In vitro regeneration of *Drosera burmannii* Vahl.: a carnivorous plant of north-east India

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Abstract An efficient in vitro regeneration protocol has been developed from shoot tips of *Drosera burmannii* Vahl., a carnivorous plant of north-east India. Various plant growth regulators were used to study their efficacy in the induction of multiple shoots and roots. Of the various treatments, the maximum number of shoots (28.8 ± 1.5) and roots (9.7 ± 0.6) was observed in one-fourth strength standard medium (MS with 50 mg/l citric acid and 10 mg/l ascorbic acid) supplemented with 4 mg/l 6-benzylaminopurine (BAP) and 4 mg/l α -naphthalene acetic acid (NAA) followed by 26.8 ± 1.4 shoots in one-fourth strength SM fortified with 4 mg/l kinetin (KN) and 4 mg/l NAA. The well-developed plantlets with shoots and roots were potted in small plastic glasses filled with a mixture of sand and farmyard manure (3:1); these plantlets when transferred to a glasshouse for hardening and acclimatization showed 90% survival.

Keywords Carnivorous plant · *Drosera burmannii* · In vitro regeneration

Introduction

Charles Darwin described carnivorous plants as ‘the most wonderful plants in the world’. Carnivorous plants show a novel way of adaptation to survive in nutrient-deficient environments. Although they follow different ways of adaptations with varying complexities, the carnivorous plants serve the same purpose. The genus *Drosera* belonging to the family Droseraceae is commonly known as sundew. The sundew is considered as the master of sticky flypaper due to the presence of long tentacles on their leaves which act as a carnivory to catch prey. The word *Drosera* is derived from the Greek word *Drosero* which means ‘glistening in the sun or dewy’. There are more than 200 known species which fall under this genus (McPherson 2010).

Drosera burmannii Vahl., usually found in acidic and sandy soils, is a tropical sundew or Burmese sundew annual carnivorous plant. It is an erect herb with short stem and rosette leaves. Each leaf is 8–10 mm long and 5–6 mm wide. The flowers are white in color, arising on a leafless scape measuring about 5–20 cm long. The plant grows to a height of about 5 cm. Because of their carnivorous nature and the beauty of their glistening traps, sundews have become favorite ornamental plants. *D. burmannii* is geographically distributed in Southeast Asia, China, India, Japan, West Africa and Australia (Nordbakken et al. 2004). The major compounds found in *D. burmannii* are naphthoquinones, plumbagin, ramanteeon, glucoside rossolide, flavonoids like quercetin and hyperoside (Wagner et al. 1984; Wang et al. 1998). The plant also exhibits anticonvulsant activity (Hema et al. 2009) and rubefacient property. The locals of Khasi and Jaintia Hills of Meghalaya, India, have reported the usage of these leaves mixed with salt to remove or heal blisters (Joseph and Joseph

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1986). This plant also forms a vital component in an Ayurvedic preparation called ‘Swarnabhasma’ (Golden ash) (Ravikumar et al. 2000). In many European countries and China, *D. burmannii* are imported to pharmaceutical industries, where the dried plants are marketed as ‘Herba Droserae’ (Didry et al. 1998). Apart from obtaining nutrients from animals, carnivorous plants sometimes show a complex interaction with them (Jennings et al. 2010; Moon et al. 2010). Carnivorous plants consume pests such as mosquitoes, midges, deer flies, horseflies and dipterans which transmit human diseases (Ellison and Gotelli 2009). Consequently, the conservation of carnivorous plants could benefit many other taxa including humans. It also helps in preventing secondary extinction of other specialist species which rely on them. As the collection of the plants increases, it ultimately leads to decline in wild populations. *D. burmannii* has been tagged as vulnerable in India (Reddy et al. 2001), and in the state of Andhra Pradesh the plant is one of the Red Listed medicinal insectivorous plants (Jayaram and Prasad 2006). Multiplication and conservation through tissue culture technique forms one of the major methods of protecting the species. Such conservation efforts would ensure continuous and ample supply of this valuable material which is in great demand by the pharmaceutical industries. In the present study, we aim to establish an efficient micropropagation protocol for *D. burmannii*, using different cultural strategies so as to help in the conservation and propagation of this plant species.

Materials and methods

Drosera burmannii Vahl. were collected from Jarain, Meghalaya, and maintained in a glasshouse at the Plant Biotechnology Laboratory, Department of Botany, North Eastern Hill University, Shillong. Shoot tips were harvested from the plants growing in the glasshouse and used as explants. Explants were washed thoroughly in running tap water and then treated with 10% sodium hypochlorite (4% active chlorine content) for 10 min and finally rinsed six times with distilled water. The shoot tips measuring about 1 cm² were inoculated on one-fourth strength standard medium (MS with 50 mg/l citric acid and 10 mg/l ascorbic acid), containing 3% (w/v) sucrose and 0.8% (w/v) agar (Himedia), and the pH was adjusted to 5.8. All media were autoclaved at 121 °C and 1.05 kg cm⁻² pressure for 15 min. Cultures were incubated in the growth room at a temperature of 25 ± 2 °C, 14/10-h photoperiod with an irradiance of 60 μmol m⁻² s⁻¹ provided by cool white fluorescent tubes.

Induction of shoots and roots

For induction of shoots and roots, shoot tip explants were excised from the in vitro raised microshoots and subcultured in different strengths of SM supplemented with varying concentrations (1–8 mg/l) of 6-benzylaminopurine (BAP), (Z) and kinetin (KN) in combination with α -naphthalene acetic acid (NAA) (Fig. 1; Tables 1, 2, 3). Data for organogenetic response and shoot multiplication were recorded after 30 days of culture inoculation. The number of roots formed was recorded after 30 days of microshoot transfer. All experiments were repeated thrice with six replicates each and data were analyzed using one-way analysis of variance (ANOVA) in JMP[®] version 7.0.1 (SAS Institute, Cary, NC). The significant differences among the means were assessed by Tukey’s test at 5% probability level.

Fig. 1 Multiple shoot formation and root induction from shoot tip explants of *D. burmannii*. **a** Initial stage of inoculation of shoot tip explants (Bar 1 cm). **b** Multiple shoot induction in one-fourth strength SM + BAP + NAA (4 mg/l) after 30 days (Bar 1 cm). **c** Multiple shoot induction in one-fourth strength SM + BAP + NAA (4 mg/l) (Bar 1 cm). **d** Root induction in one-fourth strength MS + BAP + NAA (4 mg/l) after 30 days (Bar 1 cm). **e** Well-developed roots (Bar 1 cm). **f** Regenerated hardened plantlets

Table 1 Adventitious responses of *Drosera burmannii* Vahl. from shoot tip explants cultured in various strengths of SM supplemented with different concentrations of BAP and NAA

PGR (mg/l)		After 30 days of culture					
		Full-strength SM		½ Strength SM		¼ Strength SM	
BAP	NAA	No. of shoots	No. of roots	No. of shoots	No. of roots	No. of shoots	No. of roots
Control		15.3 ± 1.1	1.5 ± 0.3	15.5 ± 1.2	2.1 ± 0.2	17.8 ± 1.3	5.3 ± 0.3
1	1	16.1 ± 1.2	2.1 ± 0.2	15.6 ± 0.8	3.5 ± 0.1	20.9 ± 1.2*	5.2 ± 0.4
1	2	16.3 ± 0.4	2.3 ± 0.4	17.2 ± 1.1*	6.3 ± 0.4*	21.5 ± 1.6*	5.5 ± 0.2
1	4	18.6 ± 0.8*	3.1 ± 0.5*	18.5 ± 1.5*	4.5 ± 0.3*	22.6 ± 1.3*	5.4 ± 0.5
1	8	16.3 ± 0.9	2.2 ± 0.2	14.7 ± 1.2	3.6 ± 0.5*	23.3 ± 1.6*	6.6 ± 0.4
2	1	16.6 ± 0.6	1.8 ± 0.7	16.3 ± 2.3	2.1 ± 0.7	19.3 ± 1.4	6.7 ± 0.4
2	2	15.6 ± 0.7	1.6 ± 0.6	17.6 ± 2.4*	2.2 ± 0.5	20.2 ± 1.6*	6.4 ± 0.5
2	4	15.3 ± 0.3	1.4 ± 0.4	16.3 ± 1.1	2.4 ± 0.6	22.6 ± 1.2*	6.2 ± 0.6
2	8	14.6 ± 0.7	1.2 ± 0.8	13.2 ± 1.3	2.5 ± 0.2	23.4 ± 1.5*	7.1 ± 0.6*
4	1	16.1 ± 0.2	1.1 ± 0.2	22.4 ± 1.4*	2.7 ± 0.4	25.7 ± 1.6*	7.3 ± 0.3*
4	2	17.6 ± 0.8*	1.1 ± 0.3	26.2 ± 1.8*	1.5 ± 0.7	26.3 ± 1.1*	8.2 ± 0.5*
4	4	16.3 ± 0.7	1 ± 0.2	25.2 ± 1.4*	1.4 ± 0.2	28.8 ± 1.5*	9.7 ± 0.6*
4	8	15.3 ± 0.8	1 ± 0.2	23.7 ± 1.6*	1.2 ± 0.3	25.9 ± 0.9*	7.5 ± 0.2*
8	1	16.3 ± 0.9	–	20.7 ± 2.1*	1.3 ± 0.4	23.4 ± 1.2*	5.3 ± 0.5
8	2	15.4 ± 0.5	–	18.4 ± 1.7*	1.5 ± 0.2	22.2 ± 0.9*	4.2 ± 0.6
8	4	14.5 ± 0.7	–	17.1 ± 2.1*	1.3 ± 0.4	20.1 ± 1.4*	4.1 ± 0.2
8	8	14.1 ± 0.8	–	15.4 ± 1.5	1.2 ± 0.5	18.7 ± 1.2	4.2 ± 0.1

Mean ± SE

* Significant difference among treatments and control ($P < 0.05$)**Table 2** Adventitious responses of *Drosera burmannii* Vahl. from shoot tip explants cultured in various strengths of SM supplemented with different concentrations of KN and NAA

PGR (mg/l)		After 30 days of culture					
		Full-strength SM		½ Strength SM		¼ Strength SM	
KN	NAA	No. of shoots	No. of roots	No. of shoots	No. of roots	No. of shoots	No. of roots
Control		15.3 ± 1.1	1.5 ± 0.3	15.5 ± 1.2	2.1 ± 0.2	17.8 ± 1.3	5.3 ± 0.3
1	1	15.1 ± 1.9	–	13.6 ± 0.9	2.3 ± 0.5	12.6 ± 1.8	5.1 ± 0.9
1	2	16.9 ± 0.7	–	17.9 ± 1.3*	2.5 ± 0.6	18.3 ± 1.8	6.3 ± 0.6
1	4	17.6 ± 0.7*	–	16.4 ± 1.9	2.6 ± 0.4	19.3 ± 1.9*	7.2 ± 0.2*
1	8	16.7 ± 0.5	–	13.3 ± 1.8*	2.8 ± 0.7	17.3 ± 1.7	5.8 ± 0.4
2	1	18.6 ± 0.4*	1.4 ± 0.3	14.8 ± 1.2	3.2 ± 0.9	16.4 ± 1.8	5.6 ± 0.6
2	2	20.4 ± 0.9*	2.2 ± 0.5	16.3 ± 1.4	3.3 ± 0.4	17.3 ± 1.8	5.4 ± 0.4
2	4	18.6 ± 0.8*	1.4 ± 0.5	14.8 ± 1.3	3.6 ± 0.2*	19.3 ± 1.2*	5.3 ± 0.9
2	8	19.4 ± 0.6*	1.3 ± 0.2	12.9 ± 1.1*	3.4 ± 0.4	21.6 ± 1.8*	5.1 ± 0.5
4	1	17.1 ± 0.7	3.2 ± 0.6*	20.3 ± 1.3*	3.7 ± 0.6*	22.1 ± 1.8*	4.8 ± 0.8
4	2	14.7 ± 0.5	1.5 ± 0.3	24.7 ± 1.7*	3.9 ± 0.3*	24.4 ± 1.1*	4.5 ± 0.5
4	4	13.5 ± 0.8	1.3 ± 0.2	23.3 ± 1.8*	3.6 ± 0.8*	26.3 ± 1.4*	4.3 ± 0.3
4	8	12.2 ± 0.7*	1.3 ± 0.1	22.5 ± 1.7*	3.4 ± 0.6	24.2 ± 1.7*	4.1 ± 0.4
8	1	14.7 ± 0.8	–	20.7 ± 1.6*	3.3 ± 0.4	23.6 ± 1.8*	3.6 ± 0.9*
8	2	16.4 ± 0.4	–	17.2 ± 1.8	2.6 ± 0.3	22.3 ± 1.9*	3.4 ± 0.5*
8	4	15.7 ± 0.6	–	14.8 ± 1.7	2.4 ± 0.2	20.1 ± 1.6*	3.2 ± 0.3*
8	8	13.1 ± 0.6*	–	12.4 ± 1.7*	2.2 ± 0.1	18.7 ± 1.9	2.3 ± 0.2*

Mean ± SE

* Significant difference among treatments and control ($P < 0.05$)

Table 3 Adventitious responses of *Drosera burmannii* Vahl. from shoot tip explants cultured in various strengths of SM supplemented with different concentrations of Z and NAA

PGR (mg/l)		No. of shoots after 30 days					
		Full-strength SM		½ Strength SM		¼ Strength SM	
Z	NAA	No. of shoots	No. of roots	No. of shoots	No. of roots	No. of shoots	No. of roots
Control		15.3 ± 1.1	1.5 ± 0.3	15.5 ± 1.2	2.1 ± 0.2	17.8 ± 1.3	5.3 ± 0.3
1	1	13.1 ± 1.2*	–	10.6 ± 1.5*	1.6 ± 0.6	18.6 ± 1.3	3.2 ± 0.6*
1	2	14.9 ± 1.7	–	11.3 ± 1.4*	1.8 ± 0.4	20.3 ± 1.3*	4.5 ± 0.7
1	4	16.8 ± 1.1	–	13.2 ± 1.1*	2.1 ± 0.5	23.5 ± 1.7*	5.1 ± 0.4
1	8	18.8 ± 1.8*	–	11.4 ± 1.3*	2.3 ± 0.3	25.3 ± 1.2*	5.3 ± 0.6
2	1	22.6 ± 1.7*	2.7 ± 0.2	15.8 ± 1.4	2.6 ± 0.8	19.4 ± 1.8	6.4 ± 0.3
2	2	24.5 ± 1.5*	3.4 ± 0.3*	17.3 ± 1.3	3.2 ± 0.5	22.3 ± 1.3*	7.2 ± 0.6*
2	4	15.1 ± 0.3	1.6 ± 0.4	20.8 ± 1.7*	4.1 ± 0.6*	21.3 ± 1.4*	6.3 ± 0.2
2	8	13.6 ± 1.5	1.8 ± 0.6	18.9 ± 1.8*	3.1 ± 0.3	22.6 ± 1.7*	5.3 ± 0.8
4	1	12.4 ± 1.8*	1.5 ± 0.8	15.3 ± 1.7	2.7 ± 0.2	19.1 ± 1.7	4.2 ± 0.7
4	2	11.8 ± 1.6*	1.3 ± 0.3	18.9 ± 1.8*	2.6 ± 0.8	22.4 ± 1.8*	3.7 ± 0.5*
4	4	13.8 ± 1.6	1.2 ± 0.3	15.3 ± 1.3	2.5 ± 0.9	17.3 ± 1.9	3.7 ± 0.4*
4	8	11.8 ± 1.8*	1.1 ± 0.1	15.5 ± 1.8	2.1 ± 0.5	15.9 ± 1.2	3.2 ± 0.3*
8	1	10.8 ± 0.9*	–	13.8 ± 1.3	1.9 ± 0.3	18.6 ± 1.5	2.8 ± 0.2*
8	2	12.6 ± 1.7*	–	16.2 ± 1.1	1.6 ± 0.1	22.9 ± 1.3*	2.6 ± 0.1*
8	4	11.8 ± 1.6*	–	13.8 ± 1.6	1.3 ± 0.3	21.1 ± 1.8*	2.5 ± 0.8*
8	8	10.1 ± 1.9*	–	11.4 ± 1.8*	1.1 ± 0.4	18.9 ± 1.4	2.2 ± 0.5*

Mean ± SE

* Significant difference among treatments and control ($P < 0.05$)

Acclimatization and transplantation

The in vitro rooted plantlets were washed thoroughly and potted in small plastic glasses filled with a mixture of sand and farmyard manure (3:1) and irrigated with water daily in a glasshouse. The plantlets were maintained under glasshouse condition at a temperature of 25 ± 2 °C, 80% relative humidity, photon flux of $100 \text{ mol m}^{-2} \text{ s}^{-1}$ and a day length of 12 h. The average survival percentage was calculated after 4 weeks of acclimatization.

Results and discussions

Plant growth regulators (PGR) have intense effect on seedling growth of many plants (Su et al. 2011). PGR cytokinins enhance shoot regeneration and auxin induces root development in shoots to make it a complete plant. In the present study, in an attempt to induce multiple shoot regeneration of *D. burmannii* from shoot tips, these were cultured on different strengths of SM supplemented with different PGR concentrations (cytokinin and auxin). Shoot induction was observed on all the tested concentrations and combinations of PGR with varied response. The highest number of shoots (28.8 ± 1.5) was observed in one-fourth

strength SM supplemented with 4 mg/l BAP and 4 mg/l NAA (Fig. 1; Table 1), which was followed by 26.8 ± 1.4 shoots in one-fourth strength SM fortified with 4 mg/l KN and 4 mg/l NAA (Table 2). The higher rate of multiple shoot induction in cultures supplemented with BAP and NAA may be largely ascribed to increased rates of cell division induced by cytokinin (BAP) in the terminal and axillary meristematic zone of explant tissues (Niranjan et al. 2010). The beneficiary effect of BAP and KN for the multiple shoot initiation has also been reported earlier (Jayaram and Prasad 2007). Considerably high number of shoots (25.3 ± 1.2) was also recorded in one-fourth strength SM supplemented with 1 mg/l Z and 8 mg/l NAA (Table 3). Most of the shoot tip explants cultured on different concentrations of MS supplemented with growth regulators grew in size and new shoots began to proliferate within 10–15 days of incubation.

Root formation was also observed on different strengths of SM with a combination of cytokinins and auxin. Although root induction was not observed on all the used concentrations and combinations of PGR, the maximum number of roots (9.7 ± 0.6) was observed on one-fourth strength SM supplemented with 4 mg/l BAP and 4 mg/l NAA. One-fourth strength SM supplemented with BAP + NAA was found to be superior to KN + NAA and

Z + NAA with respect to the induction of roots. The superiority of BAP + NAA over other PGR in in vitro propagation has also been reported in other plant species (Seyyedyousefi et al. 2013). Our data revealed that there are differences in the effect of the different concentrations of BAP + NAA, KN + NAA and Z + NAA on root induction.

One-way ANOVA of the experiment showed that there was a significant difference between the various concentrations of cytokinins and auxins and SM strength used in the study. Of the various treatments, one-fourth strength SM with BAP and NAA combination produced a maximum number of shoots and maximum root induction.

On the basis of our results, the effect of different concentrations of BAP, KN and Z in combination with NAA was evaluated; it was found that combination of BAP with NAA was found more suitable for shoot multiplication as well as root induction than KN with NAA and Z with NAA. Variation in the activity of cytokinins and auxins can be explained by differences in the uptake rate, respectively (Nikam and Savant 2007). The micropropagation protocol reported here was characterized with a rapid proliferation of shoots and rooting of *D. burmannii*. This method will be highly advantageous for large-scale production, as a uniform source of plants for commercial purposes, for pharmaceutical uses, for its conservation and a range of further biotechnological applications.

Drosera burmannii propagate mostly through seeds; however, very limited reports are available on seed germination and micropropagation. The present work demonstrated that it was possible to successfully obtain multiple shoots of *D. burmannii* from shoot tip cultures with an efficient in vitro rooting protocol. The establishment of protocols for in vitro propagation of *D. burmannii* through shoot multiplication provides a potential system for improvement, conservation and mass propagation of this amazing and medicinally important carnivorous plant.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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