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Full Length Research Paper

In vitro mass propagation of *Typhonium flagelliforme* as affected by plant growth regulators

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Tubers were used as explants in *in vitro* mass propagation of Rodent Tuber (*Typhonium flagelliforme).* The explants were obtained from sterile plantlets and placed in shoot induction medium containing basal salts of Murashige and Skoog (MS) and various concentrations of 6-benzylaminopurine (BAP) and α-naphthaleneacetic acid (NAA). Treatment containing 5 mg/l (w/v) of BAP with 1 mg/l (w/v) of NAA produced the highest number of shoots per explant (29.17) after 12 weeks of culture and also the highest mean fresh weight of shoots formed in treatment containing 5 mg/l (w/v) of BAP with 1 mg/l (w/v) of NAA. For *ex vitro* establishment, well- rooted plantlets were transferred in potting medium containing peatmoss, perlite and vermiculite (3:1:1).

Key words: *Typhonium*, 6-benzylaminopurine, α-naphthaleneacetic acid, indole-3-butyric acid, *in vitro* culture, mass propagation.

INTRODUCTION

Typhonium flagelliforme is a medicinal herb which belongs to the Araceae (Arum) family. It can be found from India to Australia and is spread northward to the sub-temperate areas of the Eastern Asia up to Sri Lanka (Nicolson and Sivadasan, 1981). In a report by World Health Organization, it was acclaimed that a high percentage of the world's population are using herbal medicine as drug and there is a growing interest in the use of traditional medicines (Tilburt and Kaptchuk, 2008). However, herbal medicines, like other natural resources, have very limited sources. Thus, artificial regeneration of herbal plants becomes important. Plant tissue culture system offers a tool for a large scale production of genetically similar plants (Wawrosch et al., 1999). Perry and Metzger (1980) reported that the people in the Philippines have been using the flowers of *T. flagelliforme* to arrest bleeding and as remedial for the treatment of injury. In addition another species of Typhonium, *T. divaricatum* is used in China for curing of internal injuries and oedema. Another report by Su et al. (2000) also showed that the rhizomes extract of *T. divaricatum* has been used for the treatment of therapeutic coughs and pulmonary conditions.

Chan et al. (2000) studied the different media for developing the tissue culture tecnique of *T. flagelliforme.* They discovered that among Murashige and Skoog (MS) (Murashige and Skoog, 1962), Nitsch and Nitsch (NN), Gamborg B5 (GB5) and White (W) medium, MS was the most suitable medium for this particular plant. The best medium for maximum shoot number, with normal complete plant, was reported on MS medium supplemented with 0.3 mg/l BA and 0.5 mg/l Indole-3-butyric acid (IBA). In 2003, Koh and Chan published a paper on *T. flagelliforme* micropropagation in immersion culture. In their study, they used 5 L fermentor containing liquid modified MS medium supplemented with 4% sucrose, 1.33 µmol/l BA and 2.46 µmol/l IBA.

The problems related to *T. flagelliforme* are sensitivity to specific growth condition in the natural environment such as moist and be in the shady area which provided the biggest need to produce the plant *in vitro* in massive amount.

This paper reports the effect of BAP either alone or in

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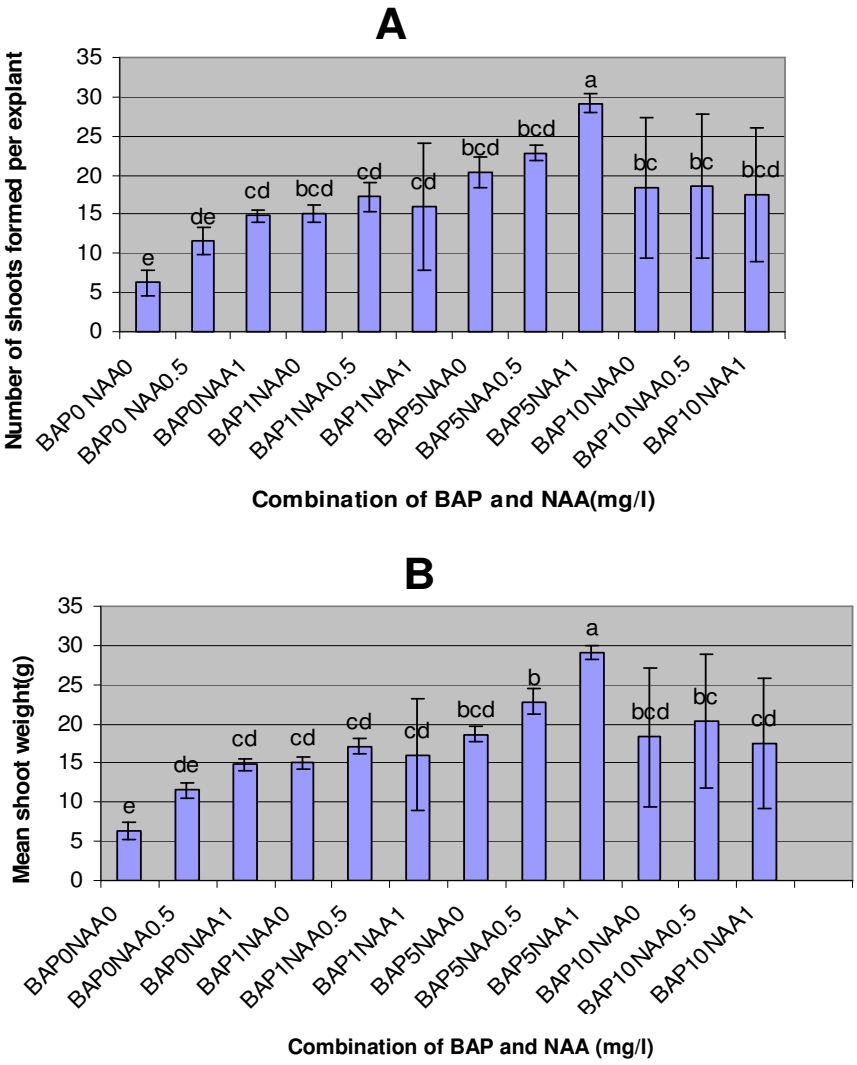

Figure 1. The effect of BAP, in combination with NAA, on mean number of shoots (A) and on mean weight of shoots (B) produced per explant after 12 weeks of culture. Means followed by the same letter(s) are not significantly different using DNMRT at α = 0.05.

combination with NAA, on adventitious shoot proliferation from tuber explants for mass production of *T. flagellaforme.*

MATERIALS AND METHODS

Plant material

The tuber explants were obtained from *in vitro* established *T. flagelliforme* plantlets and then cultured on proliferation medium containing complete MS salts supplement with 30 g/l sucrose.

Medium composition and treatment

The explants were cultured on MS medium containing different concentration and combination of BAP (0, 1, 5, 10 mg/l) and NAA (0, 0.5, 1 mg/l) for shoot induction. The pH of the medium was adjus-ted to 5.7 and MS medium without any plant growth regulator consider being the control of experiments.

Parameters recorded

The parameters taken in this study were the mean number of shoots per explant and mean fresh weight of shoots produced per explant. Data were collected every four weeks until the 12th week of culture whereas the growth condition was observed every week.

Experimental design and statistical analysis

The experiment was conducted in a Randomized Complete Block Design (RCBD). Data were analyzed using the analysis of variance (ANOVA) and Duncan New Multiple Range Test (DNMRT) at α= 5% was applied for comparison between the treatment means.

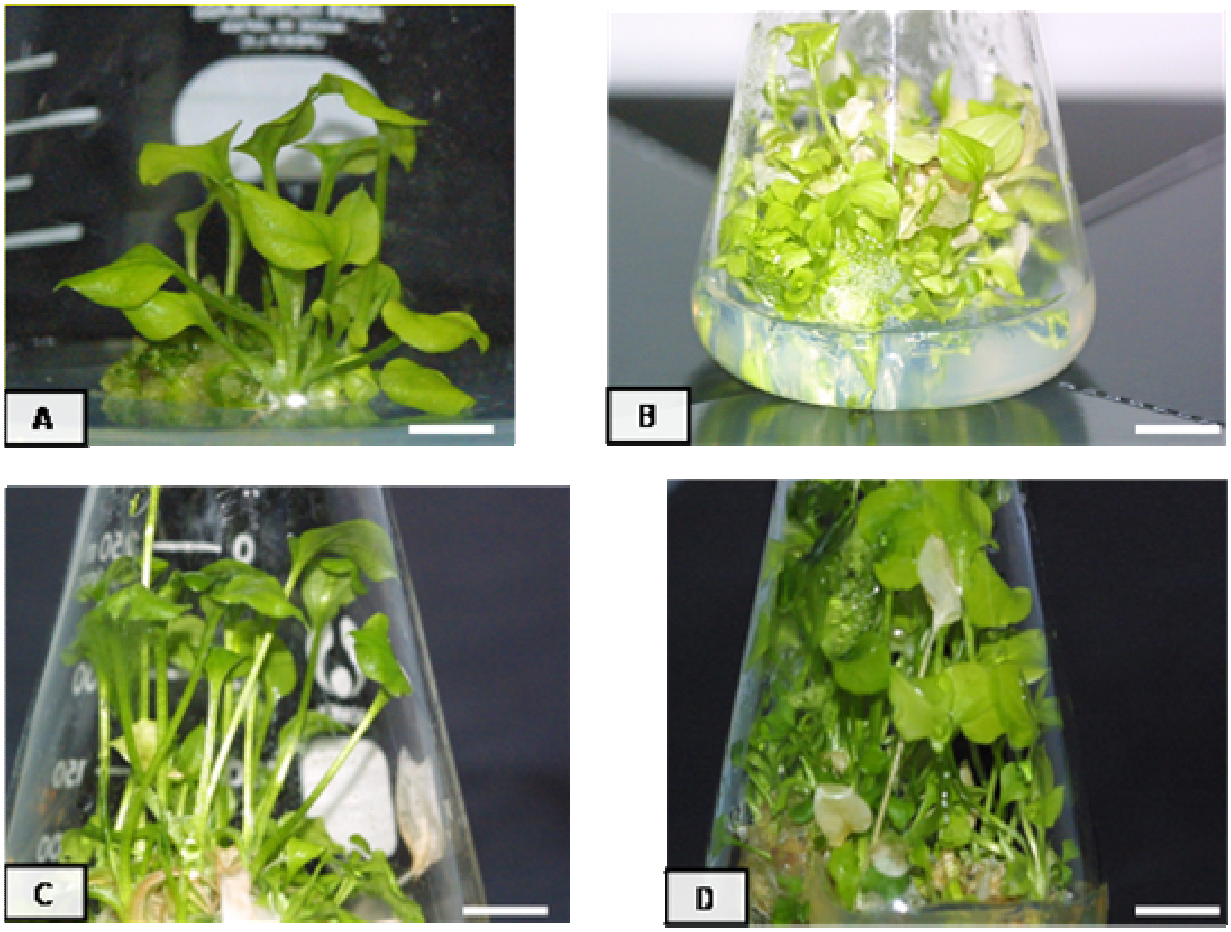

Figure 2. Shoot production, after 2(A), 4(B), 8(C) and 12(D) weeks of culture in BAP5NAA1 mgl^{-1}. Bar = 1 cm.

RESULTS AND DISCUSSION

Within two to three weeks of culture on the shoot induction medium, new shoots started to proliferate from the tuber (Figure 2A). For this plant the tuber has the main role in the propagation process.

The treatment combinations and concentrations of BAP and NAA tested were found to affect the mean number of shoots produced per explant. A high response was also detected from 9 weeks of culture until 12 weeks, while significant differences were observed among the treatments containing different combinations in terms of the number of shoots formed (Figure 1A) as well as fresh weight per explant (Figure 1B).

Treatments containing 5 mg/l (w/v) of BAP with 1 mg/l (w/v) of NAA (BAP5NAA1) produced the highest number of shoots per explant (29.17) after 12 weeks of culture (Figure 1A). The multiple shoots developed normally as shown in Figure 2.

According to Chan et al. (2000), MS medium with low concentration of BAP and NAA could cause abnormal and incomplete plantlet formation. They also found that the best medium which enabled the buds of *T. flagelliforme* to produce the most shoots was treatment with 0.5 mg/ l NAA and 0.5 mg/ l BAP, although the shoots produced were abnormal with twisted leaves and no roots.

Treatments containing BAP, either alone or in combination with NAA significantly increased the mean shoot weight formed per explant as compared to the control (BAP0NAA0), Treatments containing different combinations of BAP and NAA indicated no significant difference among them on mean shoot weight obtained per explant.

Treatment with 5 mg/l (w/v) BAP and 1 mg/l (w/v) NAA (B5N1) produced the highest mean fresh weight of explant (Figure 1B).Treatments containing 0 mg/l(w/v) NAA in combination with 0 to 10 mg/l (w/v) BAP caused no significant differences in mean fresh weight per explant.

The highest concentration of BAP at 10 mg/l (w/v) either alone or in combination with NAA 0 to 1 mg/l (w/v) (BAP10NAA0, BAP10NAA0.5, BAP10NAA1) did not differ significantly among them in terms of mean number of shoots per explant and mean shoot weight. However they were significantly different when compared to the control

(BAP0NAA0).

Rooted shoots produced simultaneously in the shoots induction medium, they were successfully acclimatized in potting medium containing peatmoss, perlite and vermin-culite (3:1:1) and grew in the natural environment. The ratio of survival plants was 90% and no observable differences appeared among the transferred.

Conclusion

The results indicated that tubers are potential explants for *in vitro* mass propagation of *Typhonium flagelliforme.* BAP alone or in combination with NAA was more effective on enhancing shoot proliferation and mean fresh weight of explants. Combination of 5 mg/l BAP and 1 mg/l NAA was selected as the most suitable concentration for the shoot initiation and multiplication as well as producing the highest mean fresh weight of shoots. Regenerated plants survived and grew normally in natural environment. The process, developed in this study, is suggested for rapid and efficient *in vitro* mass propagation of *T. flagelliforme.*

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