Keiki induction by cytokinin on *Phalaenopsis* spp.

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Abstract. A Keiki is the product of asexual propagation which naturally develop at the node of a flower stalk or cane. *Phalaenopsis* produce Keiki along the middle to end nodes on the flower stalk. Although keikis are an inefficient way to propagate orchids on a commercial scale, it is interesting to study the cytokinin hormone Benzyl Aminopurine (BAP) effect to stimulate keikis on a node along the flower stalk. Two nodes of each flower stalk were used in this experiment. Cytokinin hormone (0 mg L^{-1}, 2 000 mg L^{-1}, 4 000 mg L^{-1}, 6 000 mg L^{-1} BAP) was only applied once to the second, third, fourth, and fifth nodes of the flower stalk base. The Keiki raised from the second and third nodes of the flower stalk while the flower spike raised from the fourth to sixth basal. BAP stimulated bud break on the node of the flower stalk and could produce either flower spike or keikis depending on node position from the basal node of the flower stalk.

Keywords: Asexual propagation, hormone, important and economic orcid, Indonesian native orchid

1 Introduction

Phalaenopsis spp. is one of the important orchids and has high economic value in Indonesia and *Phalaenopsis amabilis* L. Blume is an Indonesian native orchid [1]. These orchids dominate the orchid agribusiness in the local market as well as for export purposes. Market demand continues to increase, but development constraints related to the provision of quality seeds are still the problem.

Phalaenopsis spp. generally propagated through the separation of tillers and cuttings, induction of axillary shoots, and Keiki. Keikis are common on *Phalaenopsis* and *Dendrobium moniliforme* L. orchids. On a *Phalaenopsis,* a Keiki is a small plant growing from one node along the flower stalk. The growth of Keiki may be stimulated by prolonged exposure to high temperatures during the final phase of spike growth [2]. Naturally, keikis can occur when the mother plant is struggling. Phytohormones such as cytokinin can be applied to a node on the flower stalk to stimulate the Keiki which identical clone of the

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mother plant. This method aims to produce orchid seedlings that flower quickly even though the number of propagations is less than compared to tissue culture propagation.

Phalaenopsis orchids generally bloom once a year under regular fertilizing and watering, provided that ambient temperature and light intensity are suitable. *Phalaenopsis* are tropical plants that grow well under temperatures ranging from 28 °C to 35 °C during the day and 20 °C to 24 °C at night [2]. Cytokinin such as Benzyl Aminopurine (BAP) has been used for *ex vitro* and *in vitro* propagation to promote flower bud formation on *Dendrobium* hybrid [3], *Phalaenopsis* orchid plant [4], to increase the number of spikes and flower [5], to increase the number of leaves per plant on *Phalaenopsis* hybrid cv. Fuller's sunset [6]. Moreover, Hardjo [7] reported that BAP also can use to induce calli formed somatic embryos during 30 d for *in vitro* propagation of *Vanda tricolor* Lindl. var. pallida at ½ Murashige and Skoog Medium with NAA 0.01 $mg.L^{-1}$ and BAP 0.05 $mg.L^{-1}$, also with addition of 1 % sucrose [8].

This study aimed to obtain *Phalaenopsis* orchid seedlings (Keiki) that flower quickly because they came from mature parent plants that are already flowering, by giving the treatment of BAP cytokinin growth regulators on the nodes of the flower stalk.

2 Materials and methods

Phalaenopsis flowering plants were obtained from Simanis Orchids Nursery (Lawang, East Java, Indonesia). The technical BAP grade was used. All experiments were carried out in the shade net greenhouse at PPAU (*Pusat Pembibitan Anggrek Ubaya* – Ubaya Orchid Nursery Center) IOC (Integrated Outdoor Campus), at an altitude ± 1 000 m above sea level in Trawas, East Java. Greenhouse ambient temperature was ± 30 °C/24 °C day/night.

From each plant, two nodes were selected from the base of the flower stalk to be smeared only once with BAP suspension. The sheath of the node from the *Phalaenopsis* flower stalk was carefully opened. Using the sterile toothpicks spread BAP suspension to various node positions from the base of the orchid flower stalk. There were 16 treatments in which a combination of BAP concentration (0 mg L^{-1}, 2 000 mg L^{-1}, 4 000 mg L^{-1}, 6 000 mg L^{-1}) and the position of nodes from flower stalk basal (2, 3, 4, and 5). Experiments were organized according to a completely randomized design with five replication per treatment. The observed variables were the percentage of keikis, percentage of the flower spike, Keiki length, flower spike length, and first bud emergence. The data of all variables were not a normal distribution, so analyzed statistically using Kruskal Wallis Test. Differences between treatments were identified by Mann Whitney U Test at the 5 % significance level.

3 Results and discussions

3.1 Effect of BAP to stimulate Keiki break or flower spike

The node (2nd and 3rd node) that was closest to the base of the orchid flower stalk would result in Keikis shown in Table 1). A combination of BAP treatment (6 000 mg L^{-1}) and the position of a node from the flower stalk base (2nd node) was effective in increasing the percentage and growth of Keiki, and so it was also with a flower spike. Maximum Keiki length or spike length was observed in BAP treatment (6 000 mg L^{-1}). BAP concentrations can significantly stimulate early first Keiki or flower spike, and the highest concentration of BAP (6 000 mg L^{-1}) was the fastest to stimulate bud break and growth of Keiki (Figure 1A, 1B, 1C). These results were in line with those of other workers, indicating the efficiency of BAP for bud break or flower spikes on orchid plants. BAP treatments increased the growth

of both Keiki and flower spike (Table 1). A similar response was observed in *Phalaenopsis* when orchids seedlings were treated with BAP at low temperature (30 °C/18 °C) [4] and *Phalaenopsis* hybrid cv. Fuller's Sunset [6]. The authors found BAP cytokinin was essential for bud break on nodes of the flower stalk, and it increased the growth of both Keiki and flower spike. BAP also can use for *in vitro* propagation, such as shoot multiplication of *Pogostemon cablin* Benth. var. Sidikalang at concentration 0.2 mg L^{-1} with addition of 0.2 mg L^{-1} Kinetin on Murashige and Skoog (MS) medium [9]. Applying cytokinin to the node which contained meristem tissue caused the meristem to grow to form bud. Keiki formation was caused accumulation of growth hormones at a node of the flower stalk. BAP cytokinin specifically promotes cell division, shoot multiplication, and axillary bud proliferation both for *ex vitro* and *in vitro* propagation. A similar trend of results was found by Farag [10] on *Chrysanthemum indicum* L. plants. Agustina [11] stated BAP induced faster shoot emergence from micrografting of mangosteen in vitro. Suryaningsih [12] reported that coconut water (containing cytokinin) did not affect Keiki formation and vegetative growth of *Dendrobium*, but significantly affect generative growth.

Table 1. Effect of various BAP and node of flower spike after 90 d of treatments

Combination of Treatment		Variables				
Concentration of BAP (mg L^{-1})	**Nodes of Flower Spike**	**% Keiki**	**% flower spike**	**Keiki length (cm)**	**Flower spike length (cm)**	**First bud emergence (d)**
0	2	0	0	0.00±0.000^{e}	0.00±0.000^{e}	0.00±0.000^{h}
2 000	2	20 (1/5)	0	6.6±3.130^{d}	0.00±0.000^{e}	9.2±0.837^{ef}
4 000	2	40 (2/5)	0	13.8±2.775^{b}	0.00±0.000^{e}	7.2±0.837^{fg}
6 000	2	80 (4/5)	0	27.6±2.302^{a}	0.00±0.000^{e}	5.6±1.342^{g}
0	3	0	0	0.00±0.000^{e}	0.00±0.000^{e}	0.00±0.000^{h}
2 000	3	20 (1/5)	0	8.60±2.191^{cd}	0.00±0.000^{e}	13.4±2.302^{cd}
4 000	3	20 (1/5)	0	11.6±1.517^{bc}	0.00±0.000^{e}	10.4±1.14^{de}
6 000	3	40 (2/5)	0	25.02±6.76^{a}	0.00±0.000^{e}	5.2±0.837^{g}
0	4	0	0	0.00±0.000^{e}	0.00±0.000^{e}	0.00±0.000^{h}
2 000	4	0	20 (1/5)	0.00±0.000^{e}	19.4±4.669^{cd}	20.4±2.702^{a}
4 000	4	0	20 (1/5)	0.00±0.000^{e}	26.0±9.618^{c}	20.2±1.483^{a}
6 000	4	0	20 (1/5)	0.00±0.000^{e}	70.6±8.473^{a}	8.2±0.837^{efg}
0	5	0	0	0.00±0.000^{e}	0.00±0.000^{e}	0.00±0.000^{h}
2 000	5	0	20 (1/5)	0.00±0.000^{e}	11.4±6.107^{d}	18.6±1.673^{ab}
4 000	5	0	40 (2/5)	0.00±0.000^{e}	25.4±3.647^{c}	16.2±1.304^{bc}
6 000	5	0	80 (4/5)	0.00±0.000^{e}	40.0±7.906^{b}	6.8±1.924^{fg}

Note: Means followed by the same letters within a column are not significantly different from each other at $P > 0.05$ after testing Mann Whitney U Test at $\alpha = 5$ %

Fig. 1. Development of Keiki growth on 2nd node of basal flower stalk on *Phalaenopsis* spp. A. 5 d (earliest bud emergence); B. 14 d; C. 21 d after being treated with 6 000 mg L^{-1} BAP

3.2 Effect of the position of the node from basal flower stalk to form Keiki or flower spike

The lower nodes of the flower stalk had a higher chance of giving Keiki, and the higher nodes were more likely to give a secondary spike (Figure 2). Naturally, the cytokinin content in the base of the stem is greater than at the tip. It caused the node closest to the base of the flower stalk to have a greater chance of generating Keiki than the flower spike. This condition was also supported by ambient temperature. Greenhouse ambient temperature was ± 30 °C/24 °C day/night. Under an appropriate environment, bud primordia at the node could grow to form Keiki or flower spike. According to Blanchard [13] flower initiation on *Phalaenopsis* usually was inhibited at air temperature ≥ 28 °C.

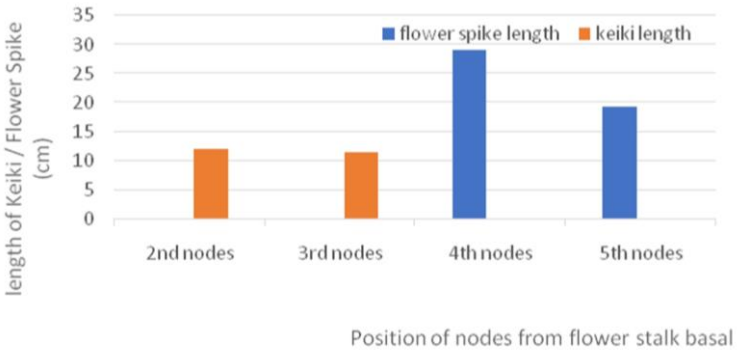

Fig. 2. Effect of nodes position from flower stalk basal on Keiki or flower spike length

Secondary keikis were obtained from basal flower spikes that emergence resulted from the 5th node of the flower stalk (Figure 3A). In this case, the spike raised and grew at the 5th node of the flower stalk, and then without applying BAP, the Keiki raised from the base and 1st node of the secondary spike. Application of BAP to the 5th node of the flower stalk had been shown to have an effect on the appearance of the new flower spike (Figure 3B). In orchids, many studies reported that BAP induced floral bud [3–6], and also being influenced by ambient temperature [13]. The position of the nodes far away from the leaves as a source of photosynthate and roots as a source of water caused a greater chance of flower spike appearance than the Keiki.

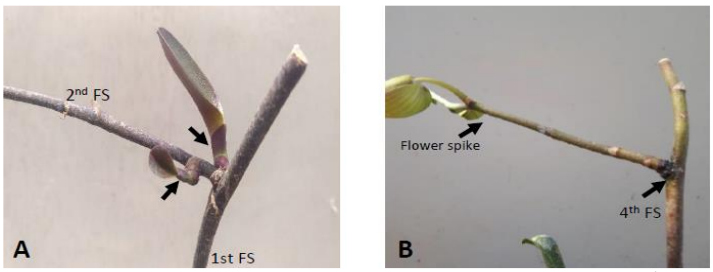

Fig. 3. Keiki was developed from a secondary flower spike that emerged at the 5th node from the flower stalk of *Phalaenopsis* spp. (A) and secondary flower spike was developed from the 4th node from flower stalk (B)
Arrow ⟶ Keiki FS: Flower stalk

4 Conclusions

The application of BAP cytokinin (2 000 mg L^{-1} to 6 000 mg L^{-1}) could stimulate Keiki or a new flower spike depending on node position from the flower spike base. The Keiki is raised from the second and third nodes of the basal flower stalk while the flower spike is raised far away from the basal flower stalk. Treated 6 000 mg L^{-1} of BAP on nodes of flower stalk was the best for stimulating Keiki or new flower spike.

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