



Effects of Explants on Plant Regeneration and Concentration of Paclobutrazol on Morphological Responses of Dwarf Water Hyssop (*Bacopa monnieri*)

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Abstract

Water hyssop is an ornamental aquatic plant which is very popular among aquarium hobbyists. The present study reports on the effect of PBZ concentration on water hyssop to produce dwarf aquatic plants. The different types of explant were cultured on MS medium supplemented with 0.5 mg/L BA for shoot and root induction. The single nodal was cut and cultured on MS supplemented with various PBZ concentrations to produce the dwarf ornamental aquatic plant. The results showed that shoot induction started within 2 weeks and the maximum average number of shoots received from the node with leaf explant was 12.11 shoots after 4 weeks of culturing. The highest concentration at 40 mg/L of PBZ retarded stems, number of leaves, number of roots, root length, and the survival rate declined within 2 weeks of culturing. Only 2.5 mg/L PBZ stimulated shoot induction at 51.85%, but 20 mg/L of PBZ the percentage of shoot induction of 48.15 was obtained. Shoot length did not increase after treating with 40 mg/L of PBZ and also resulted in the lowest average number of leaves of 1.25 leaves. The maximum concentration of PBZ caused toxic reactions, such as yellowing, stunted leaves, short roots, and death of *in vitro* cultures. The shoot length decreased with increasing concentrations of PBZ. Thus, at 2.5 mg/L of PBZ was suitable for producing dwarf water hyssop plant with the maximum shoot length, maximum number of leaves and longest root. Finally, the dwarf plantlets were acclimatized successfully to 4 weeks in aquarium conditions.

Keywords: *Bacopa monnieri*, micropropagation, paclobutrazol, dwarf aquatic plant, morphological responses

Introduction

Water hyssop (*Bacopa monnieri*) is among the aquatic plants that is very popular with aquarium hobbyists. Water hyssop is a fast proliferating aquatic plant species in wetlands (Sinha, Gupta, & Chandra, 1996). It is a very popular aquarium plant in Turkey because of its characteristics and adaptability to moderately brackish conditions (Karataş & Aasim, 2014). Water hyssop was listed as an endangered species by the IUCN long ago because of its high demand, and because of the relatively small number of species in nature (Subashri & Koilpillai, 2014). Water hyssop is well-known by aquarists and it is widespread as an ornamental aquatic plant (Laohavisuti, Ruangdej, & Wangwibulkit, 2017). It is one of the most uncomplicated and easily grown aquarium plants needing clean, clear water, sufficient nutrition, and other good conditions. It emerges upright and strong from the water surface. Furthermore, it can grow very well even with insufficient carbon dioxide (CO₂) and is resilient even in intense light. The combination of CO₂, intense light, nitrate, and phosphate will cause faster growth. In the aquarium, water hyssop has an average height of 3 to 6 cm after two months of growth in the tank (Tropica, 2019). However, it may be difficult for some aquarists to decorate in a small aquarium because of the fast growth rate. No researcher has mentioned the dwarf water hyssop as an aquarium plant although it may be among the most interesting for the aquarist when designing their home aquarium.

Plant tissue culture is very important for mass propagation and changes the morphology of ornamental plants via applying tissue culture techniques with a plant growth regulator. Hormones such as auxin,



gibberellin, and cytokinin have their own specific effects on the various cells of plants. Some hormones improve shoot proliferation and early flowering while the combination of some hormones reduces the growth ratio. Paclobutrazol (PBZ) is a plant growth regulator of the triazole family. It has been found to prevent different environmental stresses including heat radiation, drought, and chilling (Tesfahun, 2018). PBZ leads to slow plant growth, short stems, green leaf color, short stature, and leaf thickening. Most importantly, it induces flowering while increasing resistance to drought stress and high salt stress simultaneously (Indrayanti, Putri, Sedayu, & Adisyahputra, 2019). In addition, Boontiang, Chutichudet, and Chutichudet (2019) reported that PBZ can accelerate chlorophyll pigment, and nonstructural carbohydrates gathering during offseason. PBZ mainly acts by changing the number of endogenous hormones and reorienting nutrients inside the plant to stabilize the 'source-sink' balance (Kuai, Li, Yang, & Zhou 2017).

To meet medical and commercial demands for water hyssop, tissue culture was applied for consistent quality (Laohavisuti et al., 2017). The use of a biotechnical tool for water hyssop propagation is useful in light of the multiple uses of the plant, including medical uses. In these cases, dwarf water hyssop plants should be researched by *in vitro* techniques for ornamental plant production. The objectives of this study were to determine the effect of different types of explants for shoot and root induction, and to investigate the morphological response of water hyssop treated with different concentration of PBZ.

Methods and Materials

Plant material: *In vitro* explants were obtained from the Crop Biotechnology Laboratory, Agricultural Innovation and Management Division, Faculty of Natural Resources, Prince of Songkla University. They were cultured on MS medium (Murashige & Skoog, 1962) consisting of 0.5 mg/L BA, 30 g/L sucrose, solidified with 7 g/L agar, and adjusted to pH 5.7 before autoclaving at 121°C for 15 minutes. The culture was maintained at 28±0.5°C for 12h photoperiods at 12.5 µmol/m²/s for 4 weeks.

Effects of different explant types on shoot and root induction

Different explants (leaf, node with leaf, node without leaf, and internode) were cultured on MS medium supplemented with 0.5 mg/L BA, 30 g/L sucrose, solidified with 7 g/L agar, and adjusted to pH 5.7. The cultures were maintained at 28±0.5 °C under 12h photoperiods at 12.5 µmol/m²/s. After 4 weeks of culturing, the percentage of shoot induction, number of shoots, length of shoots, percentage of root induction, and number of roots were recorded.

Effects of different concentrations of Paclobutrazol on morphological responses

Single shoots were cut about 2 cm length and cultured on growth regulator-free MS medium, supplemented with 6 different concentrations of PBZ (0, 2.5, 5, 10, 20, and 40 mg/L), 30 g/L sucrose, solidified with 7 g/L agar, and adjusted to pH 5.7. The culture was maintained at 28±0.5 °C under 12h photoperiod at 12.5 µmol/m²/s. Each treatment was performed with 4 replications. After 6 weeks of culturing, the survival rate, shoot and root induction percentage, number of shoots, number of roots, number of leaves, shoot and root length were recorded and statically compared. Completed plantlets were acclimatized in a cylinder aquarium for 4 weeks to observe the survival rate.

Statistical analysis

All the experiments were conducted in a completely randomized design (CRD). The data were analyzed using one-way analysis of variance (one-way ANOVA). The statistical significances of the means of each treatment was separated by Duncan's multiple range test (DMRT) at the confidence levels of 95 and 99%.

Results

Effect of different explant types on shoot and root induction

After 4 weeks of culturing with different explants, 100% shoot induction was obtained from all types of explants. The results showed that the hormone BA was positive for shoot regeneration. Excised bud regeneration to shoot started within 2 weeks of culture on MS medium. The leaf segment exhibited small organogenesis from the plural petiolar cut edges (Figure. 1A). However, the node with leaf explant induced an adventitious shoot between 10 and 15 days (Figure. 1B), whereas the node without leaf explant produced the elongated adventitious shoot after 2 weeks of culture (Figure. 1C). Moreover, adventitious bud initiated at the apical cut edges of the internode explant (Figure. 1D) during the 2nd week of culture. Shoot regeneration occurred within 3 weeks for all types of explant.

The node with leaf explant had the highest shoot induction at 12.11 shoots (Figure. 1F), which was highly significant ($P<0.01$) compared to the other treatments. In addition, the average number of shoots which lengths above 4 cm was 0.11 shoots, from internode (Figure. 1H), though this was not significantly different from the other treatments. The highest average number of shoots which lengths between 3 and 4 cm was 0.33 shoots, from node with leaf segment, which was highly significant different ($P<0.01$) with the leaf and node without leaf explant. In contrast, the average number of shoots which lengths between 2 and 3 cm was 0.88 shoots, from a node with leaf explant, though it was not significantly different from the other treatments. The highest average number of shoots less than 2 cm was 2.44 shoots from a node with leaf explant, though this too was not significantly different from the other treatments. The average number of shoots less than 1 cm was 8.44 shoots from the node with leaf explant which was significantly different ($P<0.05$) with leaf and internode explant. As the results, the adventitious shoot less than 1 cm in length had the maximum number of shoots, compared to the other parameters.

Table 1 Effect of different explant types on shoot induction after culturing on MS medium supplemented with 0.5 mg/L BA for 4 weeks (Mean \pm SD)

Treatments	Shoot induction (%)	No. of shoots (shoot)	No. shoots in each length category (shoots)				
			> 4 (cm)	3-4 (cm)	2-3 (cm)	<2 (cm)	<1 (cm)
Leaf	100.00 \pm 0.00	9.00 \pm 0.66 ^b	0.00 \pm 0.00	0.00 \pm 0.00 ^b	0.67 \pm 0.33	1.77 \pm 1.07	6.55 \pm 0.69 ^b
Node with leaf	100.00 \pm 0.00	12.11 \pm 1.38 ^a	0.00 \pm 0.00	0.33 \pm 0.00 ^a	0.88 \pm 0.38	2.44 \pm 0.50	8.44 \pm 1.17 ^a
Node without leaf	100.00 \pm 0.00	9.22 \pm 1.26 ^b	0.00 \pm 0.00	0.00 \pm 0.00 ^b	0.44 \pm 0.39	1.55 \pm 0.19	7.22 \pm 1.26 ^{ab}
Internode	100.00 \pm 0.00	7.66 \pm 0.57 ^b	0.11 \pm 0.19	0.11 \pm 0.19 ^{ab}	0.77 \pm 0.69	1.22 \pm 0.38	5.44 \pm 0.19 ^b
F-test	ns	**	ns	**	ns	ns	*
C.V. (%)	4.26	10.91	346.41	86.60	67.88	36.05	13.49

* significantly different ($P<0.05$), ** highly significant different ($P<0.01$)

ns= not significantly different

Mean values followed by the same letters within a column are not significantly different according to DMRT.

In addition, root induction was 100% in all treatments. Root emerged between 15 and 20 days after culturing on MS basal medium supplemented with 0.5 mg/L BA. The internode explant provided the highest average number of roots at 13.66 roots whereas the lowest average number of roots was from node without leaf at 5.66 roots. Root emerged within 2 weeks in the basal medium from all type of explants. However, a statistical analysis was found that there was not significantly different in the root induction and number of roots among the explant types.

Table 2 Effect of explant types on root induction after culturing on MS medium supplemented with 0.5 mg/L BA for 4 weeks (Mean \pm SD)

Treatments	Root induction (%)	No. of root (roots/explant)
Leaf	100.00 \pm 0.00	11.56 \pm 5.50
Node with leaf	100.00 \pm 0.00	7.56 \pm 2.83
Node without leaf	100.00 \pm 0.00	5.66 \pm 2.64
Internode	100.00 \pm 0.00	13.66 \pm 5.50
F-Test	ns	ns
C.V. (%)	4.26	45.24

ns= not significantly different

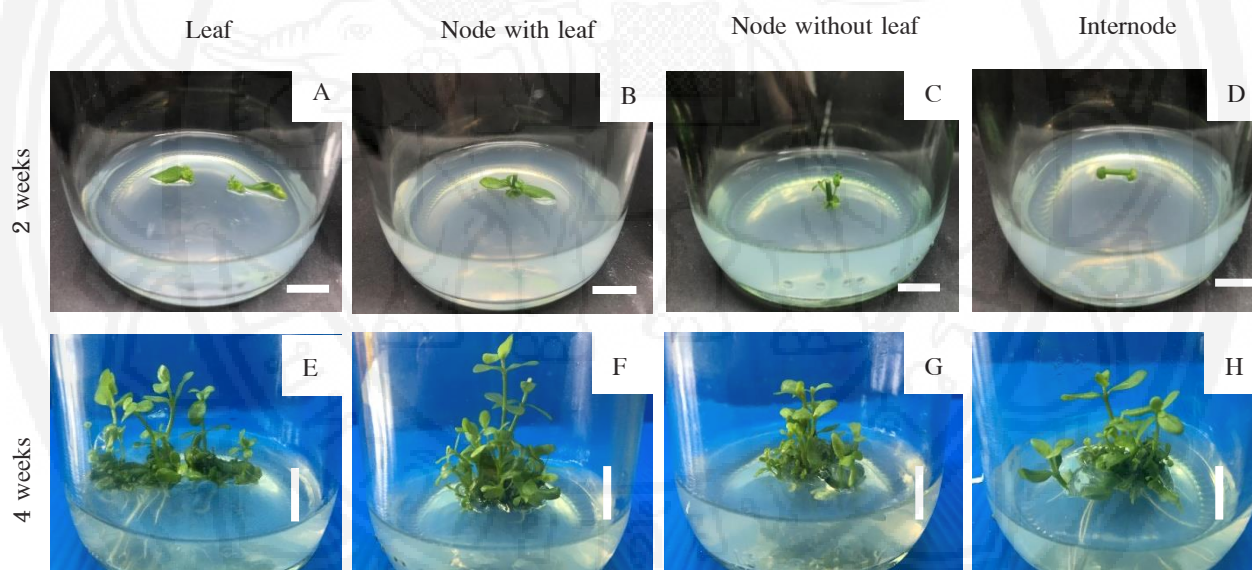


Figure 1 Characteristics of water hyssop plantlet after culturing on MS medium supplemented with 0.5 mg/L BA for 2 and 4 weeks (Bar = 1 cm)

Effect of different concentrations of Paclobutrazol on the morphology of water hyssop (*Bacopa monnieri*)

Single nodal segments were treated with different concentrations of PBZ such as 0, 2.5, 5, 10, 20, and 40 mg/L. After 6 weeks of *in vitro* culture with different concentrations of PBZ, the results showed a 100% survival rate of all treatments except the 40 mg/L PBZ treatment, which had a survival rate of 27.77%. The highest concentration at 40 mg/L of PBZ caused the water hyssop to yellow and die within 2 weeks of culturing.

The percentage of adventitious shoot induction were at 51.85, 61.11, 57.40, and 48.15%, which is highly significant ($P < 0.01$) in the range of PBZ concentration at 2.5, 5, 10, and 20 mg/L, respectively.

However, 40 mg/L PBZ provided less adventitious shoot multiplication at 3.71% due to the harmfulness of PBZ efficiency. In addition, the highest concentration of PBZ at 40 mg/L gave the lowest average number of shoots at 0.16 shoots/explant, which was highly significant ($P<0.01$) compared with other treatment excepted to the control. The average number of shoots induction were 0.78, 1.01, 1.09, and 0.76 shoots/explant from 2.5, 5, 10, and 20 mg/L PBZ, respectively. The shortest shoot length was 2 cm, which is the same as the plant's initial height. The treatment with 40 mg/L PBZ was highly significant ($P<0.01$) compared to the control. The highest average shoot length was 3.56 cm from control which was highly significant different ($P<0.01$) with other treatment excepted to 2.5 mg/L PBZ. Obviously, the treatment with 2.5 mg/L PBZ resulted the longest shoot length of 3.37 cm, which was highly significant ($P<0.01$), compared with other treatments but not when compared with the control. Consequently, the application of PBZ caused retarded stem height, shoot induction and reduced cell elongation of all treated plants.

On the other hand, the concentration of PBZ influenced the number of leaves for all treatments. The 2.5 mg/L PBZ resulted the maximum average number of leaves at 22.61 leaves/explant which was highly significant different with the other treatments. The treatment with 40 mg/L concentration had the smallest average number of leaves at 1.25 leaves/explant. PBZ affected on the smaller, thicker, and dark green leaf compared to the control. The leaves yellowed and died within 2 weeks after culturing with the overdose of PBZ.

Table 3 Effect of different concentrations of PBZ on morphology response of water hyssop (*Bacopa monnieri*) after culturing for 6 weeks (Mean \pm SD)

Concentration (mg/L)	Survival rate (%)	Shoot induction (%)	No. of shoot (shoots/explant)	Shoot length (cm)	No. of leaf (leaves/explant)
0	100.00 \pm 00.00 ^a	31.48 \pm 32.52 ^{ab}	0.61 \pm 0.48 ^{ab}	3.56 \pm 0.23 ^a	18.05 \pm 0.92 ^{bc}
2.5	100.00 \pm 00.00 ^a	51.85 \pm 11.47 ^a	0.78 \pm 0.16 ^a	3.37 \pm 0.60 ^a	22.61 \pm 0.59 ^a
5	100.00 \pm 00.00 ^a	61.11 \pm 15.31 ^a	1.01 \pm 0.22 ^a	2.56 \pm 0.25 ^b	19.29 \pm 0.38 ^b
10	100.00 \pm 00.00 ^a	57.40 \pm 19.14 ^a	1.09 \pm 0.29 ^a	2.18 \pm 0.11 ^{bc}	17.00 \pm 0.79 ^{bc}
20	100.00 \pm 00.00 ^a	48.15 \pm 20.68 ^a	0.76 \pm 0.32 ^a	2.13 \pm 0.13 ^{bc}	15.07 \pm 0.36 ^c
40	27.77 \pm 44.30 ^b	3.71 \pm 9.07 ^b	0.16 \pm 0.40 ^b	2.00 \pm 0.00 ^c	1.25 \pm 1.25 ^d
F-test	**	**	**	**	**
C.V. (%)	20.56	46.31	45.09	11.15	12.36

** highly significant different ($P<0.01$)

Mean values followed by the same letters within column are not significantly different according to DMRT.

Root induction was 100%, even though the plants were treated with different concentrations of PBZ (Table 4) excepted to 40 mg/L PBZ which had the lowest root induction at 38.88% , compared with the other treatments. The average number of roots was in the range of 0.37 to 8.40 roots/explant. The application of PBZ influenced on the number of induced roots. As a result, all the treatments with PBZ were highly significant different ($P<0.01$). The 2.5 mg/L PBZ induced the moderate average number of roots at 7.62 roots/explant while the highest concentration at 40 mg/L PBZ had the lowest average number of roots at 0.37 roots per explant.

The treated plant with 2.5, 5, 10, 20, and 40 mg/L PBZ had root length of 2.56, 2.28, 1.10, 0.83, and 0.03 cm which are highly significant ($P<0.01$). The longest root length was 2.56 cm from the treatment



applied with 2.5 mg/L PBZ, which is highly significant different ($P<0.01$) with the other treatments. While the shortest average root length at 0.03 cm, was belonged to the treatment applied with the highest concentration of 40 mg/L PBZ. The length of root induced with the highest concentration at 40 mg/L PBZ was a shorter than the length of root induced with the lowest concentration of 2.5 mg/L PBZ. Thus, the number of root induction and root length of water hyssop are reduced while applied with the higher concentration of PBZ.

Table 4 Effect of different concentrations of PBZ on root induction of water hyssop (*Bacopa monnieri*) after culturing for 6 weeks (Mean \pm SD)

Concentration (mg/L)	Root induction (%)	No. of root (roots/explant)	Root length (cm)
0	100.00 \pm 00.00 ^a	5.29 \pm 0.42 ^b	2.93 \pm 0.21 ^a
2.5	100.00 \pm 00.00 ^a	7.62 \pm 0.74 ^a	2.56 \pm 0.12 ^b
5	100.00 \pm 00.00 ^a	8.40 \pm 0.73 ^a	2.28 \pm 0.17 ^c
10	100.00 \pm 00.00 ^a	7.37 \pm 0.50 ^a	1.10 \pm 0.11 ^d
20	100.00 \pm 00.00 ^a	5.83 \pm 0.37 ^b	0.83 \pm 0.25 ^d
40	38.88 \pm 49.06 ^b	0.37 \pm 0.90 ^c	0.03 \pm 0.08 ^e
F-test	**	**	**
C.V. (%)	22.30	11.07	10.61

** highly significant different ($P<0.01$)

Mean values followed by the same letters within column are not significantly different according to DMRT.

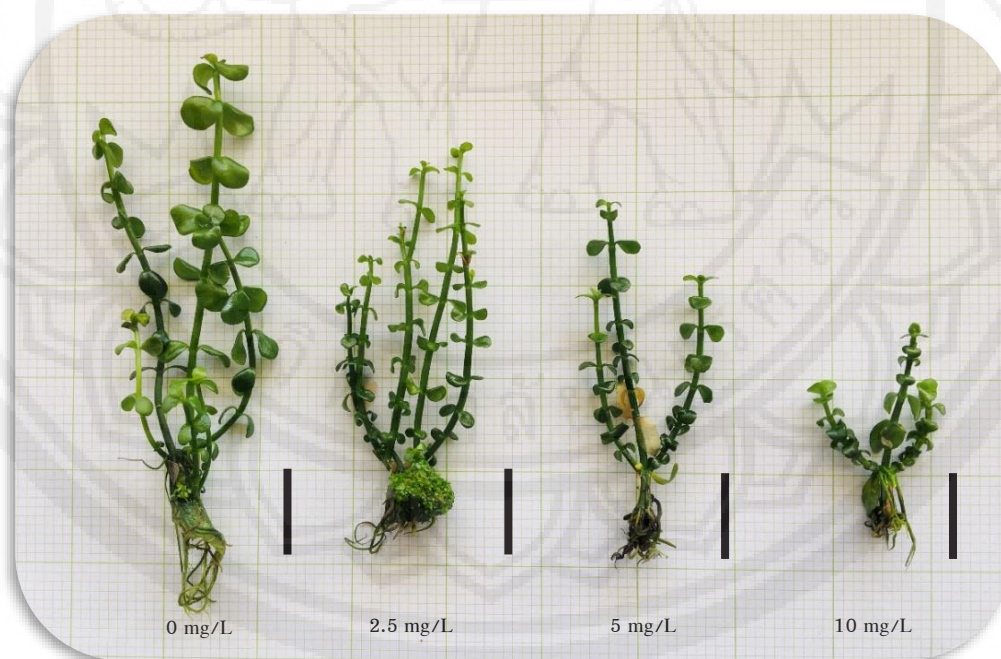


Figure 2 The characteristics of water hyssop plantlets after treated with various PBZ concentration for 20 weeks (Bars = 1 cm)

After 6 weeks of *in vitro* culture, plantlets were acclimatized to *ex vitro* by using freshwater. The plantlets were rinsed with tap water to remove the agar adhere on the roots and transferred to aquariums for

acclimatization. The plantlets adapted successfully to aquatic environmental conditions and 100% survived with healthy, strong shoots and green leaves (Figure 3).



Figure 3 The characteristics of dwarf water hyssop acclimated in the aquarium for 4 weeks

Discussion

The present study highlights the effects on adventitious shoot induction from different explants. It also aims to be a protocol for dwarf aquatic plant production, specifically for water hyssop. Micropropagation of aquatic plants is very important for production of aquarium ornamental plants. The results showed that the different explants cultured on MS medium supplemented with 0.5 mg/L BA differed in their shoot bud regeneration ability. The node with leaf segment was the best explant for shoot multiplication that was consisting of auxiliary buds at both proximal and distal of petiolar leaf, produced numerous shoots. Furthermore, the node with leaf segment had more potentially induced shoots between leaves and the bottom nodal cut edge. Similarly, Sarkar and Jha (2017) reported that shoot bud induction of water hyssop was occurred at petiolar cut edges of a leaf, and between marginal and distal cut edges of internode after culturing on MS medium for 10 to 15 days with different positions. The nodal edge placed in the agar nutrients, while the leaf margin absorbed nutrients from the surface medium. Both parts can germinate shoot bud which provided the optimum adventitious shoots, compared to the other explants. According to Joshi, Pathak, Sharma, and Singh (2010) studied on “High frequency of shoot regeneration on leaf explants of *Bacopa monnieri*” reported that the kind of wounding and placement of explants on the medium affected the rate of adventitious shoot bud regeneration. The maximum number of shoots was produced by the square leaf lamina. Exactly, the shoot bud response of water hyssop started in the second week in all sizes of explants. So, this research can suggest that the node with leaf segment has a larger surface area, hence absorbing nutrients in medium to induce the highest number of shoots.

This study found that the shoots length less than 1 cm had the maximum average number of shoots and latest induction shoots after culturing on slight of BA at 0.5 mg/L, compared to the other parameters. Conversely, Karatas, Aasim, Dogan, and Khawar (2013) reported that a concentration of BA at 0.25 mg/L BA provided maximum number of shoots/ explant and promoted early shoot formation after culturing with different internode and leaf explant on MS medium supplemented BA (0.25, 0.50, and 1.0 mg/L) and NAA



(0.25, 0.50, and 1.0 mg/L). The shoot regeneration of water hyssop was inhibited by all the combination of higher concentration of NAA and BA. The shoot from leaf explants were longer than shoot from internodes. The longer shoot regeneration obtained from leaf explant after culturing. Sharma et al. (2010) reported that the low concentration at 0.2 mg/L BA produced the maximum shoots/explant of water hyssop. According to Evans, Sharp, Ammirato, and Yamada (1983) revealed that cytokinin was very effective in promoting shoot proliferation and establishing shoot organogenesis. Similarly, Tiwari, Tiwari, and Singh (2001) suggested that the node, internode, and leaf explant were placed on various types of cytokinin for multiple shoot induction. Leaf explant sub-cultured on 0.5 mg/L of BA gave a great number of adventitious shoot buds. The results regarding on root induction showed that every type of explant induced 100% shoot induction in all treatment which cultured on MS medium supplemented with 0.5 mg/L BA. Roots emerged within 2 weeks on the basal medium from all types of explants, similar to the results on root induction by Joshi et al. (2010) who indicated that the root formation of explant began within 2 weeks. This study found that every explant of water hyssop was induced the root system within 2 weeks, thereafter, grow up until the end of the experiment.

The results of the second experiment showed that a 100% survival rate of all treated plants, except the treatment with the highest concentration at 40 mg/L PBZ. This caused plant death due to the plants' low resistance to the toxicity of the overdose concentration of PBZ. The results were in accordance with the findings of Wanderley, Faria, Ventura, and Vendrame (2014) who reported about the effects of PBZ in plant height control at 5 mg/L PBZ, but the high concentrations at 10 and 20 mg/L PBZ caused in death of new shoot due to the toxicity to the plant. The lower concentration of PBZ was a suitable practical protocol for height control of *A. graminifolia*.

The percentage of shoot induction reduced according to the increase in PBZ concentration. The PBZ application inhibited adventitious shoot regeneration of water hyssop. Indrayanti et al. (2019) reported that PBZ retarded plant growth, the percentage of plant growth rate, and led to short stems. The highest concentration of PBZ at 40 mg/L gave the lowest average number of shoots at 0.16 shoot/explant. Moreover, the number of shoots reduced, and the percentage of shoot induction decreased with increasing the concentrations of PBZ. These results were contrasted to the finding of Muangkaewngam and Te-chato (2016) who did not find any significant differences in the number of shoots/ explant and the percentage of shoot induction after application of PBZ, under *in vitro* culture of torch ginger.

The application of PBZ caused retarded stem height, reduced shoot induction, and reduced cell elongation of all treated plants. The result accordance to Kuai et al. (2017) who reported that PBZ is a plant growth regulator that changes the number of endogenous hormones and reorients the nutrients inside the plant. Indeed, this experiment discovered that PBZ at 2.5 mg/L, inhibited shoot length, so the maximum length was 3.37cm. The shoot length decreased in the same way as PBZ concentration increased. These results are consistent with the research of Muangkaewngam and Te-chato (2018) who reported that the application of PBZ changed the growth, morphology, and physiological features of the plant and retard torch ginger's height.

On the other hand, the concentrations of PBZ influenced the number of leaves for all treatments. The 2.5 mg/L PBZ had the maximum average number of leaves at 22.61, which was highly significant ($P < 0.01$), compared with the other treatments. The treatment with 40 mg/L PBZ had the smallest average number of leaves at 1.25. The plant treated with highest PBZ concentration gave a smaller, thicker, dark green leaf, compared to the control. According to Tsegaw, Hammes, and Robbertse (2005) reported that the application



of PBZ provided the potato *solanum tuberosum* L. in short stem, compact plant with the dark green, and thicker leave. In addition, Burrows, Boag, and Stewart (1992) also indicated that the application of PBZ increased chlorophyll content in leaves if it was compared with the control. The overdose of PBZ concentration made the plant get dark green leaves and the destroyed many of plants.

Root system is an important part of an *in vitro* culture. It is very important to increase the number of roots to maintain the balance between shoots and roots. The high concentration at 40 mg/L PBZ led to lowest root induction, at 38.88%. The results of root induction indicated that PBZ inhibited root formation by inhibiting gibberellin biosynthesis and increased endogenous cytokinin which like the finding of Soumya, Kumar, & Pal (2017). The highest PBZ concentration led to the fewest elongated roots, at 0.37 roots. The maximum average number of roots was 8.40 roots, after treatment with 2.5 mg/L of PBZ. Moreover, the length of root with the highest concentration (40 mg/L PBZ) was a shorter length than the root treated with the lowest concentration at 2.5 mg/L. In a similar study, El-Fadl (2017) stated that PBZ effectively stunted root length by prohibited adventitious root formation and constrained gibberellin biosynthesis. The number of endogenous cytokinin have been enhanced by the PBZ application that change cell division and give root fall off. The root achieved more deviation of photosynthate than aerial portions Salari, Saninasab, Akbari, and Rohani (2017). Consequently, the application of PBZ at 2.5 mg/L should be considered for dwarf plant root induction, which provided good quality of roots.

Conclusions and Suggestions

The node with leaf explant was selected for *in vitro* micropropagation protocol for shoot multiplication of water hyssop and for the plantlet preparation. Regarding the result of the present study, PBZ had a positively effect in changing the morphology and growth characteristics of water hyssop. PBZ at 2.5 mg/L was appropriate for dwarf plant production and plant height control this ornamental plant. This concentration could be suggested for the application of dwarf aquatic plant production on water hyssop. The different concentrations of PBZ can be applied with any aquatic plant to produce a dwarf ornamental aquatic plant and diversify for the aquarium and used for the further research protocol.

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