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The effects of BA and NAA on multiplication of Butterwort (*Pinguicula gigantea*) *in vitro*

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The effects of BA and NAA on multiplication of Butterwort (*Pinguicula gigantea*) *in vitro* was studied. Leaves were cultured on MS medium, supplemented with 0.2, 2.0, 10.0 and 20.0 mg/l BA and 0.1, 1.0, 5.0 and 10.0 mg/l NAA, 3% sucrose, 0.8% agar. The pH of the medium was adjusted to 5.7. It was found that the maximum number of shoots (21.75 shoots) were obtained from the medium with 2.0 mg/l BA and 0.1 mg/l NAA when cultured on media for 8 weeks. The heaviest weight of explants was 0.57 g. which achieved from the medium with 2.0 mg/l BA and 5.0 mg/l NAA. The MS medium with 0.2 mg/l BA and 5.0 mg/l NAA gave the average of longest shoots (0.83 cm).

Key words: Pinguicula micropropagation, BA, NAA

Introduction

The butterwort (*Pinguicula* sp.) is a group of carnivorous plants, which can be divided roughly into two main groups based on the climate in which they grow; tropical butterworts and temperate butterworts. *P. gigantean* is a homophyllous tropical butterwort species, which produces rosettes of carnivorous leaves of roughly uniform size throughout the year. The stem is short with numerous adventitious fibrous roots (Luhrs, 1995). They are used for horticultural purposes. The use of tissue culture technique has facilitated rapid mass propagation. The carnivorous species currently produces micropropagation *Pinguicula* plants, such as *P. lusitanica*. They were cultured on 1/2MS medium supplemented with 0.5 mg/l BA or 0.5 mg/l KIN to ensure a 29-fold rate of proliferation. Best rooting frequency and higher numbers and length of roots were obtained from in ¼ MS medium containing 0.2 mg/l IAA (Goncalves *et al.,* 2008). Jang *et al.*, (2003) reported that shoot tip proliferation

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of *Dionaea muscipula* was the best on MS medium but was poor on B5 medium (Gamborg et al. 1968) and the shoot number per explant was higher on MS than on B5 and LS (Linsmaier and Skoog, 1965) medium. Adams *et al.,* (1979) described a method for *Pinguicula moranensis* (Butterwort) propagation though leaf culture. Minocha (1985) reported an *in vitro* propagation method of *Dionaea muscipula* from mature leaf segments. There is no paper reporting *in vitro* propagation of *P. gigantean*. The objective of this work was to develop an efficient micropropagation method from leaves explants. The combinations of BA and NAA concentration were critically evaluated.

Materials and methods

Leaves of *P. gigantean* from a greenhouse were used as plant materials in this study. Leaves were washed in running tap water and commercial detergent. They were surface sterilized for 10 min by immersing in 3% Clorox solution containing a few drops of Tween-20, and then washed three times with sterile distilled water for 5 minutes each.

Culture media and culture condition. Leaves were cultured on MS medium supplemented with 0.2, 2.0, 10.0, and 20.0 mg/l BA (N^6-benzyladenine) and 0.1, 1.0, 5.0, and 10.0 mg/l NAA (α- naphthalene acetic acid). All media contained 30 g/l sucrose and 8 g/l agar. The pH of all culture media was adjusted to 5.7 before autoclaving. Each explant was cultured per bottle. Cultures were incubated at 25 ± 2 ^{o}C under a 16 hour photoperiod provided by cool white fluorescent lamps. Plantlets regenerated from leaves were transferred to MS medium without plant growth regulator to regenerate roots before transferring to pots.

Statistical analysis. Data were subjected to factorial in randomized complete block design and analyzed statistically using analysis of variance (ANOVA). The differences between the means were analyzed statistically using Dancan's new multiple range tests.

Results and discussion

Effect of BA

The effect of BA on multiple shoot differentiation was demonstrated in a number of cases using various explants (Lae *et al.,* 2005). The growth of butterwort leaf was summarized in Table 1. The concentration of BA (0.2, 2.0, 10.0 and 20.0 mg/l) gave shoot differentiation. The number of shoots was highest on MS medium supplemented with 2.0 mg/l BA, as this medium gave an average of 16.63 ± 1.88 shoots and the highest fresh weight of plants (0.41 g.)

was found. The highest length of shoot was found on medium supplemented with 0.2 mg/l BA. Jayaram K. and Prasad M. (2007) reported that the effect of BA at lower concentrations showed a significant effect on *Dorsera indica* with which BA at 0.1 mg/l individual developed multiple shoots within 60 days. Higher concentration of BA showed growth retardation.

Effect of NAA

The effect of NAA concentration (0.1, 1.0, 5.0 and 10.0 mg/l) is shown in Table 1. The lower concentration of NAA gave the highest number of shoots (12.47 shoots). However the higher NAA concentration gave the highest length of shoots (0.41 cm) and the heaviest weight of shoots (0.39g.). Mohd *et al.,* (2007) reported that a low concentration of auxin along with a high concentration of cytokinin was most promising for the induction and multiplication of shoots in *Tylophora indica*, and MS medium supplemented with 2.5 µM NAA proved the most effective for direct shoot regeneration.

Effect of BA and NAA

The effect of a combination of BA (0.2, 2.0, 10.0 and 20.0 mg/l) and NAA (0.1, 1.0, 5.0 and 10.0 mg/l) showed after 2 weeks of culture, and leaf explants showed swollen explant and small shoots were found at the base of the explant. Small calluses were found in explants cultured on MS medium supplemented with 2.0 mg/l BA and 5 mg/l NAA. After four weeks of culture, most of the leaf explants cultured on MS medium containing 20 mg/l BA and 10 mg/l NAA showed brown and white leaves (Fig.1a), while explants cultured on MS medium containing 0.2 mg/l BA and 0.1 mg/l NAA showed small shoots and then shoots were mature within 6 weeks (Fig. 1b). Leaf explants were cultured on MS medium supplemented with 0.2 mg/l BA and 10.0 mg/l which NAA showed small roots occurred after 6 weeks of incubation (Fig. 1c).

After eight weeks of incubation, plantlets were induced from leaf culture on MS medium supplemented with different concentrations of BA and NAA. The highest frequency of shoot regeneration (21.76 shoots per explant) occurred on MS medium with 2.0 mg/l BA and 0.1 mg/l NAA (Table 1, Fig. 1d). Shoots were regenerated directly from leaf explants. The heaviest weight of explants (0.57g.) were achieved from MS medium supplemented with 2.0 mg/l BA and 5.0 mg/l NAA (Table 1, Fig. 1e). The MS medium supplemented with 0.2 mg/l BA and 5.0 mg/l NAA gave the average of longest shoots (0.5 cm.) (Table 1, Fig. 1f). Hoque *et al.,* (1999) have also reported excellent *in vitro* shoot multiplication of ginger using BA with NAA. It has been recently reported that *Curcuma longa* cultured on MS medium supplemented with 2.0

mg/l BAP and 0.5 mg/l NAA showed highest rate of shoot multiplication. Shoots that were rooted on the 1/2MS medium supplemented with 2.0 mg/l NAA showed roots were better (Kambaska *et al.,* 2010). The direct regeneration of *Drosera* from leaf explants and shoot tips has been reported; in the *D. anglica* the highest proliferation rate was obtained on the medium supplemented with 0.05 μM BA and 0.005 μM NAA (Kawiak *et al.,* 2003.)

Plantlets with expanded leaves and developed roots were regenerated from leaves within 10 weeks (Fig 2a). The well rooted plants were transferred to pots containing peat moss for hardening (Fig 2b). After doing transferred to peat moss medium, plants started producing flower within 1 or 2 months.

Table 1. Effects of BA and NAA in MS media on average number of shoots, length of shoot and fresh weight after 8 weeks of culture.

Treatment (mg/l)		**No. of shoots ± SE[a/]**	**Length of shoot ±SE[a/] (cm)**	**Fresh weight ± SE[a/] (g)**
BA	NAA			
0.2		14.88+1.45a	0.65+0.09a	0.39+0.05
2.0		16.63+1.88a	0.43+0.05b	0.41+0.04
10.0		8.38+1.25b	0.19+0.02c	0.35+0.06
20.0		4.22+0.90c	0.10+0.02c	0.23+0.05
F-test		17.74**	24.61**	2.48ns
	0.1	12.47+1.81	0.32+0.06	0.29+0.03
	1.0	12.16+1.94	0.25+0.04	0.29+0.04
	5.0	11.06+2.02	0.39+0.08	0.41+0.07
	10.0	8.41+1.69	0.41+0.09	0.39+0.08
F-test		1.82ns	2.15ns	1.50ns
0.2	0.1	12.88±2.54	0.65±0.14	0.28±0.01
0.2	1.0	18.75±1.79	0.29±0.06	0.32±0.05
0.2	5.0	15.50±3.78	0.83±0.12	0.41±0.07
0.2	10.0	12.38±3.11	0.82±0.26	0.57±0.18
2.0	0.1	21.75±3.44	0.34±0.07	0.33±0.08
2.0	1.0	16.38±3.27	0.44±0.07	0.29±0.05
2.0	5.0	18.63±1.53	0.51±0.02	0.57±0.02
2.0	10.0	9.75±4.46	0.43±0.17	0.42±0.14
10.0	0.1	7.63±1.35	0.17±0.03	0.25±0.07
10.0	1.0	8.75±3.57	0.19±0.06	0.27±0.09
10.0	5.0	7.50±3.10	0.17±0.06	0.42±0.20
10.0	10.0	9.63±2.46	0.25±0.04	0.45±0.13
20.0	0.1	7.63±0.85	0.12±0.03	0.28±0.06
20.0	1.0	4.75±2.49	0.09±0.03	0.29±0.14
20.0	5.0	2.63±1.19	0.08±0.01	0.23±0.13
20.0	10.0	1.88±1.13	0.13±0.08	0.10±0.06
F-test		1.27ns	1.72ns	1.03ns

[a/] standard error
** result of two way ANOVA significant at 0.1
ns non significant at 0.5%

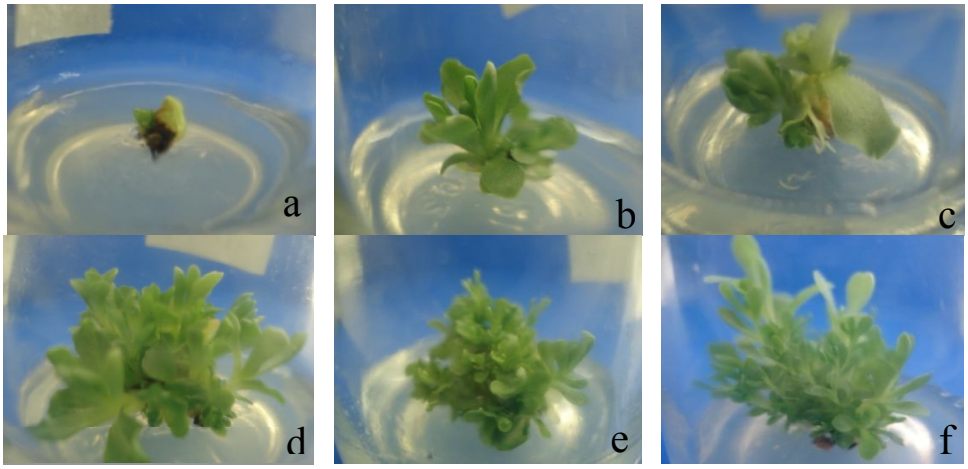

Fig. 1 Regeneration of multiple shoots from leaves on MS medium containing **(a)** 20.0 mg/l BA and 10.0 mg/l NAA after 4 weeks **(b)** 0.2 mg/l BA and 0.1 mg/l NAA after 6 weeks **(c)** 0.2 mg/l BA and 10.0 mg/l NAA after 6 weeks **(d)** 2.0 mg/l BA and 0.1 mg/l NAA after 8 weeks **(e)** 2.0 mg/l BA and 5.0 mg/l NAA after 8 weeks and **(f)** 0.2 mg/l BA and 5.0 mg/l NAA after 8 weeks

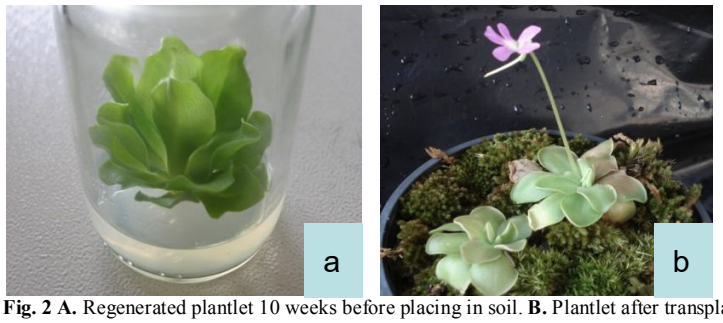

Fig. 2 A. Regenerated plantlet 10 weeks before placing in soil. **B.** Plantlet after transplanting to peat moss.

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