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Influence of paclobutrazol on growth of *Dendrobium* 'Sonia Jo Daeng' under salt stress condition in tissue culture

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ABSTRACT

Contaminated soil water in irrigation systems is one of the important problems for orchids growing in Sampran district, Nakhon Pathom province as well as Krathum Baen district, Samut Sakhon province, Thailand. Salinity reduces plant growth and productivity, therefore, this study aimed to analyze the effect of salinity and paclobutrazol (PBZ) on *in vitro* growth of *Dendrobium* 'Sonia Jo Daeng'. The first experiment involved evaluating the effect of salinity on *Dendrobium* growth in plant tissue culture condition. The results showed that high salt concentration reduced the survival rate of *Dendrobium* plantlets and the percentage of mortality. The median lethal concentration (LC_{50}) of NaCl was 193.3 mM. A higher percentage of survival rates at the PBZ concentration of 0.5 mg/L. Additional experiments from PBZ, supplemented with VW at 0.5 mg/L, have demonstrated a 100% survival of new shoots. This is probably due to the negative effect of PBZ on salt stress in *Dendrobium* 'Sonia Jo Daeng' and promoted the percentage of survival rate in tissue culture.

Keywords: triazole; environmental stress; salinity; plant tissue culture; tropical orchid

1. INTRODUCTION

Dendrobium is one of the largest genera in the *Orchidaceae* family (Kuehnle, 2006) and is very important in Thailand. Many plants of *Dendrobium* genus are precious herbs with high commercial value, especially for exporting purpose (~80% compared with other genera) (Office of Agricultural Economics, 2015). *Dendrobium* flowers have various shapes and colors; most of the commercial hybrids are free flowering throughout the year (Obsuwan et al., 2017; Obsuwan et al., 2019). Some growing areas faced salinity problems in the irrigation system, resulting in less productivity and decrease in the exported value (Department of International Trade Promotion Ministry of Commerce Thailand, 2016; Obsuwan et al., 2019). The primary salinity problem for orchid production, in some areas, was the brackish water contaminating the fresh water in the irrigation canal during the summer season (Obsuwan et al., 2019). Salt stress is one of the abiotic factors limiting plant growth and harvest (Fahad et al., 2015). Excessive concentrations of Na^+ and Cl^- ions cause a nutrient imbalance leading to oxidative stress generated by reactive oxygen species (ROS). As a result, plant growth decreases in terms of quantity and quality of productivity (Horie et al., 2012).

Nakhon Pathom province is considered the largest growing area for *Dendrobium* orchids production compared with other provinces. Moreover, some areas, especially the Sampran district, are facing contamination in the fresh water by the salt present in the water irrigation systems, thereby reducing plant production (Obsuwan et al., 2019). Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1, 2, 4-triazol)-pentan-3-ol] is a plant growth retardant that blocks gibberellin (GA) biosynthesis, leading to reduced plant height and productivity (Mehouchi et al., 1996). Moreover, PBZ also interferes with plant stress by changing the concentration of certain plant hormones, such as cytokinins, abscisic acid (ABA), and ethylene (Fletcher and Hofstra, 1988; Mackay et al., 1990). Several studies have shown how PBZ delays or postpones salt stress effects. As of now, little is known about its effect on orchid growth and development. Therefore, the present study aimed to analyze the effect of salinity on *Dendrobium* growth in tissue culture medium and the effect of PBZ supplementation, with VW medium, on delaying salt stress effect in *Dendrobium* 'Sonia Jo Daeng' using an *in vitro* system.

2. MATERIALS AND METHODS

2.1 Plant materials and culture conditions

Small plantlets of *Dendrobium* 'Sonia Jo Daeng' (0.1 cm in height and 0.1 g fresh weights) were cultured in VW (Vacin and Went, 1949) medium with 150 $ml.l^{-1}$ coconut water and kept in culture room under a 16-h photoperiod (fluorescent light at 35-40 $\mu M/m^2s$) and 25±1°C.

2.2 *Dendrobium* growth under salt stress condition

Dendrobium plantlets were cultured on VW medium supplemented with NaCl at concentrations of 0, 100, 200, and 300 mM, and then kept in a culture room under 16-h photoperiod (fluorescent light at 35-40 $\mu M/m^2s$) at 25°C±1°C for 7 weeks. The experiment was performed with 10 replicates. The following data were collected: survival rate (%), reduction rate (number of died plant/week), mortality rate (%), and median lethal concentration of NaCl (LC_{50}) were measured by probit analysis according Finney (Finney, 1952).

2.3 Use of PBZ to delay salt stress effect on *in vitro* growth of *Dendrobium* 'Sonia Jo Daeng'

Uniformed plantlets of *Dendrobium* 'Sonia Jo Daeng' (0.1 cm in height and 0.1 g fresh weights) were sub-cultured onto VW medium; 200 mM of NaCl was added to PBZ at concentrations of 0, 0.5, 1.0, 2.0, and 4.0 mg/L. After an incubation period in a culture room at 25±1°C under 16-h photoperiod (fluorescent light at 35 $\mu mol/m^2s$) for 10 weeks, data regarding the survival rate and number of new shoots were collected.

2.4 Effect of PBZ on new shoots regeneration after subculture on VW medium

New shoots from the previous experiment were transferred to VW medium and subsequently incubated in a cultured room for 10 weeks. The percentage of new shoots survival and plant growth (such as fresh and dry weight, shoots length, roots length, and number of leaves and roots) were then measured.

2.5 Statistical analysis

Experiments were conducted using a completely randomized design with 10 plants per treatment. Statistical analysis was conducted using analysis of variance using SPSS program version 18 (IBM SPSS statistics for windows, version 21.0. Armonk, NY: IBM Crop). The means of treatment results were compared using Duncan's multiple range test (DMRT) at p=0.05.

3. RESULTS AND DISCUSSION

3.1 Effect of salt stress on *in vitro* growth of *Dendrobium* 'Sonia Jo Daeng'

The effects of NaCl concentration were significantly different in *Dendrobium* 'Sonia Jo Daeng'. At various concentrations (100, 200, and 300 mM), a decrease in the percentage of survival rate was observed (86.7%, 40.0%, and 6.7%, respectively) (Table 1). Higher concentrations of NaCl led to a decrease of survival rate of *Dendrobium* 'Sonia Jo Daeng' in tissue culture. NaCl at concentrations of 100, 200, and 300 mM increased plant reductions per week (Table 1); similarly, the mortality rate of *Dendrobium* increased to 6.7%, 60.0%, and 93.3%, respectively (Table 1). The excessive Na^+ and Cl^- ions in plants may cause water potential imbalance, nutritional imbalance, water loss in plant cells, and decrease in photosynthetic activity. In addition, this will lead to reduction of leaf greenness due to thylakoid membrane degradation and will generate ROS, resulting in reducing quantity and quality of plant yield (Gupta et al., 1995; Xie et al., 2015). Similar effect was found at higher concentration of NaCl *in vitro* conditions, which have shown negative effect on the survival rate of protocorm-like-bodies in two *Dendrobium* cultivars: *Dendrobium* 'Sonia Earsakul' and *Dendrobium* 'Miss Orchid' (Khamtae et al., 2018). Previous research revealed that the salinity concentration at 0.00005–0.00010 mM (NaCl) or more is responsible for inhibiting the growth of *Dendrobium* 'Sonia Earsakul' under greenhouse conditions by reducing the number and dry weight of leaf as well as the flower size (Khamtae et al., 2018). Similar results were found in sorgum. The results showed that high concentration of NaCl decreased fresh and dry weight of stem, root length, and water content (Forghani et al., 2018). In a high salinity level of 0.00065 mM NaCl (within 30 days), plants lost nearly 50% of their leaf dry weight and flower quality (Abdullakasim et al., 2018). Similarly, in tomato, the plant dry mass is reduced by approximately 50% during a 0.00035 mM NaCl treatment (Maggio et al., 2007). We additionally measured the LC_{50} of *Dendrobium* plants cultured on VW medium containing 0, 100, 200, and 300 mM of NaCl and found that NaCl at a concentration of 193.3 mM killed 50% of orchid plants (Figure 1). In a different experiment, we added NaCl at a concentration of 200 mM to evaluate the efficacy of PBZ to alleviate salt stress effect in *Dendrobium*.

3.2 Effect of PBZ delaying salt stress effect on *in vitro* growth of *Dendrobium* 'Sonia Jo Daeng'

Dendrobium plantlets were cultured on VW medium with 200 mM NaCl supplemented with PBZ at the concentrations of 0, 0.5, 1, 2, and 4 mg/L for 10 weeks. The results showed that *Dendrobium* plantlets cultured on VW with 200 mM NaCl supplemented with 0.5 mg/L PBZ showed a strong percentage of survival up to 40% (Figure 2). Moreover, when PBZ was higher than 0.5 mg/L, the survival rates were decreased (Figure 2). This may be due to the role of PBZ at appropriate concentration, which could reverse the negative effect, such as imbalance of plant nutrients, osmotic stress, and imbalance of specific ion uptake (Flowers et al., 1991). In wheat, a salt tolerance cultivar has lower rates of Na^+ uptake and higher rates of K^+ or Ca^+ uptake than that of Na^+ (Schachtman and Munns, 1992). In mango seedlings grown under salinity stress, the results showed that 1,500 mg/L PBZ reduces the stress from NaCl by decreasing ion leakage up to 64% when compared with the non-PBZ-treated plants (Srivastava et al., 2010). In *Phaseolus vulgaris*, PBZ treatment under salt stress condition in soil improves free proline content, which prevents negative effect in terms of less membrane damage (Mackay et al., 1990). In wheat, PBZ could enhance salt tolerance by adding osmoprotectant chemicals especially in salt-sensitive cultivar (Hajihashemi et al., 2006). In peach and olive plants under salt stress condition, PBZ treatment have reduced Na^+ and Cl^- ions level (Abou El-Khashab et al., 1997). In wheat planted under salt stress condition, PBZ can enhance adaptive systems, such as increased shoot and roots' fresh weight according to increase in the water content (Fletcher et al., 2000).

Table 1. Survival rate, plant reduction and mortality rate of *Dendrobium* 'Sonia Jo Daeng' cultured on VW medium supplemented with NaCl at concentrations of 0, 100, 200, and 300 mM

Concentration of NaCl (mM)	Survival rate (%)	Plant reduction (number of died plants/week)	Mortality (%)
0	100.0	0	0
100	86.7	0.2	6.7
200	40.0	1.8	60.0
300	6.7	2.3	93.3

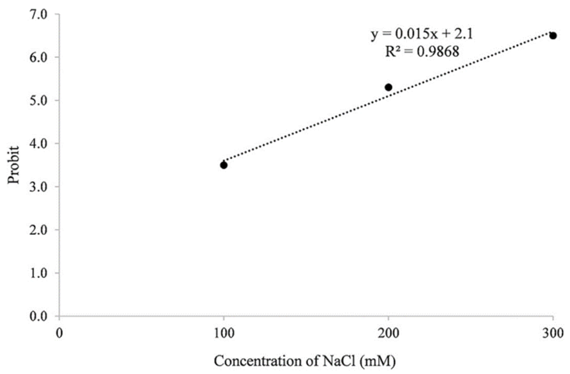

Figure 1. Median lethal concentration (LC_{50}) of NaCl on VW medium; y=0.015x+2.1; R^2=0.9868; y=5.00; LC_{50}=193.3 mM

3.3 Effect of PBZ on new shoot regeneration after subculture in VW medium

Our analysis found that two different concentrations (0.5 and 1 mg/L) of PBZ were optimal for producing new shoots (Table 2). Characteristics of new shoot derived from PBZ treatment showed that the shorter new shoot has thicker leaves (Figure 3). PBZ has been reported to decrease plant growth by reducing internodal elongation with thicker and darker leaves (Grossman, 1988). PBZ prevents gibberellin (GA) biosynthesis, resulting in lower plant growth and development (Mehouchi et al., 1996). In rose plants, for example, the application of PBZ causes a 10% total wax increase and changes the proportion of certain wax constituents in the rose leaves within 11 days (Jenks et al., 2001). Our experiment found that PBZ at the concentrations of 0.5 and 1 mg/L were appropriate for new shoot induction, and this may be due to increase in PBZ signaling of cytokinin in *Dendrobium* plants.

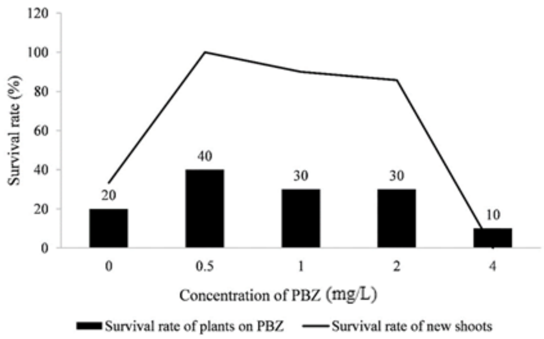

Figure 2. Percentage of *Dendrobium* 'Sonia Jo Daeng' survived on VW medium with 200 mM NaCl supplemented with paclobutrazol (PBZ) at the concentrations of 0, 0.5, 1, 2, and 4 mg/L for 10 weeks and the percentage survival of new shoots

Table 2. Number of new shoots' fresh and dry weight of *Dendrobium* 'Sonia Jo Daeng' cultured on VW medium with 200 mM of NaCl supplemented with paclobutrazol (PBZ) at concentrations of 0, 0.5, 1, 2, and 4 mg/L for 10 weeks

Concentration of PBZ (mg/L)	Number of new shoots	Fresh weight	Dry weight
0.0	0.3±0.21^{bc}	0.1±0.00^{ab}	0.009±0.00^{b}
0.5	1.0±0.15^{a}	0.2±0.03^{a}	0.015±0.00^{a}
1.0	1.0±0.26^{a}	0.2±0.02^{a}	0.019±0.00$^{a.}$
2.0	0.7±0.21^{ab}	0.1±0.04^{ab}	0.012±0.00^{ab}
4.0	0.^{c}	0^{c}	0^{c}

Note: Mean comparisons were made using Duncan's multiple range test (DMRT) at *p*=0.05. Different letters indicate significant differences between treatments.

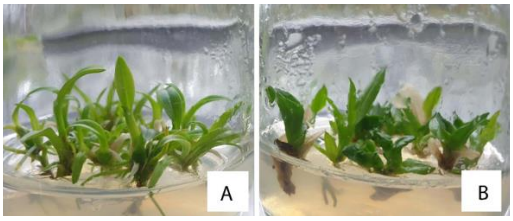

Figure 3. New shoot regeneration of *Dendrobium* 'Sonia Jo Daeng' on VW medium without PBZ (A) and with PBZ (B)

After subculture of new shoots onto VW medium for 10 weeks, the results showed that new shoots transferred from VW medium supplemented with PBZ 0.5 mg/L exhibited a maximum survival rate of 100% while new shoots transferred from the control treatment without PBZ treatment had only 33.3% survival rate (Figure 2). In salt stress condition, growth and development in plants are reduced due to different aspects, such as osmotic or nutritional imbalance (Flowers et al., 1991). A study of the effect of PBZ in two cultivars of wheat under salt stress condition has shown that plants treated with PBZ have a significant increase in root and shoots' fresh weight (Hajihashemi and Kiarostami, 2007). The present study showed that all new shoots obtained from VW (200 mM NaCl) at all PBZ concentrations had higher values of fresh weight, dry weight, shoots length, roots length number of leaves, and roots, except PBZ at a concentration of 4.0 mg/L (Table 2). Fletcher et al. (2000) found that PBZ strengthens the root diameter and length in wheat cultivar Ghods under salinity condition (Fletcher et al., 2000). Our experiment revealed that the number of roots from new shoots on a VW medium after NaCl treatment with 1.0 mg/L PBZ increased the root formation and length compared with other treatments (Table 3). PBZ treatment appeared to increase the length of fibrous roots and lateral root formation in *Catharanthus roseus* species (Jaleel et al., 2007). Diverse experiments indicated that PBZ helps in the detoxification process of active oxygen (Upadhyaya et al., 1990; Kraus and Fletcher, 1994), increases proline levels (Mackay et al., 1990) as well as antioxidants (Senaratna et al., 1988), including chlorophyll content (Fletcher and Hofstra, 1988). The mechanism of PBZ-treated plants has been responsible for a slower growth rate in plants as well as increased the water soluble content in some others (Abou El-Khashab et al., 1997; Van den Driessche, 1996). Further research will help evaluate the growth and development of new shoots in PBZ-treated plants after they are transplanted on to the field.

Table 3. Shoot length, leaf number, root length, and root number from new shoot of *Dendrobium* 'Sonia Jo Daeng' on VW medium from salt stress condition with PBZ at concentrations of 0, 0.5, 1, 2, and 4 mg/L

Concentration of PBZ (mg/L)	Shoot length	Number of leaves	Root length	Number of roots
0.0	0.5±0.00^{a}	3.0±0.00^{a}	0.7±0.00^{a}	8.0±0.00^{ab}
0.5	0.8±0.09^{a}	3.5±0.17^{a}	0.9±0.06^{a}	8.2±0.52^{ab}
1.0	0.7±0.07^{a}	3.8±0.15^{a}	0.9±0.05^{a}	9.7±0.72^{a}
2.0	0.6±0.03^{a}	3.7±0.21^{a}	0.7±0.08^{a}	5.7±0.96^{b}
4.0	0^{b}	0^{b}	0^{b}	0^{c}

Note: Mean comparisons were made using Duncan's multiple range test (DMRT) at p=0.05. Different letters indicate significant differences between treatments.

4. CONCLUSION

High concentration of NaCl decreased the survival rate of *Dendrobium* 'Sonia Jo Daeng'. The application of 0.5 mg/L PBZ can increase the survival rate of *Dendrobium* 'Sonia Jo Daeng' under salt stress conditions in tissue culture because it promotes the survival new shoot when transferred to regular condition on VW medium. Hence, in the future, the application of PBZ may be valuable to alleviate the salt stress effect in *Dendrobium* plants.

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