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Paclobutrazol enhance budbreak and flowering of Friederick's Dendrobium orchid *In Vitro*

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In vitro shoot of Friederick's Dendrobium orchid at length of 2-3 cm consisted of 3-4 nodes was excised and transferred to culture on MS medium supplemented with 3% sucrose, and four concentrations of PBZ (0.025, 0.05, 0.075 and 0.1 mg/l). PBZ at concentration ranging from 0.025 to 0.075 mg/l promoted budbreak at all nodes at high percentage of 40 to 50 significant difference to high concentration tested (0.1 mg/l). PBZ at 0.05 mg/l gave the highest percentage of floral bud induction at 29% significantly difference to other concentrations. Flowering obtained in all PBZ concentrations containing medium was normal in morphology. The flowers were yellow in color with a diameter of 3-4 cm and consisted of three sepals, two petals, one lip and male and female organ.

Key words: paclobutrazol, budbreak, flowering, *in vitro*, dendrobium

Introduction

The orchid genus includes a large number of species. Besides their economic interest, orchids present also ecological interests by the diversity of their modes of pollination. Most of orchid species such as *Dendrobium* found in the Asiatic region need to be protected from the danger of extermination through deforestation.

Plant growth retardants generally reduce elongation of the internodes of higher plants *in vitro*. Similar results are also observed in reduction in leaf size, dark green color of leaves and thickening of root (Graebe, 1987). There have been few reports on the effect of growth retardants on plantlets grown *in vitro*. Those are focus on the preparation of *in vitro plant* to soil (Smith *et al.*, 1990), induction of drought tolerance during nursery period (Fletcher and Hofstra,

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1985; Swietik and Miller, 1983; Wieland and Wample, 1985). Flowering is a complex mechanism involved in developmental and physiological science. These processes are sensitive to the environment. The ability of explants to produce flowers *in vitro* depends upon various factors such as internal and external, chemical and physical factors which can not be predicted. In term of chemical, plant growth regulators play an important role in this activity. There have been some reports on *in vitro* flowering of orchid but they were species-dependent. Most of them were stimulated by cytokinin (Kostenyuk *et al.*, 1999). In addition, growth retardant was also reported to be superior to induction of flower *in vitro* (Nujeen and Te-chato, 2007).

Paclobutrazol[(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4 triazol-1-yl) pentan-3-ol] is one of growth retardant which inhibits kaurene oxidase and thus blocks the oxidative reactions from ent-kaurene to ent-kaurenoic acid in the pathway leading to gibberellic acid (Graebe, 1987; Radimacher *et al.*, 1984). It is active as a growth retardant in broad spectrum of species (Dalziel and Lawence, 1984; Lever *et al.*, 1982) especially in *Chrysanthemum x morifolium* (Barrett, 1982; McDaniel, 1983; Menhenett, 1984). It is also widely used to induce mild stress tolerance in seedlings and adult plants (Asare-Boamah *et al.*, 1986; Marshall *et al.*, 1991; 2000; Fletcher *et al.*, 2000). The mode of action of PBZ has been associated with a decrease in transpiration, plant height, biomass and leaf area and increase in stomatal resistance. In case of induction of organ formation, there still have little attention. The aim of this study was to document the changes in lateral bud, root induction and floral morphological development of Friederick's Dendrobium orchid.

Materials and methods

Plant material

Multiple shoots induced from culturing shoot with one nodal explant of Friederick's Dendrobium orchid on MS medium supplemented with 0.5 mg/l NAA and 2.5 mg/l BA were used as source of explant. The cultures were maintained under 20 mol/m^2/sec 14 h photoperiod at 26±4^{o}C.

Culture medium and PBZ

Basal MS medium was used without the supplement of growth regulators. The medium was supplemented with 3% sucrose and PBZ at final concentration of 0.025, 0.05, 0.075 and 0.1 mg/l.

Effect of PBZ on response of cultured shoot

Individual shoot of Friederick's Dendrobium orchid at length of 3 cm consisted of 3-4 nodes was excised and transferred to culture on MS medium supplemented with 3% sucrose, and four concentrations of PBZ as mention above. Each concentration of PBZ was replicated three times and each replication consisted of 20 bottles. The cultures were carried out in 8 oz bottle containing 25 ml of culture medium and kept at 26±2^{o}C under 1600 lux illumination for 14 h. After culture for 3 months the responses of shoots in terms of percentage budbreak, average number of shoots/node and average number of roots/shoot were examined.

Effect of PBZ on floral bud induction

Single shoot of Friederick's Dendrobium orchid at length of 2 cm consisted of 2 nodes was excised and transferred to culture on MS medium supplemented with 3% sucrose, and four concentrations of PBZ as mention above. Each concentration of PBZ was replicated three times and each replication consisted of 20 bottles. The cultures were carried out in 8 oz bottle containing 25 ml of culture medium and kept at 26±2^{o}C under 1600 lux illumination for 14 h. After culture for 3 months the floral bud induction in each concentration of PBZ was examined. Significant among PBZ was analysed by completely randomized design and mean was separated by Duncant's multiple range test (DMRT).

Results

Effect of PBZ on response of cultured shoot

PBZ at concentration ranging from 0.025 to 0.075 mg/l promoted budbreak at all nodes at high percentage of 40 to 50 significant difference to high concentration tested (0.1 mg/l) (Fig. 1A). An average number of two shoots were obtained from 0.05-0.75 mg/l PBZ while other concentrations gave only one shoot (Fig. 1B). An average number of roots per shoot were recorded to be 1 to 6 depended on the concentration of PBZ (Fig. 1C). High concentration of PBZ caused the bigger, thicker, shorter and broader leaves. Similar result was obtained in stem development. The stem became bigger and shorter than that of control (Fig. 2A). All the shoots developed from each node were big in size and produce root simultaneously (Fig. 2B). High concentration seemed to promote root formation. However, concentration higher than 0.05 mg/l played inhibition role on number of root.

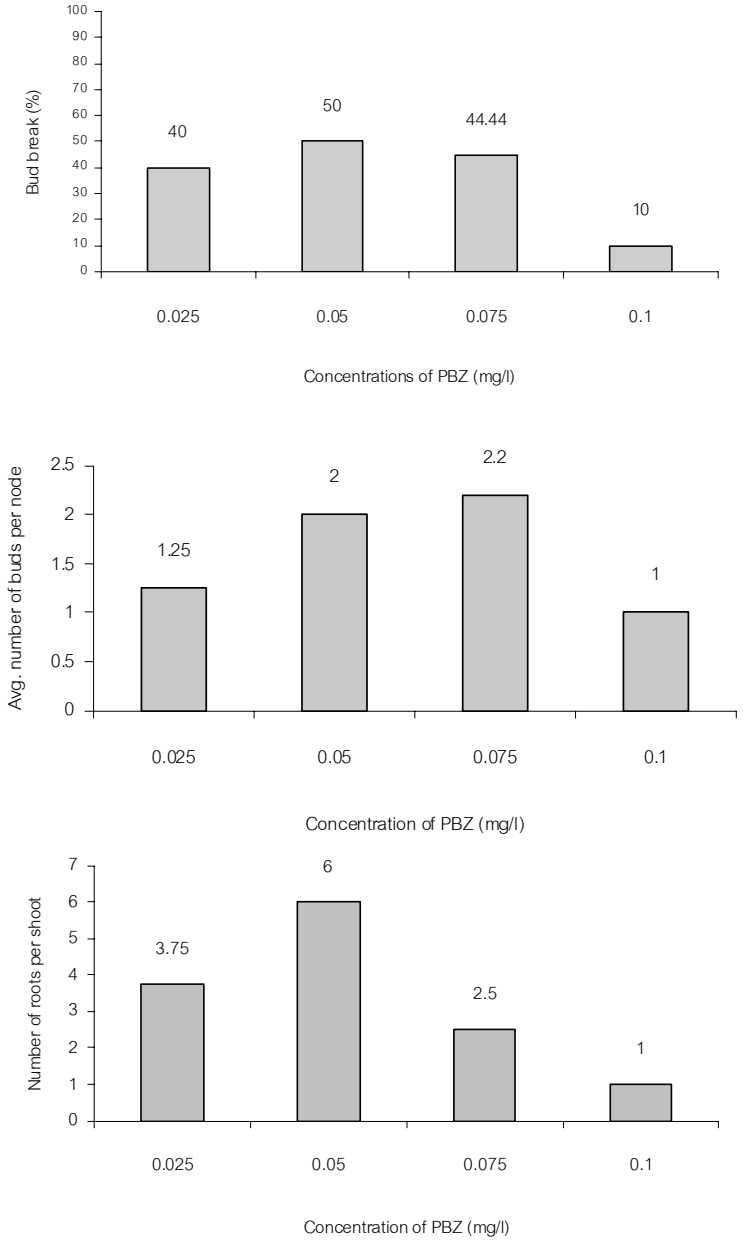

Fig. 1. Effects of PBZ containing in MS hormone-free medium supplemented with 3% sucrose on response of cultured shoot after 3 months of culture.

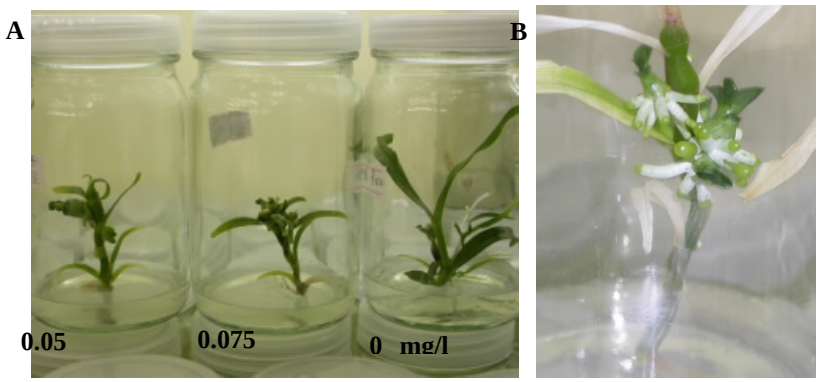

Fig. 2. The response of cultured shoot (3-4 nodes) on PBZ containing in MS hormone-free medium supplemented with 3% sucrose after 3 months of culture (A) subsequent to root formation (B).

Effect of PBZ on floral bud induction

Without supplementation of PBZ in culture medium floral bud induction was not found. A low concentration of PBZ at 0.025 mg/l promoted a small number of floral bud (10%). PBZ at 0.05 mg/l gave the highest percentage of floral bud induction at 29% significantly difference to other concentrations (Fig. 3). However, The highest concentration of PBZ (0.1 mg/l) provided the lowest result (6.95 floral bud induction). Flowering of Friederick's Dendrobium orchid obtained in all PBZ concentrations containing medium was normal in morphology (Fig. 4A). The flowers were yellow in color with a diameter of 3-4 cm and consisted of three sepals, two petals, one lip and male and female organ (Fig. 4B).

Discussion

PBZ is one of plant growth regulator which is widespread in agriculture both foliage and flowering plant production. The changes that PBZ application induces in various plant parts e.g. thicker leaves and larger diameter of stem. In this present study leaves from PBZ-treated plants were smaller, thicker and also observed to be darker green than those from untreated plants. Similar results were also reported by Wood (1984) and Ziv *et al*. (1986). In case of stem length and diameter, all PBZ-treated plants gave shorter internode and bigger stem lead to the strength of stem. Contrary result was obtained in poinsettia stem which McDaniel *et al*. (1990) found that PBZ caused a weak stem. The effect of PBZ on this evident might be due to the increment and

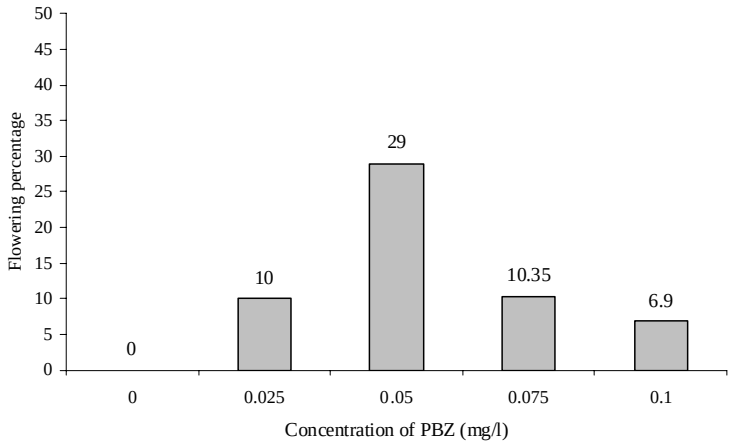

Fig. 3. Effects of PBZ containing in MS hormone-free medium supplemented with 3% sucrose on floral bud induction from cultured shoots (2 nodes) after 3 months of culture.

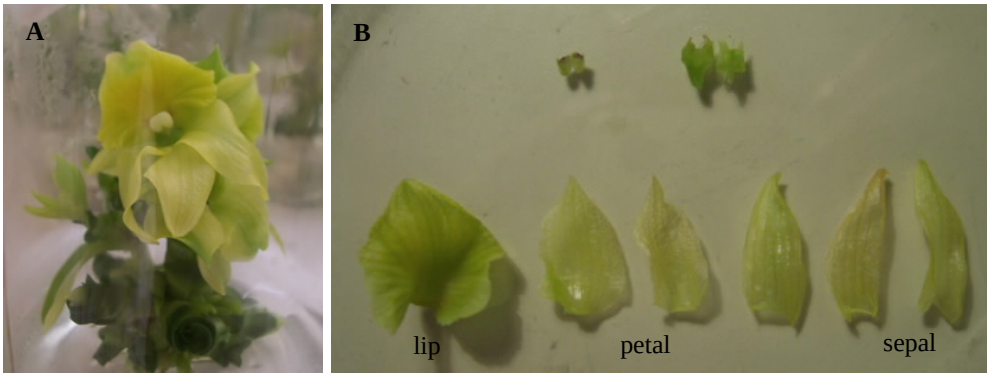

Fig.4. *In vitro* flowering of Friederick's Dendrobium orchid in 0.05 mg/l PBZ containing medium with normal morphology (A) and structural parts (B) after 3 months of culture. (bar=1cm)

decrement in width of bundle sheath or storage of starch in parenchymatous tissue (Aguirre and Blanco, 1990). This evident was clearly found in the stem of new budbreak (Fig. 2B). It is possible that PBZ plays both functions (increase in bundle sheath and starch in parenchymatous tissue). Furthermore, thickening of the roots from new shoot was observed. Eventhough there had a report on the inhibition of lateral root formation (Bausher and Yelenosky, 1987) this present study suggests the opposite result to this observation. Both number of roots and their diameter increased far greater than the above studies.

PBZ is azole derivative which had been found to promote the shoot inducing capability. PBZ at high concentration of 3 mg/l gave the highest shoot number of 3.5 in korarima [Aframomum corrorima (Braun) Jansen] (Tefera and Wannakrairoj, 2006) while a low concentration at 0.05-0.075 mg/l gave the best result in this present study. All axillary buds along the cultured shoot were promoted to develop a new emerging shoot at an average of 2.2 shoots/cultured shoot (Fig. 1B). The different response to concentration of PBZ might be species specific. In addition, starting explant used for culture is different lead to the difference in physiological status, especially the balance of indigenous phytohormones. In case of herbaceous gloxinia, it could tolerate to very high concentration of PBZ at 500 mg/l (Te-cahto and Chudecha, 2006). However, PBZ in combination with some cytokinins, BA or TDZ was reported to have synergistic effects on shoot proliferation (Werbrouck and Debergh, 1996; Tefera and Wannakrairoj, 2006). Unfortunately, cytokinins were not systematically used in combination with PBZ in this experiment. So, their synergistic effect on proliferation of the orchid species in this study can not be postulated. It is very interesting to investigate this effect in the next experiment.

PBZ had been reported to induce shortening of the internodes, reduction of leaf size, an intensification of green coloration of leaves and thickening of roots (Graebe, 1987). For floral bud induction in vitro there still had few reports (Te-cahto and Chudecha, 2006). Mainly use of PBZ for this purpose were reported in field production of both fruit trees and ornamental pot plants. Floral bud induction in vitro in almost all orchid species was performed by manipulation the culture media with BA and NAA (Sim *et al*., 2007). Our previous study also success in floral bud induction in Friederick's Dendrobium orchid with BA but abnormal flowers were obtained (Nujeen and Te-chato, 2007). Replacing BA with PBZ at low concentration at 0.05 mg/l promoted normal flowering (Fig. 4). The function of PBZ on normal flowering development was not clearly understood. The suggestion from this investigation is PBZ might block the activity of some cytokinins which induce abnormality of flowering in vitro.

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