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EFFECTS OF NPK AND 6-BENZYLAMINOPURINE ON VEGETATIVE GROWTH AND FLORAL INDUCTION IN TWO *DENDROBIUM* SPECIES

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Abstract

In the present study, the effects of Nitrogen (N), Phosphorus (P), Potassium (K), and 6-Benzylaminopurine (BAP) on vegetative growth and floral induction under green house conditions in two *Dendrobium* species namely *Dendrobium chrysotoxum* and *D. williamsonii* were observed. The dosages of N, P and K fertilizer used were 1:1:1 for T_1, T_2, and T_3, 2:1:1.5 for T_4, T_5, and T_6 and 3:1:1.5 for T_7, T_8, and T_9 treatments. In another experiment, different concentrations (15, 25, 50, 75, 100, 125,150,175 and 200mgl^{-1}) of growth hormone (BAP) were also used in the above treatments, respectively with a view to assessing the time taken for flowering and the other related parameters. In *Dendrobium chrysotoxum*, the plants responded to the higher N ratio (T_7) resulting in optimal plant height (13 cm), width of leaf (3.4 cm), leaf length (10.7cm) and the number of leaves per plant (4). In *Dendrobium williamsonii* with the application of NPK, the increased ratio (T_7) was proved optimal to produce more vegetative growth; at the time of harvest, the highest plant height (11.8cm), leaf length (9.7cm), leaf width (2.1cm) and number of leaves (5.1) were recorded in treatment T_7. Positive effects of exogenous application in general, of BAP were also observed on plants in promoting early flowering in both the species.

Introduction

DENDROBIUM ORCHIDS are most liked commercial plants which are used both as cut-flowers and potted-plants. Apart from the floricultural value, these have medicinal properties as well. Over 60 species of *Dendrobium* are found in China, and many studies have confirmed their importance as antioxidant, immunity enhancer, and antitumor agents (Ye and Zhao, 2002). Amongst dendrobiums of medicinal importance, *D. chrysotoxum* known as gold orchid is the most commonly used herb in the Chinese medicine (Li *et al.*, 2005). It is also known to be used in cosmetics as an active agent for preventing or delaying the appearance of the signs of skin ageing by promoting cell or tissue longevity. Leaves and stem of *Dendrobium williamsonii* are used for healing fractured bones by the Minpa tribal (Medhi and Chakrabarti, 2009). *Dendrobium* was reported to be the second most valuable orchid genus sold in Japan in 2002 which had a market share of 20% only behind *Phalaenopsis* Blume (Laws, 2004; Wang, 2004).

As compared to other crops, the studies on effect of fertilizers on orchids are scarce. Fertilizer deficiency is a widespread disorder in many crops including orchids that can lead to death of the plants if supplementation with appropriate nutrients is not done in time. Fertigation in orchids has always been a controversial subject and often confusing. According to Scully (1951), orchid plants require little fertilizer because of their slow growth, but Adams (1970) recommended five different fertilizers monthly for best growth and flowering. Davidson (1957) observed that deficiencies of Nitrogen (N) and Phosphorus (P) deteriorated growth of *Cattleya* more drastically than did Potassium (K), Calcium (Ca) or Magnesium (Mg) in gravel culture and they responded efficiently to applications of N and P. Lunt and Kofranck (1961) stated that high rates of N fertilizer in liquid form when applied weekly, promoted vegetative growth at the expense of flowering in *Cymbidium* species grown in two grades of fir bark. According to Bernier *et al.* (1993), cytokinins act as one of the multifactorial components that function as the floral stimulus. The effects of exogenous supply of cytokinins in inducing *in vitro* flowering were also observed in *Dendrobium* sonia17 (Tee *et al.*, 2008).

There are very few reports regarding improvement in the production of *Dendrobium* orchids. This investigation indicates that growth, development and flower induction in two species of *Dendrobium* could be regulated by the NPK fertilizer and growth regulator 6-Benzylaminopurine (BAP). The objective of this study was to determine the optimal ratio of NPK with or without BAP for assessing the vegetative growth and the time taken for first flower opening, of *Dendrobium chrysotoxum* and *D. williamsonii*.

Materials and Methods

The experimental site is situated at a latitude of 25°14'.27" North and longitude of 93°59'.913" East and at an elevation of 1197m above Mean Sea Level

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(MSL). The range of temperature variation is between 32°C and 3°C. The relative humidity ranges between 60 - 90% and the average annual rainfall is about 1505 mm. Live plants of the investigated species were collected from two different localities from Manipur *i.e.*, Willong (N-25°27'959", E-93°56'926" and Elevation 1028m), and Hengbung hills (N-24°50'886", E-94°30'732" and Elevation 1598 m) of Senapati. Plants with an average of 2 – 3 leaves were planted into clay pots (28x24cm) containing broken pieces of bricks, charcoal, and leaf mould in a ratio of 1:1:1. Plants were maintained in the polyhouse and were watered at alternate days during winters and daily in summers.

Pot experiment was conducted using 3x3x4 factorial treatment combinations with 9 replicates in a completely randomized block design. The experimental factors were i) three ratios of N, P and K fertilizer *i.e.;* (1:1:1), (2:1:1.5) and (3:1:1.5) in three dosages for each ratio and ii) Four intervals of timing. Hence, the dosages of N, P and K fertilizer were 1:1:1 for T_1, T_2 and T_3, 2:1:1.5 for T_4, T_5 and T_6 and 3:1:1.5 for T_7, T_8 and T_9 treatments. In another experiment, different concentrations (15, 25, 50, 75, 100, 125,150,175 and 200mgl $^{-1}$) of growth hormone (BAP) were also used in the above treatments, respectively with a view to assess the time taken for flowering and the other related parameters. The experiment was carried out in a randomized completely block design with three replicates for each treatment.

These experiments were conducted for six months from (Dec. 2012-May 2013). Observations were recorded at 20 days intervals. The parameters *i.e.*, plant height, leaf length, leaf width, leaf number/plant, days from inflorescence emergence to first flower opening, length of inflorescence, number of flower per inflorescence, flower width and flowering duration (days) were measured and recorded. Width of the flower was measured as the length from one end of the sepal to the other end of another sepal. The Data was analyzed by two way and one way analysis of variance (ANOVA) and means were compared using the post-hoc test at $p \leq 0.05$.

Results and Discussion

Vegetative Growth

N, P and K fertilizer mixture of 1:1:1, 2:1:1.5 and 3:1:1.5 at 1g, 2g and 3g l^{-1} were used respectively. The significant differences were observed among different treatments. The NPK ratio showed the significant results on growth performance of *Dendrobium chrysotoxum* (Table1). All the treatments showed significant result on growth of the plant. On the day 80, after plantation, the plants responded to the higher N ratio (T_7) resulting in optimal plant height (13 cm), width of leaf (3.4 cm), leaf length (10.7cm) and the number of leaves per plant (4). Significant variations were also observed in the vegetative growth of *Dendrobium williamsonii* with the application of NPK. The increased ratio (T_7) was proved optimal to produce

Table.1. Effects of NPK fertilizer ratio on height (cm), leaf length and width (cm), and number of leaves (per plant) in *Dendrobium chrysotoxum.*

Parameters	Fertilizer treatment	Time (Year 2012-13) 20(days)	40(days)	60(days)	80(days)
Plant height (cm)	Control	7.1^{a}	7.1^{a}	7.9^{a}	8.9^{a}
	T_1	8.3^{b}	8.6^{ab}	9.4^{b}	11.2^{b}
	T_2	8.8^{b}	9.1^{b}	9.6^{b}	11.3^{b}
	T_3	9.6^{c}	10.6^{c}	10.8^{cc}	12.3^{c}
	T_4	7.2^{a}	7.8^{a}	8.2^{a}	10.6^{b}
	T_5	9.2^{c}	10.6^{c}	11.4^{d}	13.0^{c}
	T_6	8.7^{b}	8.7^{b}	9.6^{b}	11.2^{b}
	T_7	10.6^{d}	10.9^{c}	11.6^{d}	13.0^{c}
	T_8	9.1^{bc}	10.1^{c}	10.3^{c}	11.5^{bc}
	T_9	8.5^{b}	8.5^{b}	9.4^{b}	11.2^{b}
Significance		***	***	***	***

Table.1. Effects of NPK fertilizer ratio on height (cm), leaf length and width (cm), and number of leaves (per plant) in *Dendrobium chrysotoxum* (contd.).

Parameter	Fertilizer treatment	Time (Year 2012-13) 20(days)	40(days)	60(days)	80(days)
Leaf length (cm)	Control	5.2[a]	5.3[a]	6.1[a]	7.7[a]
	T_1	5.7[b]	6.2[b]	7.9[bc]	9.2[c]
	T_2	6.9[cd]	7.2[c]	8.3[c]	9.9[c]
	T_3	7.5[cd]	7.5[c]	8.5[c]	9.6[c]
	T_4	6.0[bc]	7.1[c]	7.9[b]	8.6[b]
	T_5	8.2[d]	8.8[d]	9.1[d]	10.6[d]
	T_6	6.5[bc]	6.9[bc]	7.1[b]	8.3[b]
	T_7	9.0[e]	9.4[d]	9.3[d]	10.7[d]
	T_8	7.6[d]	7.6[c]	8.4[c]	10.3[d]
	T_9	6.7[c]	7.0[c]	7.7[bc]	8.8[b]
Significance		***	***	***	***
Leaf width (cm)	Control	1.4[a]	1.5[a]	1.9[a]	2.6[a]
	T_1	2.0[d]	2.1[cd]	2.6[c]	2.9[b]
	T_2	1.9[cd]	2.0[c]	2.5[c]	2.9[b]
	T_3	1.5[ab]	1.6[ab]	2.0[a]	2.6[a]
	T_4	1.6[b]	1.7[b]	2.2[b]	2.8[b]
	T_5	1.8[c]	2.3[d]	2.8[d]	3.0[c]
	T_6	1.9[cd]	2.0[c]	2.6[c]	3.2[d]
	T_7	1.9[cd]	2.2[d]	2.8[d]	3.4[e]
	T_8	1.9[cd]	2.1[cd]	2.7[cd]	3.2[d]
	T_9	2.0[d]	2.1[cd]	2.7[cd]	3.0[c]
Significance		***	***	***	***
Leaf number (per plant)	Control	2.0[a]	2.1[a]	2.6[a]	2.7[a]
	T_1	2.3[b]	2.4[b]	2.8[b]	3.4[c]
	T_2	2.1[a]	2.3[b]	2.7[ab]	3.1[bc]
	T_3	2.3[b]	2.4[b]	2.7[ab]	2.9[b]
	T_4	2.3[b]	2.7[c]	3.1[c]	3.3[c]
	T_5	2.4[b]	2.9[d]	3.3[d]	3.7[d]
	T_6	2.3[b]	2.7[c]	3.1[c]	3.3[c]
	T_7	2.8[c]	3.4[e]	3.7[e]	4.0[e]
	T_8	2.3[b]	2.7[c]	3.1[c]	3.2[c]
	T_9	2.4[b]	2.7[c]	2.9[b]	3.0[b]
Significance		***	***	***	***

more vegetative growth; at the time of harvest, the highest plant height (11.8cm), leaf length (9.7cm), leaf width (2.1cm) and number of leaves (5.1) were recorded in treatment T_7 (Table 2).

Promotion of vegetative growth by N, P and K fertilizer was clearly visible in the experiments. These findings are in agreement with the earlier work by some authors (Lee and Lin, 1987; Wang and Gregg, 1994). Wang (1996) determined that higher concentration of N is

Table 2. Effects of NPK fertilizer ratio on height (cm), leaf length and width (cm), and number of leaves (per plant) in *Dendrobium williamsonii*.

Parameters	Fertilizer treatment	Time (Year 2012-13) 20(days)	40(days)	60(days)	80(days)
Plant height (cm)	Control	5.7[a]	6.0[a]	6.6[a]	8.5[a]
	T_1	6.9[b]	7.1[b]	7.7[b]	9.6[b]
	T_2	7.8[c]	8.4[c]	9.1[cd]	10.7[c]
	T_3	7.6[bc]	8.0[c]	9.2[cd]	10.3[b]
	T_4	7.8[c]	7.8[bc]	7.9[b]	9.4[b]
	T_5	8.2[c]	8.5[cd]	9.6[d]	10.7[c]
	T_6	7.5[bc]	7.9[c]	8.7[c]	10.0[b]
	T_7	9.1[d]	9.2[d]	9.8[d]	11.8[c]
	T_8	7.9[c]	8.2[c]	9.2[cd]	9.8[b]
	T_9	7.4[bc]	7.7[bc]	8.5[c]	10.0[b]
Significance		***	***	***	***
Leaf length (cm)	Control	4.7 [a]	4.7 [a]	5.5 [a]	7.3 [a]
	T_1	6.4 [e]	6.4 [d]	7.1 [d]	8.3 [e]
	T_2	4.9 [b]	5.9 [bc]	6.4 [b]	7.6 [b]
	T_3	6.5 [e]	7.2 [f]	7.7 [f]	8.8 [f]
	T_4	5.9 [d]	6.3 [d]	7.4 [e]	8.1 [d]
	T_5	7.0 [f]	7.4 [g]	8.0 [g]	8.8 [f]
	T_6	5.7 [c]	5.8 [b]	6.5 [b]	7.8 [c]
	T_7	7.8 [g]	7.9 [h]	8.2 [h]	9.7 [g]
	T_8	5.8 [cd]	6.8 [e]	6.8 [c]	8.2 [de]
	T_9	5.6[c]	6.0 [c]	6.7 [c]	8.3 [e]
Significance		***	***	***	***
Leaf width (cm)	control	0.7 [a]	0.8. [a]	1.1 [a]	1.7 [a]
	T_1	0.9 [b]	1.0 [b]	1.5 [c]	1.8 [ab]
	T_2	1.0 [bc]	1.0 [b]	1.6 [cd]	2.0 [bc]
	T_3	1.4 [d]	1.4 [d]	1.9 [e]	2.0 [bc]
	T_4	1.1[c]	1.2[c]	1.6	1.9[b]
	T_5	0.9[b]	1.1[bc]	1.5 [c]	1.9[b]
	T_6	0.9[b]	1.0[b]	1.5[c]	1.8[ab]

Table 2. Effects of NPK fertilizer ratio on height (cm), leaf length and width (cm), and number of leaves (per plant) in *Dendrobium williamsonii* (contd.).

Parameters	Fertilizer treatment	Time (Year 2012-13) 20(days)	40(days)	60(days)	80(days)
	T_7	0.9^{b}	1.2^{c}	1.7^{d}	2.1^{c}
	T_8	0.7^{a}	0.9^{ab}	1.3^{b}	1.8^{ab}
	T_9	0.7^{a}	0.9^{ab}	1.2^{ab}	1.8^{ab}
Significance		***	***	***	***
Leaf number (per plant)	Control	2.2^{a}	2.3^{a}	2.8^{a}	3.2^{a}
	T_1	2.6^{c}	2.8^{cd}	2.9^{a}	3.4^{b}
	T_2	2.4^{b}	2.5^{bc}	2.8^{a}	3.4^{b}
	T_3	2.6^{c}	2.7^{c}	2.9^{a}	3.3^{a}
	T_4	2.9^{d}	2.9^{d}	3.2^{b}	3.7^{c}
	T_5	2.4^{b}	2.4^{ab}	3.2^{b}	3.9^{d}
	T_6	2.6^{c}	2.9^{d}	2.9^{a}	3.3^{a}
	T_7	3.0^{e}	3.2^{e}	4.2^{d}	5.1^{f}
	T_8	2.9^{d}	3.2^{e}	3.4^{c}	4.4^{a}
	T_9	2.8^{d}	2.8^{cd}	3.2^{b}	3.7^{c}
Significance		***	***	***	***

more critical than P or K in promoting vegetative growth in a medium consisting of 80% Douglas fir bark and 20% peat. In the current study, the low P and high N and K fertilizer increased vegetative growth indicating thereby less requirement of P in the vegetative growth. Hence, continuous application of an adequate level of N and K fertilizers may be more important than increased P for optimal vegetative growth. In both the present species, the most suitable fertilizer ratio was 3:1:1.5 with optimal dose 1 gl^{-1} followed by 2:1:1.5 with optimal dose 2gl^{-1} as per the experiment. Thus, it may be suggested that use of fertilizers at optimal proportions results in noticeably better growth.

Floral Induction

Gordon (1990) recommended that a fertilizer with high P content should be used prior to and during the development of the inflorescence for best flowering. Since all plants used in the experiment were presently fertigated with 1:1:1, 2:1:1.5 and 3:1:1.5 NPK fertilizers until flower bud initiation. The present experiment confirms that exogenous application of BAP has significant effect in flowering. Table 3 shows the positive effects of exogenous application in general, of BAP on plants in promoting early flowering in *Dendrobium chrysotoxum* and *D. williamsonii*. In *Dendrobium chrysotoxum* Treatment T_9 exhibited the shortest time interval (33 days) from inflorescence emergence to first flower opening; largest inflorescence (22cm) was observed in treatment T_7; highest number of flowers per inflorescence (23.3) was observed in treatment T_7; and the highest width of flower (4.7cm) was recorded in treatment T_4. Zepeda *et al.* (2006) had reported the same kind of results with the application of cytokinins causing an increase in the ovary diameter of flowers of seedless grapes. Similar results were also obtained presently in *D. williamsonii*; shortest time interval (51 days) from inflorescence emergence to first flower opening was recorded in treatment T_9; inflorescence length was highest (6.9cm) in treatment T_7; number of flowers per inflorescence was recorded largest (3) in treatment T_7; and highest width of flower (6.3cm) was recorded in treatment T_4. Plant exposure to all the concentrations of BAP tested (15-200 mgl^{-1}) in the form of foliar spray along with NPK initiated early flowering as compared to the control plants. Plants treated with 200mgl^{-1} BAP (T_9) were the earliest to bloom followed by 100 mg^{-1} of BAP while control plants took the longest number of days. These observations are in line with the earlier work of Nisha *et al.*, (2012) that plants treated with higher BAP concentration significantly reduced the number of days needed to

Table.3. Effect of NPK fertilizer along with BAP on initiation of flower opening, length of inflorescence, number of flowers/ inflorescence, width of flower, and flower duration in *Dendrobium chrysotoxum* and *D. williamsonii*..

Parameters		Treatment of NPK + BAP									
		Control	T_1*	T_2*	T_3*	T_4*	T_5*	T_6*	T_7*	T_8*	T_9*	Significance level
Dendrobium chrysotoxum	1st floral blooming	43^{d}	39.3^{c}	38^{c}	37.7^{bc}	39^{c}	34^{a}	36.3^{b}	36.3^{b}	35.7^{b}	33^{a}	***
	Length of inflorescence	9.6^{a}	11.5^{a}	9.6^{a}	9.6^{a}	16.0^{c}	14.0^{b}	12.2^{b}	22.0^{d}	13.3^{b}	12.7^{b}	***
	Number of flowers/ inflorescence	8.7^{a}	14.0^{bc}	12.3^{b}	11.3^{ab}	18.3^{cd}	20.7^{d}	14.0^{bc}	23.3^{d}	16.3^{c}	11.3^{ab}	***
	Flower width	2.8^{a}	3.7^{c}	3.7^{c}	3.5^{cb}	4.7^{f}	4^{d}	3.7^{c}	4.5^{e}	3.3^{b}	3.1^{b}	***
	Flower duration	7^{d}	6.3^{d}	6^{c}	5.3^{c}	6^{c}	6^{c}	5.3^{c}	5.3^{c}	4^{b}	3^{a}	***
Dendrobium williamsonii	1st floral blooming	59^{e}	56.7^{d}	56^{d}	55.3^{d}	56.7^{d}	53^{ba}	55.3^{c}	56.3^{d}	55.3^{c}	51^{a}	***
	Length of inflorescence	5.1^{a}	5.7^{c}	6.0^{d}	5.8^{c}	6.3^{d}	6.0^{d}	5.8^{c}	6.9^{e}	5.6^{bc}	5.6^{bc}	***
	Number of flowers/ inflorescence	1.3^{a}	1.7^{a}	2^{ab}	2^{ab}	2.7^{b}	2^{ab}	1.7^{a}	3^{b}	2^{ab}	1.7^{a}	***
	Flower width	3.9^{a}	4.2^{a}	4.1^{a}	4.0^{a}	6.3^{b}	5.8^{b}	5.4^{b}	6.1^{b}	5.2^{b}	4.9^{a}	***
	Flower duration	23.3^{g}	21.7^{f}	20.3^{e}	19.7^{d}	22.3^{f}	19.7^{d}	18.7^{c}	20.3^{e}	18.7^{b}	17^{a}	***

* Treatments with BAP;*** Significant at $p \leq 0.001$, for each row, Values with the same letter are not significantly different ($p \leq 0.05$)

bloom. Although BAP treatment was shown to be useful for shortening the days required for blooming, the flowers of the plants treated with higher concentration of BAP became yellow and started withering before these could open fully. Lorteau *et al.* (2001) also showed that BAP could stimulate ethylene production in plants. In the present study, the wilting happened in some plants at early stage when the first flower started to open indicating thereby the effect of ethylene production. However, the increased bud abortion observed by Lin (1994) was not observed in this study. Incidentally, plants treated with high concentration of BAP showed shorter flowering duration as compared to those treated with its lower concentration and control plants.

Hence, it is concluded that treatment of NPK and BAP affected both vegetative and floral development in both the presently investigated species. Present data suggest that, water soluble fertilizer NPK with high N and K ratio (3:1:1.5) promotes vegetative growth and BAP at 200 mgl^{-1} has great potential in inducing early initiation of flowering in both the orchid species namely *Dendrobium chrysotoxum* and *D. williamsonii*.

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