EFFECT OF BENZYLADENINE ON FLOWERING OF A *Dendrobium sonia* ORCHID IN CASE OF GREENHOUSE CONDITION

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SUMMARY

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. The greenhouse experiment were conducted on *Dendrobium sonia* orchid and arranged in Randomized Complete Block Design with three replications in nethouse condition at Ho Chi Minh city. In this study, the potential effect of Benzyladenine (BA) on inducing inflorescence production of *Dendrobium* hybrid (*Dendrobium sonia*) was investigated. *Dendorobium sonia* plantlets were subjected to spray containing different BA concentrations (30 ml/plant) at concentration from 45 to 120 ppm. The results indicated that the application of BA 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. The the application of BA at 90 ppm was suitable in improvement the percentage of visible inflorescence, flower diameter, number of flowers on the first inflorescence, number of flowers on pseudo-bulb and flower life. This study showed that BA is a potential plant growth regulator that can speed up the flowering process of *Dendrobium sonia* in greenhouse condition at Ho Chi Minh city, Viet Nam.

Keywords: **Benzyladenine, cytokinin, *Dendrobium*, flowering, foliar spray, inflorescence.**

1. 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, Vietnam, 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 evergrowing 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 effects of supplying exogenous cytokinin in inducing *in vitro* flowering were also observed in *D.* Chao Praya Smile (Hee et al., 2007), *Cymbidium niveomarginatum* Mak (Kostenyuk et al., 1999) and *Phalaenopsis* Pink Leopard 'Petra' (Duan and Yazawa, 1995).

The objective of this study was to clarify the effect of BA in the form of foliar spray on inducing early flowering in a local commercial *Dendrobium* hybrid known as *Dendrobium sonia*.

2. RESEARCH METHODOLOGY

Plant materials and maintenance

Nine month old cloned *Dendrobium sonia* 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 coconut fiber powder at an equal ratio of 1:1. Plants were maintained in a greenhouse at $28^0 \pm 2^0$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. The Boom Flower foliar fertilizer were sprayed on the plants two times a week.

BAP application

Plants were exposed to foliar spray of various BA concentrations ranging from 0 (control) to 120 ppm (0, 45, 60, 75, 90, 105 and 120 ppm). Each plant was sprayed with 30 ml of freshly prepared BA at concentrations mentioned above. Control plants were sprayed with 30 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 (from Dec 2020 to June 2021) at Ho Chi Minh city, Viet Nam.

Data collection

The experiment was set up as one - way factorial design (Randomized Complete Block Design) with 3 replications, each spot has 3 pots (Fig. 1). Observation was carried out weekly. The following parameters were measured and recorded: percentage of visible inflorescence, days taken for the first inflorescence emergence and first floral bloom, diameter of first flower, average diameter of flowers per inflorescence, number of flowers per inflorescence, length of inflorescence (cm), number of flowers per inflorescence, total number of inflorescence per plant, flower life.

Statistical analysis

The experiment was a completely randomized design with seven experimental treatments, three replications of each and 10 pots per replication. Data were analysed by analysis of variance, using a one-way analysis of variance (GLM procedure in SAS version 9.2, SAS Institute Ltd., North Carolina, USA). Tukey's HSD multiple range test results were considered signifcant at $p<0.01$.

Fig. 1. The overview of experiment

3. RESULTS AND DISCUSSION

Effect of BA concentrations on number of days required for inflorescence and flowering production

The experimental results showed that the earliest inflorescence appeared when spraying BA concentrations of 45, 90 and 105 ppm was 6 days after application (DAA). This was also consistent with the experimental results of Goh (1978) when inflorescence appeared at 9 or 10 days after he injected BA on the pseudo - bulb *Dendrobium* Louise Dark at two concentrations of 10^{-4}M and 10^{-3}M. Tee el all (2008) also gave similar results in the *in vitro Dendrobium sonia* 17 flowering induction experiment, in which explants in medium containing BA appeared to flower after 6 months of culture while other explants in BA-free medium did not produce flowers. In addition, Blanchard and Runkle (2008) found that *Doritaenopsis* and *Phalaenopsis* sprayed with BA concentrations of 200, 400 ppm produced flowers earlier than control from 3 to 9 DAA.

The results (Table 1) showed that there was a significant difference ($P<0.01$) in visible inflorescence production at different BA concentrations. This results was also consistent with the study of Goh and Yang (1978), Goh (1979), Tee et al. (2008), Sakai and Ichihara (2000). Experiments by Goh and Yang (1978) on two varieties *Dendrobium* Lady Hochoy and *Dendrobium* Buddy Shepler x

Dendrobium Peggy Shaw concluded that the flowering of these two orchids requires cytokinin. When further experiments on *Dendrobium* Louisae, Goh (1979) found that the induction of inflorescence occurred in treatments applied BA at a concentration of 225 ppm while the control did not appeared within 3 weeks after application. Experimental results of Tee et al. (2008) concluded that *Dendrobium* Sonia 17 was induced to flower in 1/2 MS medium containing 20 µM BA. Similarly, BA was also found to induce flowering in *Dendrobium* Red Emperor at concentrations of 450, 900 and 4,500 ppm, while the treatments without BA application did not have any flower.

Table 1. Effect of BA concentrations on visible inflorescence and flowering production of *Dendrobium sonia*

BA concentrations (ppm)	Visible inflorescence production (DAA)	Ratio of visible inflorescence production at 30 DAA (%)	Flowering production (DAA)
0	0.00 b[a]	0.00 b[b]	0.00 b a[a]
45	6.00 a	77.78 a	58.00 a
60	8.00 a	77.78 a	60.00 a
75	9.00 a	77.78 a	56.00 a
90	6.00 a	88.89 a	56.00 a
105	6.00 a	88.89 a	55.00 a
120	10.00 a	88.89 a	63.00 a
Cv (%)	21.63	30.38	3.32
P	**	**	**

[a]: The values are converted to lg(x +1); [b]: The values are converted to arcsin√% angle.

In the same average group, the values with the same accompanying characters do not have statistical significance P <0.05; ns: none significant; [*]significant difference (p < 0.05); [**]significant difference (p < 0.01).

The rate of inflorescence appearance at 30 days after application was highest in the treatment sprayed with BA at concentrations of 75, 90, 105 ppm (88.89%) compared to control (0%). The treatment at 45, 90 and 105 ppm gave the earliest flowers, followed by treatments at 60 and 75 ppm.

Effect of BA concentrations on flower size and number of flowers per inflorescence

Flower size is one of the important parameters of flower quality, especially for *Dendrobium sonia* flowers which harvested by cutting inflorescence. One of the criteria for evaluating beautiful or ugly flowers is how big or small the petals are. The beautifull inflorescence of *Dendrobium sonia* requires many flowers and large flowers.

The results showed that the first bloom diameter was the largest in the treatment sprayed with BA concentration of 90 ppm but there was no statistical difference with the treatments sprayed with BA concentration of 45, 60, 75, 105 ppm.

The mean inflorescence diameter of the inflorescence was the largest in treatments at 90 and 105 ppm concentrations and this difference was statistically significant (P < 0.01). Thus, BA concentration has an effect on flower size in *Dendrobium sonia* orchids. This result was also consistent with the experimental results of Anna and Danuta (2003), Moazzam et al. (2011). Experiments on *Dianthus caryophyllus* L. by Anna and Danuta (2003) showed that kinetin spraying at concentrations of 0.05 mM and 0.1 mM also increased flower diameter in *Carnations*. Similarly, the experiment on *Polianthes tuberose* L. by Moazzam et al. (2011) also showed that the 50 ppm BA treatment had the largest flower diameter compared with the 100 ppm BA treatment and the untreated control. Cytokinins have also been reported to increase the size of transgenic *Pectunia* flowers (Nishijima et al., 2006).

Table 2. Effect of BA concentrations on the first bloom flower diameter, average flower diameter of inflorescence and number of flowers of inlorescence

BA concentrations (ppm)	Diameter of first bloom (cm)	Average flower diameter of inflorescence (cm)	No. flowers of inflorescence
0	0.00 b[a]	0.00 d[a]	0.00 c[a]
45	9.20 a	9.04 b	2.67 ab
60	9.23 a	9.10 ab	2.67 ab
75	9.33 a	9.03 b	2.33 b
90	9.52 a	9.38 a	4.00 a
105	9.52 a	9.11 ab	2.67 ab
120	9.17 a	8.90 c	2.33 b
Cv (%)	0.96	1.07	8.43
P	**	**	**

[a]: The values are converted to $y = (x + 0,5)^{1/2}$;

In the same average group, the values with the same accompanying characters do not have statistical significance P <0.05; ns: none significant; [*]significant difference (p<0.05); [**]significant difference (p<0.01).

Effect of BA concentrations on inflorescence size

The size of the inflorescence is one of the factors that determine the quality of flowers of *Dendrobium sonia* potted and cut flowers because it is related to the needs of consumers. Consumers generally prefer *Dendrobium sonia* inflorescence long and numerous flowers. The study results showed that the longest inflorescence was in the treatment with BA concentration of 60 ppm (17.33 cm) (Fig. 2) but there was no statistical difference for the other treatments sprayed with BA. Thus, BA has an effect on inflorescence length. This result was also consistent with the experimental results of Goh (1978) on *Dendrobium* Louise Dark when the average inflorescence length in the BA-treated treatment was 64 cm, while the inflorescence length in the treatments was not handle is 50 cm. However, when plants were treated with BA at high concentrations, the inflorescence length was reduced and caused the abnormal flower pattern (Sakai et al., 2000). The length of the inflorescence was also shown in Table 3. The inflorescence in the treatment at BA concentration of 15 ppm was longer than that of the other treatments, the treatment with 120 ppm BA had the shortest inflorescence.

Table 3. Effect of BA concentrations on inflorescence size of *Dendrobium sonia*

BA concentrations (ppm)	Inflorescence lenght (cm)	Section length bring flowers (cm)	Inflorescence perimeter (mm)
0	0.00 b[a]	0.00 c[b]	0.00 c[a]
45	15.33 a	5.20 a	12.67 b
60	17.33 a	5.22 a	13.67 ab
75	16.00 a	5.25 a	13.00 b
90	16.67 a	4.72 a	14.00 a
105	16.23 a	4.25 ab	13.72 ab
120	14.00 a	3.60 b	13.17 b
Cv (%)	4.17	12.00	2.76
P	**	**	**

[a]: The values are converted to lg (x +1), [b]: The values are converted to $(x + 0,5)^{1/2}$

In the same average group, the values with the same accompanying characters do not have statistical significance P <0.05; ns: none significant; [*]significant difference (p<0.05); [**]significant difference (p<0.01).

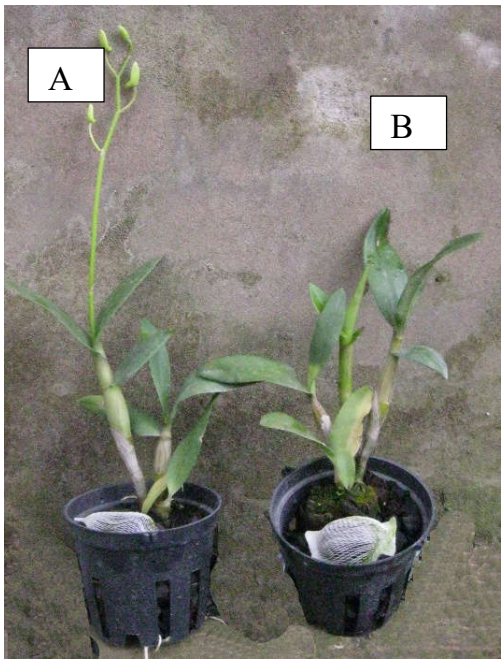

Fig. 2. Inflorescence appeared in orchids treated with BA (A) and did not appear in orchids not treated with BA (B)

Effect of BA concentration on number of inflorescence and total flower on pseudo-bulb

The total number of inflorescences and the total number of flowers on the pseudobulbs are two important parameters, showing the flower yield of orchids. The higher the yield of the orchid, the faster the grower recovers capital and profits. In addition, consumers often prefer orchid pots with many inflorescences on the same pseudobulb.

The research data showed that the highest number of inflorescences were in the treatment with BA concentrations of 90, 120 ppm and this difference was very significant compared with the other treatments. This was similar to the experimental results of Sakai et al. (1998) when BA application on *Dendrobium* Jap Hawaii "Uniwai Pearl" formed 1.70 inflorescences, more than twice that of the untreated treatments (0.80 inflorescence). The research results of Blanchard and Runkle (2008) on *Phalaenopsis* Golden Treasure '470' also showed that the number of inflorescences increased when BA was treated in the concentration range of 100-400 ppm.

The total number of flowers on pseudobulbs was highest at the concentration of 90 ppm ($p \leq 0.01$). Thus, BA had a significant effect on the total number of flowers on pseudobulbs. This result was also consistent with the experimental results of Sakai et al. (2000) on *Dendrobium* Jaquelyn Thomas at one year old. Sakai el all,. (2000) found that the number of flowers in the treatment with 100 mM BA was more than that in the untreated treatment (8.92 and 0.52 flowers respectively), the treatments with 100 mM BA and 10 mM in 2-year-old plants produced more flowers than the untreated treatment (6.32, 4.00 and 0.24 flowers, respectively). The experiment of Blanchard and Runkle (2008) on *Doritaenopsis* also gave similar results that the BA treatments at 200, 400 ppm gave more flowers than the control from 3 to 8 flowers.

In summary, through our study, we found that BA treatment at a concentration of 90 ppm for 9-month-old *Dendrobium sonia* orchids gave the best yield and flower quality.

Bảng 4. Effect of BA concentration on number of inflorescence and total flower on pseudo-bulb of *Dendrobium sonia*

BA concentration (ppm)	No. inflorescence on pseudo-bulb	Total flowers on pseudo-bulb
0	0.00 c[b]	0.00 d[b]
45	1.67 ab	2.67 bc
60	2.00 a	4.33 b
75	2.00 a	3.33 bc
90	2.27 a	5.67 a
105	2.00 a	3.33 bc
120	2.67 a	2.33 c
Cv (%)	9.01	9.21
P	**	**

[a]: The values are converted to lg (x +1), [b]: The values are converted to $(x + 0.5)^{1/2}$

In the same average group, the values with the same accompanying characters do not have statistical significance P < 0.05; ns: none significant; [*]significant difference (p<0.05); [**]significant difference (p < 0.01).

4. CONCLUSION

The application of BA 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. The the application of BA at 90 ppm was suitable in improvement the percentage of visible inflorescence, flower diameter, number of flowers on the first inflorescence, number of flowers on pseudo-bulb and flower life.

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ẢNH HƯỞNG CỦA NỒNG ĐỘ CHẤT ĐIỀU HÒA SINH TRƯỞNG BA ĐẾN QUÁ TRÌNH RA HOA CỦA LAN *Dendrobium sonia* TRONG ĐIỀU KIỆN NHÀ MÀNG

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TÓM TẮT

Dendrobium là chi lan được biết đến như một loại hoa thu hoạch bằng cách cắt cành bởi chúng rất đa dạng về màu sắc, cho hoa nhiều trên bông và cho năng suất bông cao. Một trong những câu hỏi đặt ra làm thế nào thúc đẩy quá trình ra hoa sớm, rút ngắn thời gian chăm sóc đối với loài hoa lan có tính thương mại cao này là vấn đề đang rất được quan tâm. Thí nghiệm được thực hiện theo kiểu khối đầy đủ ngẫu nhiên một yếu tố, ba lần lặp lại trong điều kiện nhà màng tại Thành phố Hồ Chí Minh với mục đích xác định hiệu quả và nồng độ thích hợp của chất điều hòa sinh trưởng BA trên lan *Dendrobium sonia*. Mỗi nghiệm thức được phun với liều lượng 30 ml BA/cây ở nồng độ từ 45 – 120 ppm. Kết quả cho thấy việc xử lý BA đã làm tăng tỷ lệ xuất hiện phát hoa, cảm ứng ra hoa sớm, làm tăng chiều dài phát hoa và chất lượng hoa lan *Dendrobium sonia*. Trong đó, xử lý BA ở nồng độ 90 ppm là thích hợp để cải thiện tỷ lệ xuất hiện phát hoa, đường kính hoa, số hoa trên bông, số hoa trên giả hành và tuổi thọ của hoa.

Từ khóa: Benzyladenine, cytokinin, *Dendrobium*, kích thích ra hoa, phát hoa.

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