INDUCTION OF PLANTLETS ON INFLORESCENCE OF PHALAENOPSIS BY APPLICATION OF N-6-BENZYL ADENINE[1]

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ABSTRACT

Clonal propagation of *Phalaenopsis* can be accomplished by division of plantlets spontaneously formed on the inflorescence node and tip, or on root in some of *Phalaenopsis* species and hybrids. Plantlets can also be induced by top cutting. These current asexual methods yielded only a few plants of a clone per year. In an attempt to induce plantlet formation on the inflorescences, various concentrations of N-6-benzyl adenine (BA) in lanolin were applied on the dormant buds after removing the bracts, or at the tip of inflorescences. The most effective method was the application of 2000 ppm BA on dormant buds of the lower nodes of inflorescence after removal of the bract.

Of the entire family of Orchidaceae, *Phalaenopsis* is one of a popular cultivated plants. *Phalaenopsis* (Greek, *phaluna* meaning moth,-*opsis* meaning resembling) received its name from the similarity in appearance of its flowers to some tropical moths. The moth orchids and their hybrids are grown in great quantities for cut flower trade, the blossom of the white forms are especially utilized for wedding bouquets. The genus *Phalaenopsis* was established in 1825 by Blume, changing from a species previously described as *Epidendrum amabiles* by Linneaus, and *Cymbidium amabile* by Roxburgh. The same species was already mentioned in 1970 by Rumphius as *Angraecum album majus* (Northen, 1970)

The number of species of the genus *Phalaenopsis* varies considerably according to various authors. Rolfe (1886) enumerates thirty-four species, Pfitzer (1889) mentions only ten species, while Schlecter (1927) accepts forty species as a reasonable estimation.Finally Quisumbing (1957) presents a list of seventy species. Irrespective of the fact that many species-names may be synonyms, some species have been changed recently to other or new genera. In general the exact number of species is not well defined (Northen, 1970). They are found from Taiwan down to Queensland. Some are quite rare and still use in hybridization.The flowers are not outstanding but they remain in flower from two to five months.

Species of *Phalaenopsis* have a monopodial type of growth with a characteristic vegetative appearance. The foliage is attractive. The long, broad curling leaves may be shiny and leathery,

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plain green or mottled with greyish green, often purple underneath. They are ten to forty centimeters long. The plants are slow growing and one or two new leaves are produced each year. Mature plants attain an average height of twelve to fifteen centimeters, although a few individuals may grow taller. Large flattened roots emerge from the stem between mature leaves and may grow as long as one meter. The inflorescences emerge from the axils of the leaves. They may be long and branching out in most species, the white species are usually unbranched. In some plants all flower buds on inflorescences develop at about the same time so that the whole spray opens at once. On the others, the basal flowers open first, while the tip of inflorescence continues to form more buds. Plants with the latter habit can remain in flower for most of the year, individual flowers last only for several weeks but new ones open to replace those that fade. In many of the species and hybrids, if the flower stem is cut just below the node that produced the first flower, a branch stem may emerge from one of the inflorescence node, giving a second spray of flowers.

In some species *(Phal. equestris, Phal. intermedia,* and *Phal schillerana),* plantlets develop spontaneously at the tip of inflorescence after flowering. In other species *(Phal. lueddemanniana)* plantlets form on nodes of the inflorescences. In the case of *Phal. stuartiana* and some of its hybrids, plantlets may form on the roots. (Northen, 1970)

Traditionally *Phalaenopsis propagation has been accomplished by two methods; sexual and asexual. The sexual is by seeds, but since Phalaenopsis* is highly heterozygous, it is difficult to obtain large numbers of uniform plants from seedling populations. Vegetative or assxual propagation of a clone is done, most commonly, by division or cuttings of plantlets. A clone can be defined as "genetically uniform material derived from a single individual."

Phalaenopsis do not lend themselves as well to vegetative propagation as some other genera. However, it is possible to get additional plants by :

(1) Removing side shoots that developed at the base of the plant.

(2) Cutting off the upper half of plant thus breaking apical dominance. One or two plantlets will then be produced on the upper buds.

(3) Removing and growing separately of plantlets formed on the inflorescence.

Since these current asexual methods yields only a few plants of a clone per year. The main objective in this study is to develop new techniques for rapid clonal propagation of *Phalaenopsis.* In trials to induce plantlet formation on the inflorescences of plants grown in the greenhouse, various concentrations of N-6-benzyl adenine (BA) in lanolin were applied on the dormant buds with or without bracts, or at the tip of inflorescences.

MATERIALS AND METHODS

Plants used in this study were *Phalaenopsis* and related hybrids.

1. **Spontaneous plantlet formation**

The sits of spontaneous plantlet production at the tip or node of inflorescence after flowering or at the root of *Phalaenopsis* species and hybrids were observed.

2. **Topping**

Terminal cuttings of *Phalaenopsis* species and hybrids were made. Number of plantlets formed on the remaining basal stem were recorded after four months.

3. **Application of BA to inflorescences**

BA in lanolin paste was prepared by the method of Thompson and Jacobs (1966) incorporating 500, 1000 and 2000 ppm and applied to the node with or without bracts or at the tip of inflorescence. Number of plantlets formed in each treatments were recorded after one, two and six months.

RESULTS

1. Spontaneous plantlet formation

Observations of a large number of plants showed that some *Phalaenopsis* species and interspecific hybrids, as shown in Table, spontaneously produced plantlets at the node or tip of inflorescence after flowering, or no roots.

In *Phal.amabilis, Phal.intermedia* and *Phal.equestris* (Fig.1) plantlets developed at the tip of the inflorescence after flowering. In other species,*Phal. cornu-cervi* and *Phal.lueddemanniana* (Fig.2) plantlets formed on the nodes of the inflorescence. In the interspecific hybrid. *Phal.amabilis* x *Phal.lueddemanniana* plantlets formed on the nodes as well as on the tip of the inflorescence. Meanwhile a plant of *Phal.stuartiana* with an extensive root system on the surface of a plumeria tree has plantlets arising from root in many places (Fig. 3)

2. Topping

When terminal cuttings of plants with a visible length of stem axis of *Phal.amabilis, Phal.* Clara Knight, *Phal.* Chieftain, *Phal.* Chieftain x *Phal.* Susan Merkel, *Phal.stuartiana nobilis, Dtn.* Coral Sand x *Phal.* Zada, *Phal.* Arcadia x *Phal.cochleris,* and *Phal.* Zada x *D. pulcherrima* were removed (topping), these cuttings were readily established into plants within a few weeks. After four months two shoots were produced on the remaining basal stem of *Phal.amabilis* (Fig.4) and one plantlet on the other plants.

3. Application of BA to inflorescences

3.1 Determination of concentration

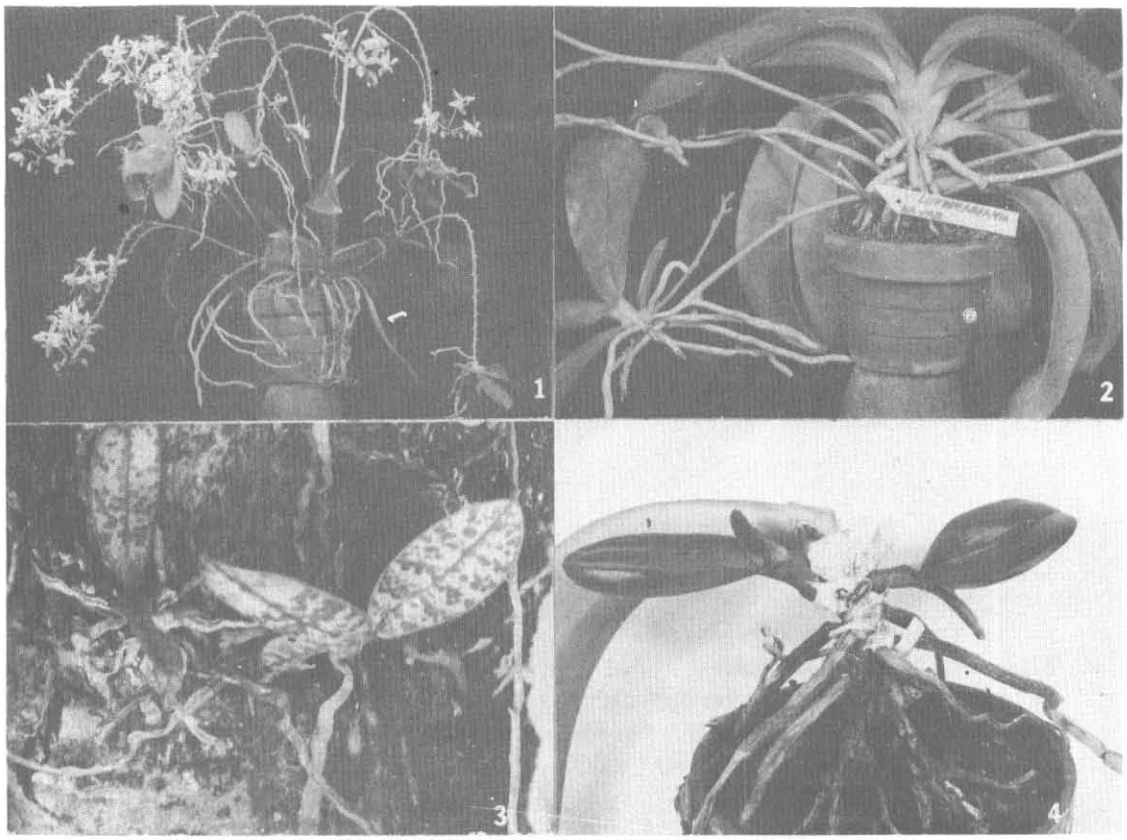
In some *Phalaenopsis* species and interspecific hybrids, plantlets are formed spontaneously at the nodes or at the tips of inflorescence. This phenomenon rarely occurs in *Phalaenopsis* hybrids. Buds found on inflorescence node remain dormant and do not naturally form plantlets. This investigation was carried out whether BA could be used to break bud dormancy and induce plantlet formation. The average shoot lengths, 2 months after application of 0, 500, 1000 and 2000 ppm BA in lanolin paste to the two lowest nodes of inflorescences of *D.purcherrima* without bract were shown in Table 2. BA at 500, 1000, and 2000 ppm broke bud dormancy, whereas the control buds remained dormant. Since application of BA at 1000 and 2000 ppm were more effective than at 500 ppm, application of 500 ppm was omitted in the subsequent experiments.

3.2 Determination of penetration

This experiment was conducted to determine the effect when applied to nodes without removal of bract. The result obtained one month after application of 200 ppm of BA in lanolin paste to the lowermost node of 20 inflorescences of *Phal.intermedia* with and without bracts is shown in Fig.5. When 2000 ppm BA was applied to the bud with bracts (Fig.5 Br +) the bud showed no growth but when the bract was removed prior to application of BA growth was observed (Fig.5 Br −).

3.3 Determination of translocation

This experiment was carried out to determine whether BA would be translocated acropetally or not. The result after one month of application of 2000 ppm BA to the lowermost node of 10 inflorescences of *D. pulcherrima* after removing the bract was shown in Fig. 6. The bud which was treated with 2000 ppm BA grew. whereas the untreated acropetal buds showed no growth.



Spontaneous production of plantlets

Figure 1. At tips of inflorecences after flowering in *Phal.equestris.* O.2 x

Figure 2. On the node of inflorescence of *Phal.lueddemanniana.* 0.2 x

Figure 3. On the root of *Phal.stuartiana.* 0.2 x

Induction of plantlets by topping

Figure 4. Two shoots formed on the basal stem of *Phal.amabilis* 4 months after topping. 0.4 x

Table I Sites of spontaneous plantlet production

Plant	Site of Plantlet		
	Inflorescence		Root
	Tip	Node	
Phal.amabilis	+		
Phal.cornu-cervi		+	
Phal.equestris	+		
Phal.intermedia	+		
Phal.lueddemanniana		+	
Phal.stuartiana			+
Phal.amabilis x Phal.lueddemanniana	+	+	
Phal.cornu-cervi x Phal.lueddeminniana		+	
Phal.equestris x Phal.lueddemanniana	+		
Phal.intermedia x Phal.sanderana		+	
Phal intermedia x Phal.sanderana		+	
Phal.lueddemanniana x Phal.speciosa		+	
Phal.lueddemanniana x phal.stuartiana		+	

Table 2 Shoot length of *D. pulcherrima* two months after application of BA

BA (ppm)	No. of treated shoots	Ave. shoot length (cm)
0	20	0.1
500	20	0.3
1000	20	0.7
2000	20	0.7

Explanation of figures

Effect of BA on inflorescences

Figure 5. One month after application on buds of *Phal.intermadia*. Growth occured only on bud without bract (−Br). lx.

Figure 6. One month after aplication on lowermost buds of *D.pulcherrima*. Only treated buds enlarged, other buds were uneffected. 0.3x.

Figure 7. Two months after application on tip *of Phal.amabilis* x *Phal.lueddemanniana,* inflorescence elongated and continued to flower. 0.3x.

Figure 8. Six months after application on tip of *Phal.amabilis* x *Phal.lueddemanniana,* inflorescence elongated and either continued to flower or produced plantlets Horizontal line marks point of BA application of nodal bud of *Phal amabilis* x *Phal.lueddmanniana* showing single or multiple shoots. 0.6 x.

Figure 10. Six months after application on nodal bud of *Phal.ambilis* x *Phal.lueddemanniana* showing single or multiple shoots. 0.6x.

Figure II. Variations in growth of *Phal.amabilis* x *Phal.lueddemanniana* six months after application of 1000 ppm BA. 0.6x

Figure 12. Variations in growth of *Phal.amabilis* x *Phal.lueddemanniana* six months after application of 2000 ppm BA. 0.6x.

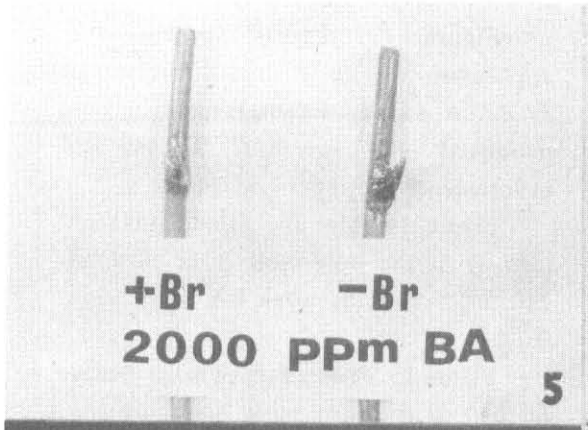
3.4 Effect of BA application on tip and nodal bud of inflorescences

This experiment was carried out to induce plantlet formation at the tips of inflorescences. The result of 1, 2 and 6 months after applications of each 0, 1000 and 2000 ppm of BA to the tip of inflorescences on Phal.amabilis x Phal.lueddemanniana are shown in Figures 7, 8 and 16.

The untreated tips elongated only 1.8 cm in 6 months, whereas the tip elongated to an average of 10.2 cm at 1000 ppm BA and 17.3 cm at 2000 ppm BA (Fig.16)

When BA was applied to the tip of an inflorescence, the control remained dormant after a period of 2 months, while more flowers resulted in BA treatments of 1000 and 2000 ppm (Fig.7). After 6 months the untreated tips still remained dormant where as at 1000 and 2000 ppm BA the tips elongated, and either plantlets formed at the tip or more flowers resulted (Fig.8). At the point of BA application, the inflorescence axis curved and became swollen (arrow). In some cases abnormal flowers resulted on treated tips, e.g.flower without lip (arrow).

In the control treatment, two tips reproduced flowers and none formed



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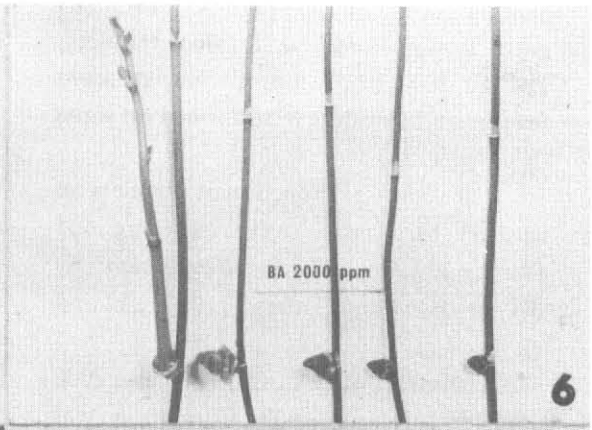

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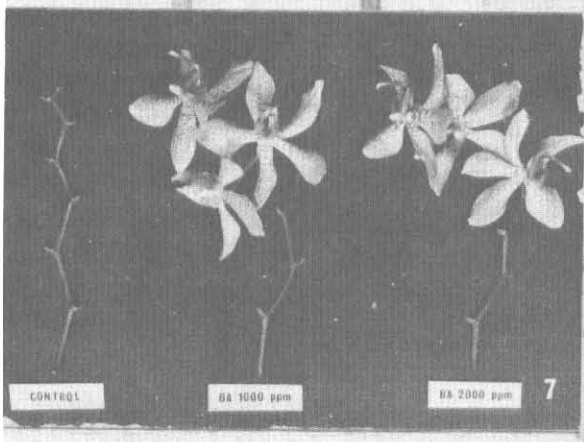

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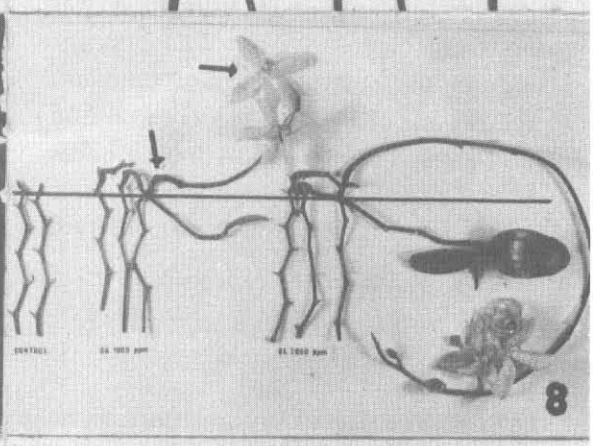

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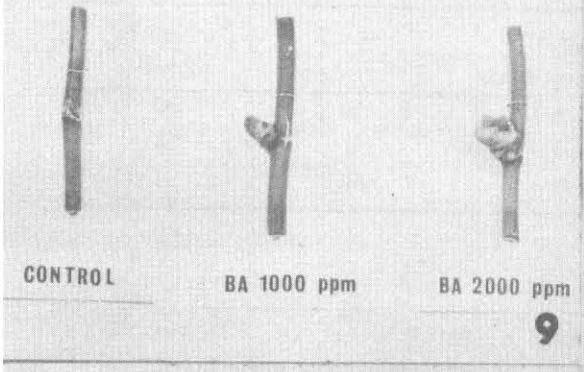

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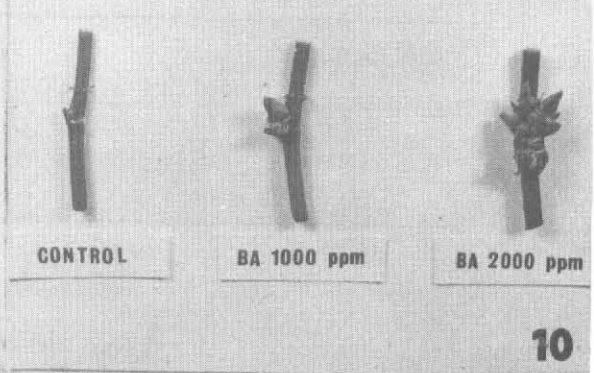

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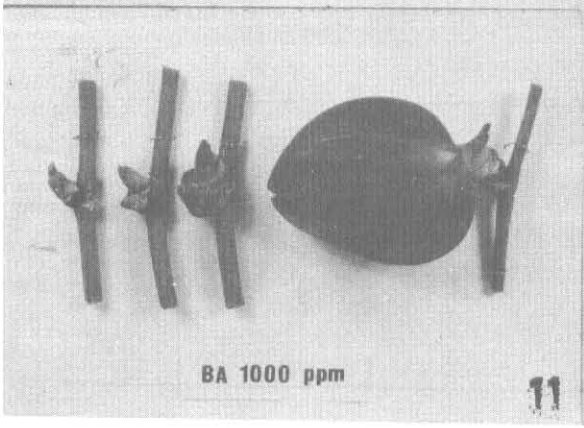

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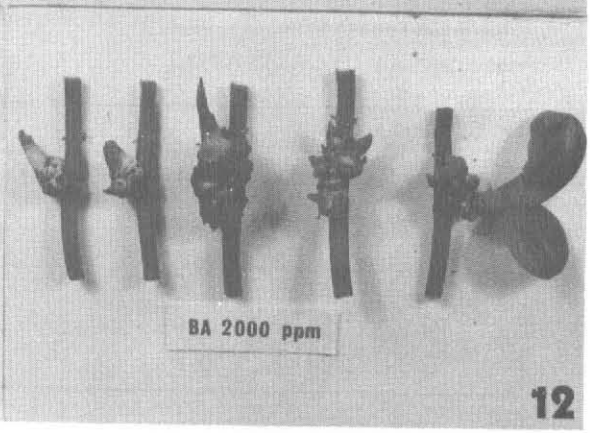

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plantlets; while at 1000 ppm two produced more flowers and five formed plantlets; and at 2000 ppm four produced more flowers and plantlets were formed on three tips (Table 3).

The results of induction of plantlets on nodal bud of inflorescence of *Phal.amabilis* x *Phal.lueddemanniant* by BA are shown on Figures 9, 10, 11, 12 and Table 4.

The effect of BA of 0, 1000 and 2000 ppm on multiple shoot formation was shown on Figure 9. After 2 months the buds in the control remained dormant, whereas one or multiple shoots formed on the 1000 and 2000 ppm BA treated nodes After 6 months, the multiple shoot became dormant; not much growth was observed after 2 months (Fig.10).

Effect of BA on inflorescence

Figure 13. Eight months after application on nodal buds (arrows) of *Phal.*Terri Cook, two single plantlets were produced at lowermost nodes and inflorescences at upper nodes. 0.lx.

Figure 14. Multiple shoots produced (arrow) at the lowermost node of *Phal.* Lucifer, 8 months after BA application. 0.3x.

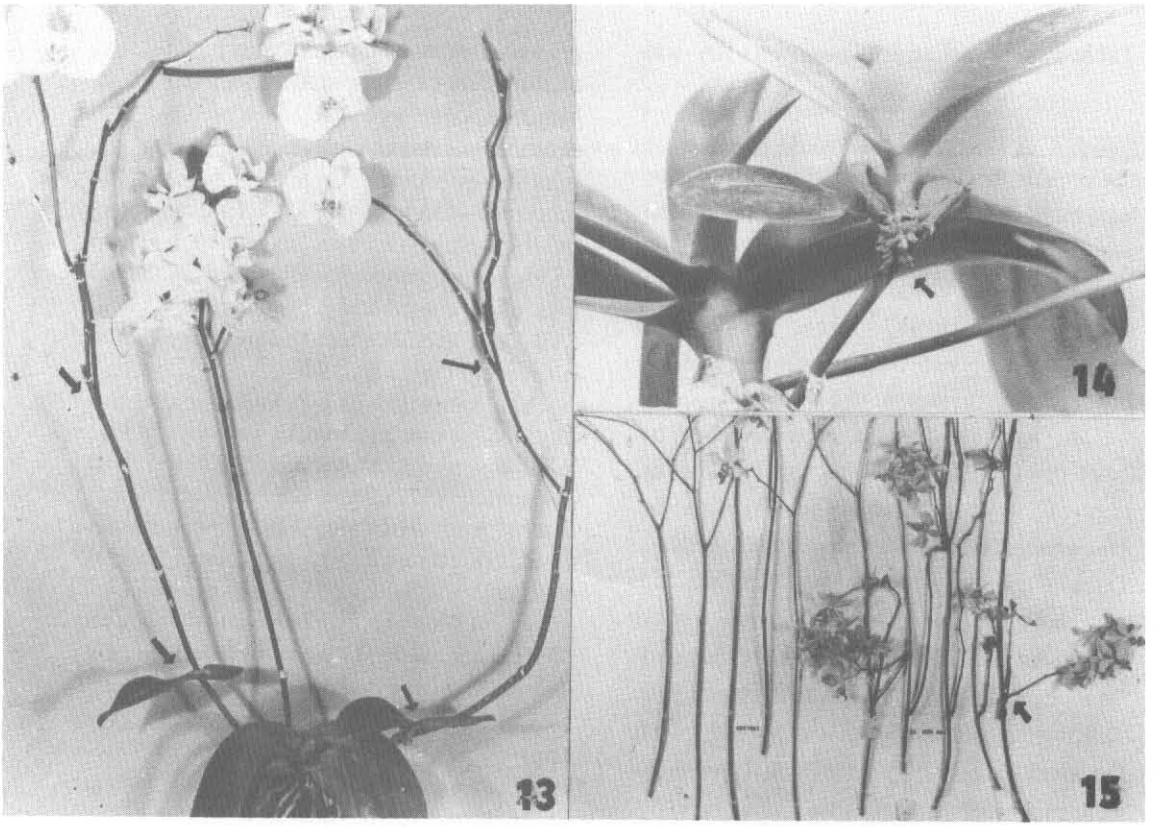
Figure 15. Production of inflorescence (arrow) at the lowermost nodes of *Dtn.* Dorette, three months after BA application. 0.lx.

TABLE 3. Effect of BA on inflorescence tip of *Phal.ambilis* x *Phal.lueddemanniana* after six months.

BA (ppm)	No.of treated tips	No.of dormant buds	No.of inflorescence	No.of plantlets
0	10	8	2	0
1000	10	3	2	5
2000	10	3	4	3

Table 4. Induction of shoots on the node of inflorescence of *Phal.amabilis* x *Phal.lueddemanniana** six months after treated with BA. (*an interspcific hybrid which spontaneously forms plantlets.)

BA(ppm)	No.of treated nodes	dormant buds	No.of nodes with one shoot	one plantlet	multiple shots	multiple shoot & one plantlet
0	20	18	1	1	0	0
1000	20	0	7	0	11	2
2000	20	0	4	0	12	4



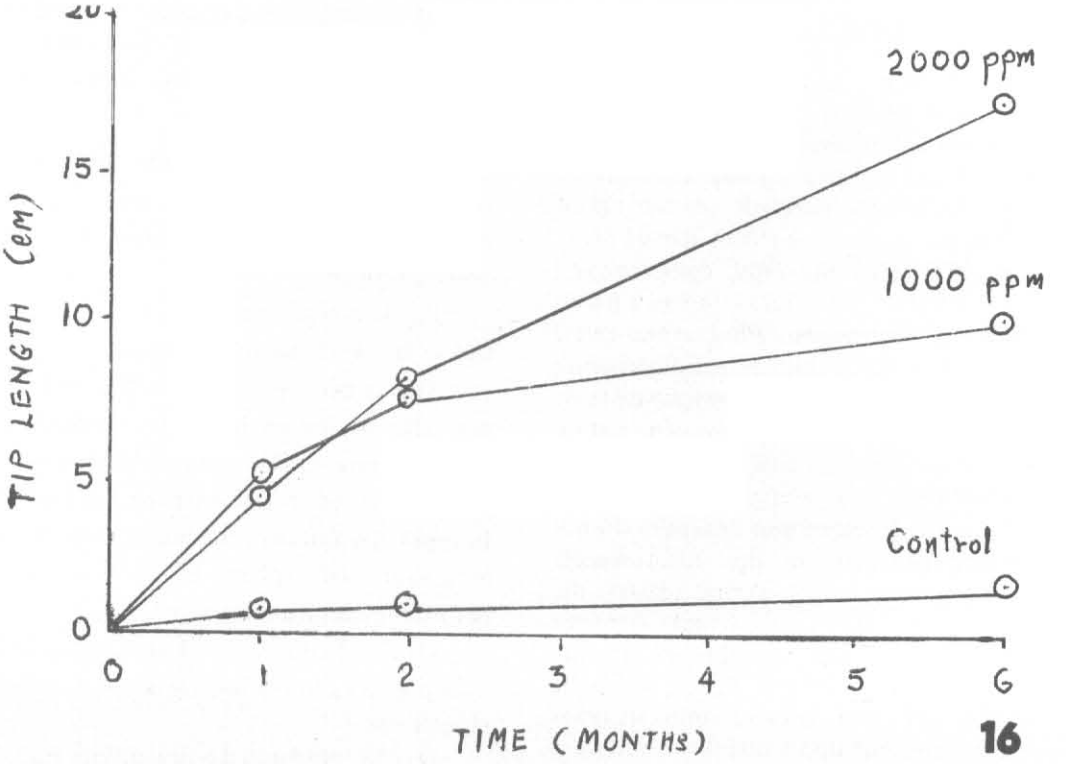


Figure 16. Effect of BA on growth of inflorescence tip of *Phal.amabilis* x *Phal.lueddemanniana*.

Table 4 shows that application of BA was successful in breaking dormancy of all nodal buds while most of the control buds remained dormant. Also, multiple shoot formation was obtained only on those nodes treated with BA. Few plantlets were obtained from multiple shoot formation. After an initial stimulation. most of the shoots fail to proceed any further in growth.

In addition BA at concentration of 2000 ppm was applied to the buds after removal of bracts of other hybrids of *Phalaenopsis*. In 8 months, buds of *Phal.* Terri Cook produced plantlets at the lowermost nodes and inflorescences at the top nodes as shown in Figure 13. Buds of *Phal.* Lucifer (Fig. 14) produced multiple shoots at a single node, while with *Dtn.* Dorette (Fig. 15) more inflorescences were produced in 3 months.

DISCUSSION

Vegetative, asexual, or clonal propagation of *Phalaenopsis* is accomplished by division of plantlets which are induced by top cutting (TableI) or which form spontaneously on the tip of inflorescences *(Phal.amabilis. Phal.intermedia.* and *Phal. equestris),* on the node of inflorescences *(Phal.lueddemanniana, Phal. cornu-cervi)* on the tip as well as along the inflorescence *(Phal.amabilis* x *Phal.lueddemanniana),* or on the roots *(Phal.stuartiana).* The nature or spontaneous plantlet formation in *Phalaenopsis* and interspecific hybrids is not known. *Phalaenopsis* generally do not produce plantlets on the inflorescence. Usually during the flowering season, the buds on the inflorescence remain dormant and tip continues to flower. If the inflorescence is cut just below the node bearing the first flower, only spikelets emerge from the upper nodes to produce a second spray of flowers (Northen, 1970).

Failure of vegetative buds on *Phalaenopsis* inflorescence to produce plantlets suggests an internal block of the growth process. The onset and termination of dormancy are apparently regulated by a balance of growth inhibitors and promoters. At the dormancy stage, the inhibitor-promoter balance favors the inhibitor components. This condition may be the result of either excessive level of endogenous inhibitor substances such as phenolic compounds or ascorbic acid (ABA) or the absence or deficiency of the promoters (Walker, 1970)

The application of cytokinins to axillary buds of apple (Chvojka *et al.,* 1961; Williams and Stahly, 1968; Kender and Carpenter, 1972). Peas and *Helianthus* sp. (Sachs and Thimann, 1964) overcomes apical dominance and stimulates lateral bud growth. Also dormancy of buds of grape (Weaver, 1963) and peach (Weiberger, 1969) were terminated by treatment with cytokinin.

When N-6-benzyl adenine (BA) was applied to dormant buds on the inflorescence node after removal of bracts, shoot growth was initiated because the addition of an external growth promoter caused the inhibitor-promoter balance to shift in favour of promoters. Four types of growth were observed upon the application of BA:

(1) The growth of the bud was stimulated during the first one or two months after which this bud became dormant again.

(2) The bud developed into multiple shoots but later became dormant.

This is because the BA is depleted, or degraded by the environment, or leached by watering. The inhibitor-promoter balance shifted in favor of the inhibitors. In types one and two, the buds can be used as a source of explants in aseptic culture before they return into dormancy.

(3) The bud developed into a plantlet. This plantlet can be directly planted in the greenhouse.

(4) The bud formed a new inflorscence,-

The bud on the newly formed inflorescence could be treated repeatedly, causing more branching, so that more

inflorescence nodes were suitable for use in inflorescence node propagation.

The type of growth of buds on the inflorescence node is dependent upon the hybrid, BA concentration, position on inflorescence and possibly season of the year. The application of BA at the lower nodes produced plantlets, whereas application at the upper nodes produced more inflorescence (Fig.13). The level of flowering hormones was possibly optimal near the tip. The tendency of producing plantlets at the lower nodes and inflorescences at the upper nodes is supported by *in vitro* experiments (Intuwong, 1974)

By application of BA it is possible to induce plantlets, but it is not recommended for commercial use, because only a small number of plantlets is obtained; however, it increases the number of plantlets that result from inflorescence node propagation by aseptic methods.

Heide (1965) found cytokinin treatment of *Bryophyllum diagremontianum* leaves greatly increased the number of buds, but at the same time inhibited root formation. When BA was applied to *Phalaenopsis* buds, multiple shoots with swollen base were obtained from a single node. This appears to be caused by mobilization of protein reserve food and photosynthetic products in the treated area. This mobilizing effect has also been reported in grapes (Letham, 1969, Quinlan and Weaver, 1969).

BA in lanolin paste is not translocated acropetally from the point of application (Fig. 10) and cannot penetrate through bracts (Fig. 9). To be effective in induction of plantlets, BA had to be applied on the bud. This method resulted in limited success because of the uncontrolled and unknown factors in the plants themselves or due to the influence of the environment.

SUMMARY

Clonal propagation of *Phalaenopsis* can be accomplished by division of plantlets spontaneously formed on the inflorescence node, inflorescence tip, or root in some of *Phalaenopsis* species and hybrids. Plantlets can also be induced by top cutting or application of 2000 ppm N-6-benzyl adenine (BA) in lanolin paste on dormant bud of the lower node of inflorescence after removal of bract.

LITERATURE CITED

Chvojka, L.,K..Veres and J.Kozel 1961. The effect of kinins on the growth of apple-tree buds and incorparation of radioactive phosphate. *Biol.Plant.* 3:140-147.

Heide, O.M. 1965. Effects of 6-Benzylaminopurine and 1 napthaleneacetic acid and epiphyllous bud formation in Bryophyllum. *Planta* 67: 281-296.

Intuwong, O. 1974.*Clonal propagation of Phalaenopsis*. Ph.D. Dissertation, Univ. of Hawaii.

Kender, W.J. and S. Carpenter. 1972. Stimulation of lateral bud growth of apple tree by 6-benzylaminopurine. *J.Amer. Soc. Hort. Sci.* 97:377-380.

Letham, D.S. 1969. Cytokinins and their relation to other phytohormones. *Bio. Sci.* 19:309-316.

Northen, R.T. 1970. *Home Orchid growing*. 3rd. ed D. Van Nostrand Co. Inc. Princeton, New Jersey. 374 p.

Quinlan, J.D. and R.J. Weaver.1969.

Influence of benzyladeine, leaf darkening, and ringing on movement of ^{14}C-labelled assimilates into expanded leaves on *Vitis vinifera*. *Plant Physiol.* 44:1247-1252.

Sachs, T. and K.V. Thimaun. 1964. Release of lateral buds from apical dominance. *Nature* 201:939-940

Thompson. N.P. and W.P. Jacobs. 1966. Polarity of IAA effect on sievetube and xylem regeneration in *Coleus* and tomato stems. *Plant Physiol.*41:673-682.

Walker, D.R. 1970. Growth substances in dormant fruit buds and seeds. *Hort. Sci.*5:414-417.

Weaver, R.J.1963. Use of kinin in breaking rest of buds of *Vitis vinifera*. *Nature* 198:207-208.

Weiberger, J.H. 1969. The stimulation of dormant peach buds by cytokinin. *Hort. Sci.* 4:125-126.-

Williams, M.W. and E.A. Stahly. 1968. Effect of cytokinins on apple shoot development from axillary-buds. *Hort.Sci.* 3:68-69.