BAP tested (100-300 mg/L) in the form of foliar spray decreased the days needed for the first floral bloom when compared with control plants. Although BAP treatment was shown to be useful for shortening the days required for blooming, there seemed to be some drawbacks too. One main problem faced by this hybrid mostly in plants treated with higher concentration of BAP was the yellowing and withering of the flower bud before it could bloom. Lorteau et al. (2001) showed that BAP could stimulate ethylene production in plants. In the present study, the yellowing happened in some occasion at a very early stage when the first bud started to form. This could be because of ethylene formation in plants that were treated with BAP especially when higher concentrations were used. Previous study showed the requirement of benzyladenine for normal development of floral buds in *in vitro* plants of roses (Nguyen et al., 2006) whereby the buds started to wilt when they are about 20mm in length before yellowing and finally drop before they could bloom. In *Bougainvillea*, cytokinin application successfully reduced the occurrence of bud abscission before blooming which was a common and serious problem otherwise (Moneruzzaman et al., 2010).

BAP treatment and length of inflorescence

The effect of BAP on the mean length of inflorescence is represented in Fig. 4. Mean length of inflorescence was the highest in plants treated with 200 mg/L of BAP giving an average length of 43.3 cm, followed by plants treated with 150, 100, 250 and 300 mg/L of BAP showing an average length of 36.9, 32.3, 30.7 and 28.3 cm, respectively. Control plants produced inflorescence stalk at an average length of 29.71 cm. Plants treated with 200 mg/L of BAP produced almost 50% longer inflorescence when compared with the control whereas the mean length of inflorescence stalk produced on *D.* Angel White exposed to high concentration of BAP (250 and 300 mg/L) were reduced and not significantly different from plants treated with 100 mg/L and control treatment. The reason behind this phenomenon is still not clear.

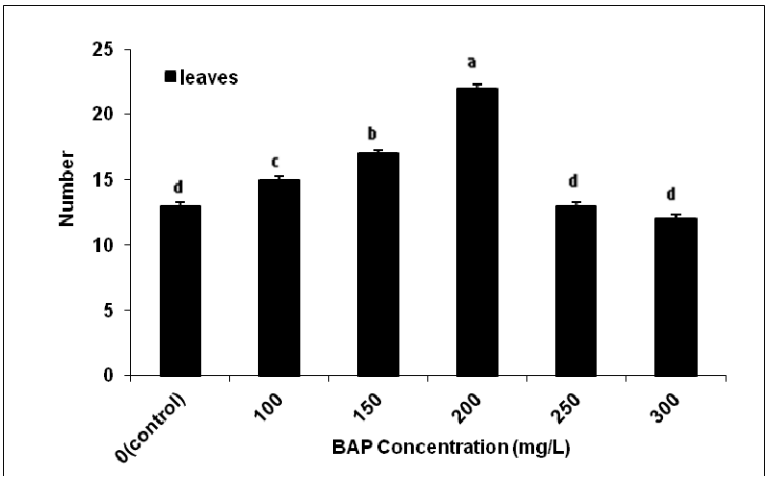

Fig 5. The effect of different concentrations of BAP on number of leaves per plant in *D.* Angel White. Bar indicates the standard error of mean. The different letters indicate that the values are significantly different (p≤o.o5).

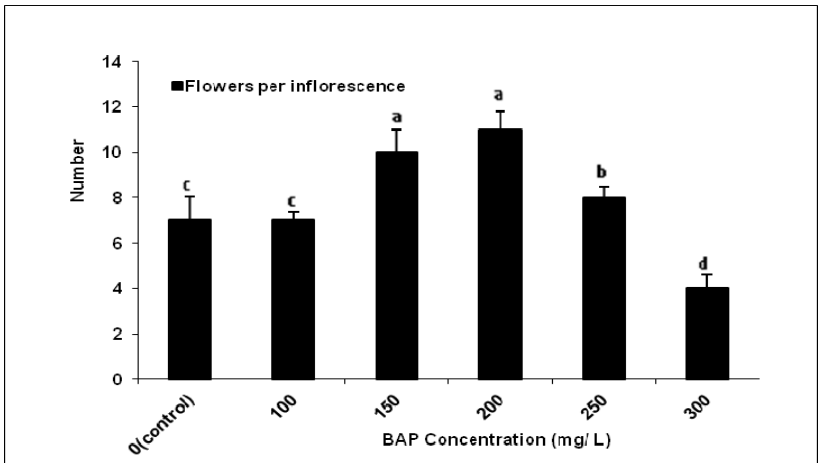

Fig 6. The effect of different concentrations of BAP on number of flowers per inflorescence in *D*. Angel White. Bar indicates the standard error of mean. The different letters indicate that the values are significantly different (p≤o.o5).

BAP treatment and number of leaves

Furthermore, BAP treatments increased the total number of leaves in the plant and are shown in Fig. 5. Plants that were not sprayed with BAP (control) developed approximately 13 leaves per plant, whereas those sprayed with 200 mg/L BAP produced the highest number of leaves followed by plants treated with 150 mg/L of BAP by producing 40.9 % and 23.6 % more leaves than the control plants. These results indicate that the appropriate concentration of BAP (150-200 mg/L) have the effect to increase the number of leaves. Similar response was observed in *Cajanus cajan* L. leaves when the number of leaves and their area (cm^2) from plants treated with cytokinin (Kinetin) was higher than control (Mukherjee and Kumar, 2007). Foliar application of growth retardants in *Bougainvillea* plants successfully produced more lateral branches by suppressing apical dominance (El- Quesni et al., 2007). The formation of new leaves in DAW plants could be due to apical dominance suppression as it is important to note that the term young leaves used in this study refers to increase in number of leaves from new plants formed via lateral branching from the main plant. This result is in agreement with the findings of Hafiz et al. (2009) who reported that the application of exogenous cytokinin could increase the lateral branching of *Jatropha curcas* plant maintained under glasshouse condition. Leaves function as the core site for the production of sugar which will be further translocated to other part of the plants. Blanchard and Runkle (2008) had reported that plants with bigger leaf during the reproduction stage produce more inflorescence and flowers per plant than smaller plants comparatively. However, the relationship between the flowering response and the number of leaves and their sizes in this study remains unclear. A study carried out by Moneruzzaman et al. (2010) in *Bougainvillea* plant proved the effect of defoliation in flowering plants. In future, the leaf size, plant height and endogenous cytokinin changes should be taken into consideration in order to relate number of leaves with the growth of DAW plant and its flowering response.

BAP treatment and number of flowers

The highest number of flowers per inflorescence (14) as recorded for the plants treated with 200 mg/L BAP followed by plants treated with 150 mg/L BAP which showed 31% less number of flowers than those with 200 mg/L (Fig. 4). Both control plants and those exposed to 100 mg/L BAP

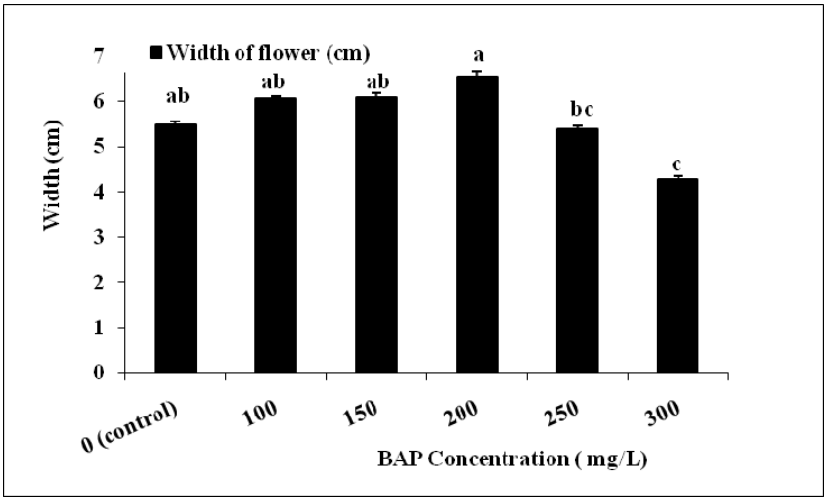

Fig 7. The effect of different concentrations of BAP on the flower width of *D.* Angel White. Bar indicates the standard error of mean. The different letters indicate that the values are significantly different (p≤o.o5).

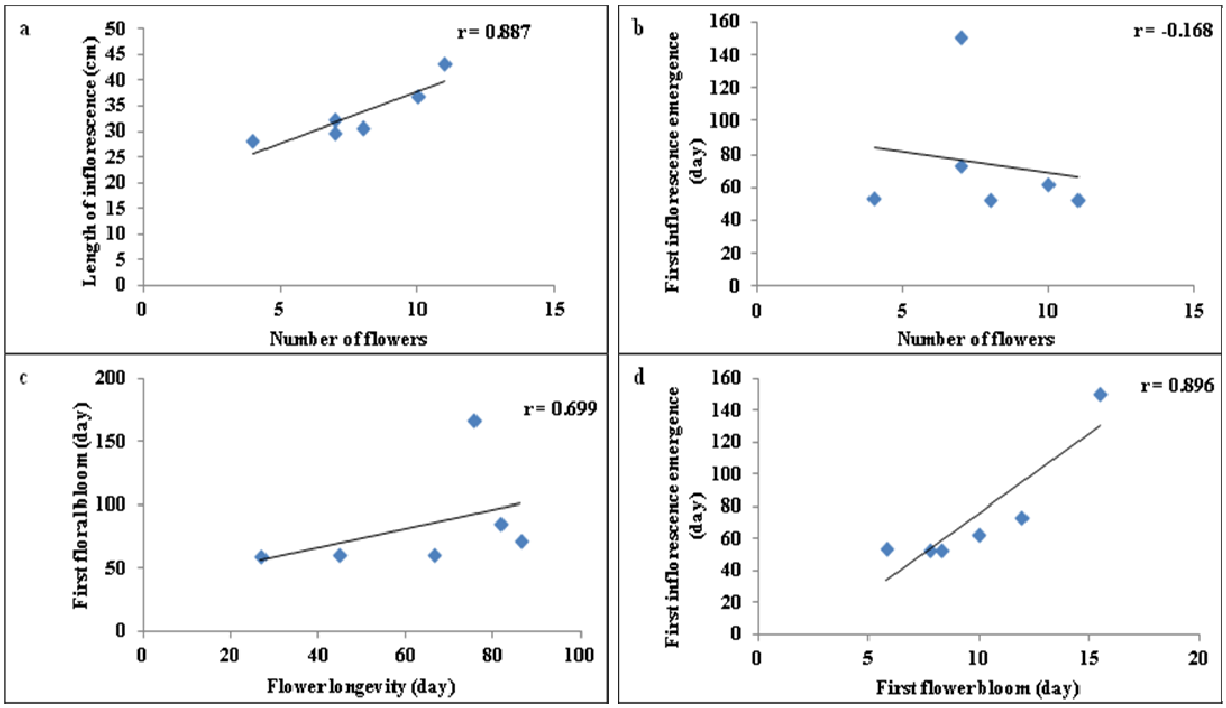

Fig 8. Linear regression analysis of the average value from each BAP concentration for (a) length of inflorescence (cm) versus the number of flowers produced. (b) number of days taken for the first inflorescence emergence versus number of flowers produced. (c) number of days taken for the first flower to bloom versus the longevity of flower and (d) number of days taken for the first inflorescence emergence versus number of days taken for the first floral bloom.

produced an average of 7 flowers. Plants sprayed with 250 mg/L and 300 mg/L of BAP produced an average of 9 and 5 of flowers, respectively. When comparing all the treatments, the lowest number of flowers was produced from plants that were treated with the highest concentration of BAP (300 mg/L), where the flowers began to wither at early stage if compared to the flowers from all the other treatments. Other studies have also shown the same type of result when the application of cytokinin enhanced the number of flower buds on inflorescences in other *Dendrobium* (Sakai, 2000). In a study carried out by Bang and Zeng (2010), number of flowers in *Jatropha curcas* increased when exposed to exogenous benzyladenine treatment, which was believed to be due to the ability of cytokinin to regulate the activity in inflorescence meristem. Combination of cytokinin treatment and branch defoliation was effective in increasing the number of floral buds in *Bougainvillea* plant (Moneruzzaman et al., 2010).

BAP treatment and flower width

The average widths of the flowers from the treated *D.* Angel White plants were shown in Fig. 7. The mean width of flower is the biggest for plants treated with 200 mg/L of BAP (6.2 cm). Plants treated with 300 mg/L of BAP produced flowers with the lowest mean width (4.3 cm) whereas the highest width (6.1 cm) at 150 and 100 mg/L of BAP treatments

Fig 9. The effect of BAP treatments on flowering of *D*. Angel White plants. Flowers from plants treated with BAP at 100 mg/L (a), 150 mg/L (b), 200 mg/L (c),250 mg/L (d), and 300 mg/L (e), respectively.

followed by 250 mg/L BAP (5.39 cm) and control plants (5.9 cm). Zepeda et al. (2006) had reported the same kind of situation when the application of cytokinin bioregulator caused an increase in the ovary diameter of flowers of seedless table grapes. In the present study, it is also possible that the change of ovary size affected the width of flower although the diameter of the ovary from the flowers was not measured.

Regression analysis

A linear regression analysis (Fig. 6) was carried out between the length of inflorescence (cm) and number of flowers, number of days taken for first inflorescence emergence and number of flowers, first floral bloom and flower longevity and finally between number of days for first inflorescence emergence and first floral bloom. This analysis was performed to investigate the relationship between the parameters mentioned above. It is important to understand the correlation of these characters as they can be used as a selection tool to predict flowering performance of a plant. There was a positive correlation (r=0.887) between the length of inflorescences and the number of flowers that were formed (Fig. 6a).The number of flowers, however was not influenced by the number of days taken to induce the first inflorescence stalk since no clear correlation (r=-0.168) was found between the first inflorescence emergence (day) and number of flowers per inflorescence (Fig.6b). Therefore, in DAW, an early emergence of inflorescence cannot be used as an indicator to expect a huge floral spray. The correlation between the number of days for first inflorescence emergence and first floral bloom was relatively high (r=0.896).

Materials and methods

Plant materials and maintenance

One year old cloned *D.* Angel White hybrids were used in this experiment. Young and healthy plants uniform in height 15cm with an average of six leaves were obtained from a local nursery. The plants were repotted into ceramic pots (15cm x 13cm) containing charcoal and broken pieces of bricks at an equal ratio of 1:1. Plants were maintained in a greenhouse at $24^{o} \pm 2^{o}$C under a natural photoperiod for 60 days to acclimatize the young plantlets to the new environment before exposing them to any treatment. The plants were watered once daily. Two types of fertilizers, Garden Foliar Fertilizer 67 and 63 (13 N: 27 P: 27 K and 21 N: 21 P: 21 K;YMWOO Corporation, Malaysia) were sprayed (as instructed) on the plants after watering at an interval of two days.

BAP application

Plants were exposed to foliar spray of various BAP (Sigma-Aldrich, Germany) concentrations ranging from 0 (control) to 300 mg/L. (i.e., 0,100, 150, 200, 250, and 300 mg/L). Each plant was sprayed with ten ml of freshly prepared BAP at concentrations mentioned above. Control plants were sprayed with ten ml of distilled water. Spraying was conducted in the dusk on a weekly basis for the first month, followed by application of every two weeks in the subsequent months. All the plants were maintained under greenhouse conditions as previously mentioned. This experiment was conducted for six months.

Data collection

Observation was carried out weekly. The following parameters were measured and recorded: percentage of inflorescence production (%), days taken for the first inflorescence emergence and first floral bloom, length of inflorescence (cm), number of flowers per inflorescence, total number of leaves per plant and the width of flowers (cm) produced. Width of the flower is measured as the length from one end of the petal to the other end of another petal.

Statistical analysis

The experiment was a completely randomized design with six experimental treatments, three replications of each and 10 pots per replication. Data were analysed by analysis of variance, and treatments were compared using the least significance difference (LSD) test at P=0.05.

Conclusion

This study indicates great potential for speeding up flowering process in *Dendrobium* orchids by the application of plant growth regulators especially cytokinin and to aid in further improvements through traditional breeding techniques. In future, plant growth regulators would be able to induce synchronous flowering in orchids which can help in maintaining a steady position in the floriculture industry.

Acknowledgements

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