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Effect of paclobutrazol on three different aquatic macrophytes under *in vitro* monoculture or polyculture conditions

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Three aquatic plants, coontail (*Ceratophyllum demersum* L.), hydrilla [*Hydrilla verticillata* (L. f.) Royle] and giant duckweed [*Spirodela polyrhiza* (L.) Schleiden], were successfully surface sterilized and cultured on liquid basal MS (Murashige and Skoog, 1962) medium under aseptic conditions. Shoot explants obtained from these plants were transferred to basal MS medium supplemented with 0, 0.25 and 0.5 mg/l paclobutrazol (PBZ) under *in vitro* monoculture or polyculture conditions. There were some differences in the patterns of fresh weight increases of the three aquatic plants under monoculture and polyculture conditions. Among the three macrophytes studied, coontail was the most sensitive to 0.25 or 0.5 mg/l PBZ as its fresh weights did not increase at these PBZ concentrations during eight weeks under both monoculture and polyculture conditions. Giant duckweed were relatively more sensitive than hydrilla in response to addition of PBZ to the growth medium under both monoculture or polyculture conditions suggesting that PBZ might not be an effective aquatic pest control agent for hydrilla. The dominance of giant duckweed over hydrilla was effectively overturned with the addition of 0.5 mg/l PBZ to the polyculture medium.

Key words: Aquatic plants, coontail, giant duckweed, hydrilla, plant growth retardant.

INTRODUCTION

Coontail (*Ceratophyllum demersum* L.) and hydrilla [*Hydrilla verticillata* (L. f.) Royle] are submergent perennial fresh-water plants that are well known for being used as decoration and oxygen production in a fish aquarium. The giant duckweed [*Spirodela polyrhiza* (L.) Schleiden] is a free-floating macrophyte found in natural fresh waters. Under natural conditions, these plants provide many beneficial ecological services, but they could also be problematic weedy species; particularly, hydrilla is known to be highly invasive and difficult to control (Sousa, 2011). More research is needed to help better management of these aquatic weedy plants in the natural environments. Gibberellin synthesis inhibitors including paclobutrazol (PBZ) have been suggested as promising herbicides for limiting excessive stem growth of submerged aquatic weeds without reducing plant viability

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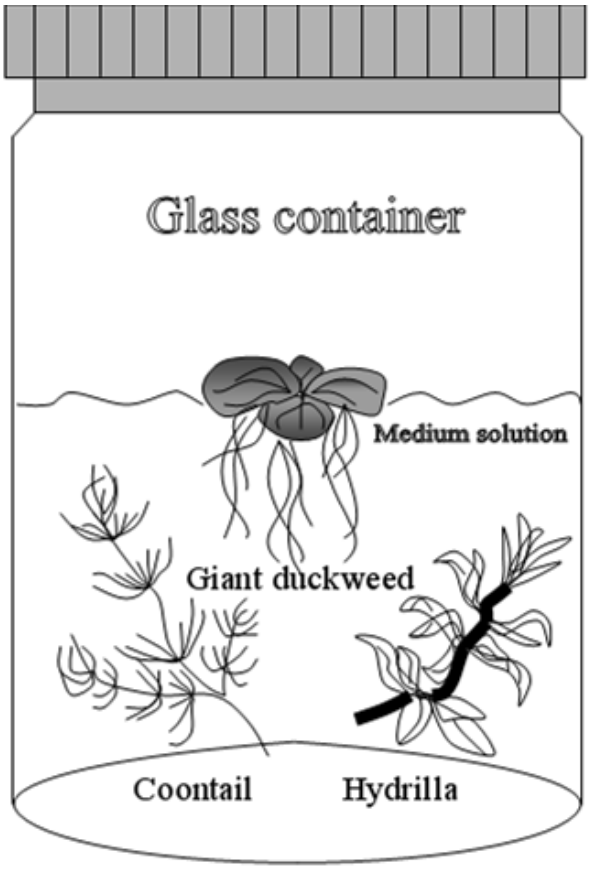

Figure 1. A schematic drawing showing the concept of *in vitro* polyculture of three aquatic macrophytes.

(Lembi and Chand, 1992; Van, 1988). Many factors including rainfall and degradative activities of micro-organisms could complicate the interpretation of the results obtained from trials of these herbicides under natural conditions. Plant tissue culture has been used in various investigations with a range of objectives including micropropagation (Thorpe, 2007) and application to aid assessment of some ecological questions (Hughes, 1981; Kauth and Kane, 2009).

The main objective of the present study was to compare growth (fresh weight changes) of the three different aquatic macrophytes when cultured singly (*in vitro* monoculture) in the absence or presence of PBZ as this had not been investigated before under highly controllable and aseptic environmental conditions. In addition, the relative sensitivity (differences in growth or fresh weight changes) of these three plants to different concentrations of PBZ was also investigated under *in vitro* polyculture conditions (Figure 1) as response of aquatic macrophytes with different genetic propensities placed within the same environment to PBZ was not known. This was the first study to grow different types of plants in the same culture vessel *in vitro*; although the principles and practice of polyculture of crops in cultivated fields are not new (Geno and Geno, 2001).

MATERIALS AND METHODS

Establishment of *in vitro* stock plant cultures

Coontail and hydrilla plants were purchased from the Chatuchak market in Bangkok, Thailand while giant duckweed plants were collected from natural waters in the Trat province, Thailand. In the laboratory, the plants were washed with clean running tap water for 15 min to remove unwanted matters. Then, all the leaves were removed from the long stem before it was cut into small pieces as explants (each about 3 cm length with 3 nodes) for establishing *in vitro* stock cultures. All these explants were rinsed briefly 3 times in distilled water. Surface sterilization began by immersing 50 pieces of explants from each species in 15% (v/v) Clorox (a commercial bleach solution containing 5.25%, w/w, sodium hypochlorite as available chlorine) to which 2 to 3 drops of Tween-20 were added and washed in distilled water and when required, immersed in Clorox again as shown in Table 1. After surface sterilization, nodal explants of coontail, hydrilla or whole giant duckweed plants were cultured separately in culture vessels containing basal MS medium (Murashige and Skoog, 1962) without addition of agar or any plant growth regulator for 4 weeks to establish stock plant cultures. All the culture media used in this study were adjusted to pH 5.7 before they were autoclaved at 121°C and 15 psi for 20 min.

Glass containers (4.5 cm diameter × 8.5 cm height) were used as culture vessels. All cultures were kept in a growth room at 25±2°C under 16 h of illumination with white fluorescent lamps (47.31 μmol m^{-2} s^{-1} light intensity) and 8 h of darkness.

Monoculture and polyculture experiments

The new shoots developed from coontail and hydrilla nodal explants of the stock cultures were excised into 3 cm long pieces. The three macrophytes (1 excised shoot of coontail, hydrilla or 3 giant duckweed plants) from their respective stock cultures were placed separately in a culture vessel containing 35 ml of basal MS medium supplemented with 0, 0.25 or 0.5 mg/l PBZ (monoculture experiment) or all placed together in a culture vessel (polyculture experiment) containing 100 ml of basal MS medium supplemented with the same range of PBZ concentrations. There were four replicate culture vessels for each concentration of PBZ.

Data analysis

Fresh weights of the plant materials during culture were determined at 0, 2, 4, 6 and 8 weeks of the monoculture and polyculture experiments. Mean percentages of fresh weight of the three macrophytes were analysed and 1-way ANOVA was first performed at the significance level of $P < 0.05$. After this, when appropriate, Duncan comparison of means was carried out at $P < 0.05$.

RESULTS AND DISCUSSION

Surface sterilization and preliminary observations

An objective of this study was to use plant tissue culture techniques to aid the study of the effects of PBZ on the three selected aquatic plants and not to micropropagate them. There was no prior report on *in vitro* culture of coontail and hydrilla and the previous tissue culture protocol of giant duckweed (Li et al., 2004) was not appropriate for the present purpose. Therefore, some preliminary tissue culture investigations were necessary.

Table 1. Time and sequence of steps (from top to bottom) for surface sterilization of shoot explants of the three aquatic macrophytes to establish *in vitro* stock cultures.

Steps Involved	Time for each step (min)		
	Coontail	Hydrilla	Giant duckweed
15% (v/v) clorox	2	2	1.30
Distilled water	3	3	-
15% (v/v) clorox	2	2	-
Distilled water	3	3	-
15% (v/v) clorox	2	2	-
Distilled water	3	3	3
Distilled water	3	3	3
Distilled water	3	3	3

In particular, surface-sterilization of explants from the three aquatic plants was a challenging problem. It was found that the plant parts of the three macrophytes changed into transparent or white-pale structures following surface sterilization based on a protocol previously used in our laboratory (Bodhipadma et al., 2010). Thus, to minimize this from occurring, the time of immersing the explants in a bleach solution was reduced and this step had to be repeated several times, particularly as far as coontail and hydrilla were concerned (Table 1). In addition, it was also important to remove all the leaves from the explants.

Once the stock plants were free of any contamination, surface-sterilization of experimental materials taken from the stock plants was unnecessary anymore. All three macrophytes when cultured separately were able to grow on liquid basal MS medium with 3% sucrose.

Monoculture experiment

Under monoculture conditions in the absence or presence of PBZ, there were little or no changes in the fresh weights of the three macrophytes in the first two weeks (Figure 2A). Giant duckweed exhibited a different pattern of increase in fresh weight from those of hydrilla and coontail. The main period of increase in the fresh weight of giant duckweed was between weeks 2 and 4 before the rate of increase started to slow down. In contrast, that of hydrilla and coontail was between weeks 6 and 8. In response to medium supplemented with 0.25 mg/l PBZ, the main period of increase in the fresh weight of giant duckweed was delayed to between weeks 4 and 6 while that of hydrilla was not changed compared to culture in the absence of PBZ (Figure 2B). In response to medium supplemented with 0.5 mg/l PBZ, both giant duckweed and hydrilla exhibited close to linear increases in their fresh weights between weeks 2 to 8 and weeks 4 to 8, respectively (Figure 2C). Among the three macrophytes, coontail was most sensitive to PBZ as the growth of coontail was inhibited in the medium supplemented with 0.25 or 0.5 mg/l PBZ (Figure 2B and C). Giant duckweed was more sensitive to 0.25 mg/l PBZ than hydrilla. The fresh weight of giant duckweed after 8 weeks of culture in the absence of PBZ was more than double than in the presence of 0.25 mg/l PBZ (compare Figure 2A and B). By contrast, the fresh weight of hydrilla at the end of experiment (8 weeks of culture) was the same in the absence or presence of 0.25 mg/l PBZ (Figure 2B).

In response to 0.5 mg/l PBZ, the fresh weights of both giant duckweed and hydrilla were lower than those at 0.25 mg/l PBZ (Figure 2C).

Polyculture experiment

Under polyculture conditions without any added plant growth regulator, the most notable change was that the fresh weight increase of giant duckweed was already evident at week 2 and the increase continued almost linearly throughout the experiment (Figure 2D). In contrast, the increase in the fresh weight of hydrilla or coontail was only evident from week 4 and then leveled off after week 6 (Figure 2D). This suggested that giant duckweed was more successful than hydrilla and coontail when all three species were under *in vitro* polyculture conditions. This might be related to the observation that giant duckweed plants exhibited an inconspicuous adjustment (lag) period following subculture onto fresh medium than the other two plants. This could give giant duckweed a competitive advantage under *in vitro* polyculture over the other two aquatic plants in the present study. The fresh weight increases of giant duckweed, when cultured under polyculture conditions and in the presence of 0.25 mg/l PBZ, were severely curtailed (Figure 2E) compared to polyculture in the absence of PBZ (Figure 2D). Most of the fresh weight increase of giant duckweed occurred between weeks 2 and 4 in the absence of any plant growth regulator (Figure 2D) but that in the presence of 0.25 mg/l PBZ occurred between 4 and 6 weeks instead (Figure 2E).

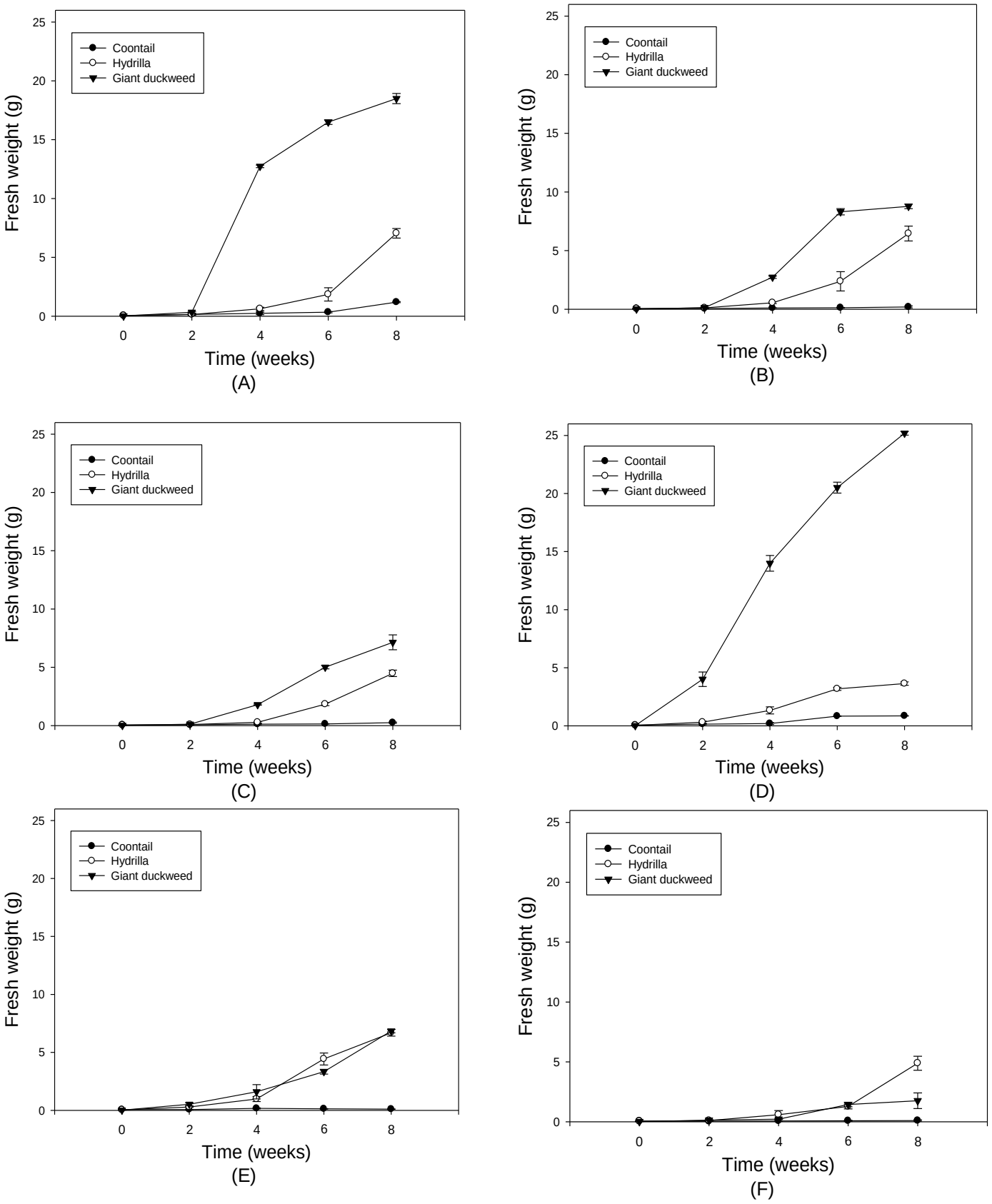

Figure 2. Fresh weight changes during *in vitro* monoculture (A to C) and during *in vitro* polyculture (D to F) of three aquatic plants on basal MS medium supplemented with 0, 0.25 or 0.5 mg/l of paclobutrazol, respectively.

Under polyculture conditions, the fresh weight increases exhibited by hydrilla were similar in the presence or absence of 0.25 mg/l PBZ in MS medium (Figure 2D and E). When the concentration of PBZ was increased to 0.5 mg/l and under polyculture conditions, there were no differences in fresh weights of giant duckweed and hydrilla in the first 6 weeks of culture (Figure 2F). However, by the end of the experiment (8 weeks), hydrilla had a higher fresh weight than giant duckweed (Figure 2F). This was also the only treatment among all the experiments in which the fresh weight of giant duckweed was less than that of hydrilla. Similar to the under monoculture conditions, the fresh weight of coontail did not increase throughout the experiment under polyculture conditions in the presence of 0.25 or 0.5 mg/l PBZ.

From the results obtained, it became clear that both giant duckweed and coontail were sensitive to 0.25 and 0.5 mg/l PBZ but hydrilla was relatively insensitive to these two concentrations of PBZ under polyculture conditions. The patterns in the changes of fresh weights of the three macrophytes under monoculture or polyculture conditions in the absence or presence of PBZ appeared to be different. However, the monoculture and polyculture conditions studied here were probably not ideal to permit direct comparison of the performance of the three macrophytes under monoculture and polyculture conditions as the volume of culture medium in the polyculture experiment was three times that of the monoculture experiment taking into consideration that all three plants were cultured together compared to when each plant was cultured individually, respectively. Nevertheless, it would seem that coontail was most sensitive while hydrilla was relatively insensitive to 0.25 or 5 mg/l PBZ under both monoculture and polyculture conditions. This is consistent with other studies showing that sensitivity to applied PBZ concentrations is dependent on the plant species (Million et al., 2002). Furthermore, the effect of PBZ on giant duckweed might be more severe under polyculture than monoculture conditions. The present results also broadly support the potential use of plant growth retardants such as PBZ and others to aid control and management of aquatic macrophytes (Chand and Lembi, 1994; Fox et al., 1994; van and Vandiver, 1994).

In conclusion, these findings from the present tissue culture studies under highly controllable environmental and aseptic conditions have implications for PBZ application in the control of different aquatic macrophytes being co-present under natural conditions.

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