J. Plant Physiol. Vol. 139. pp. 246–248 (1991)

Short Communication

In Vitro Regeneration of Pitcher Plant (*Nepenthes khasiana* Hook. f.) – A rare Insectivorous Plant of India

T. S. Rathore[1], P. Tandon[2], and N. S. Shekhawat[1]

[1] Plant Biotechnology Laboratory, Department of Botany, Jodhpur University, Jodhpur 342 001, India

[2] Department of Botany, N.E. Hill University, Shillong 793 014, India

Received June 6, 1991 · Accepted July 30, 1991

Summary

Nepenthes khasiana, a rare insectivorous plant, was regenerated from nodal stem segments. Multiple shoots (10–12) were differentiated on Murashige and Skoog medium containing 0.1 mg · L^{-1} IAA and 2.0 mg · L^{-1} BAP within 5–6 weeks. Addition of 50.0 mg · L^{-1} ascorbic acid, 25.0 mg · L^{-1} citric acid, 50.0 mg · L^{-1} arginine and 25.0 mg · L^{-1} adenine sulphate to the culture medium increased the number of shoots. Browning of cultures could be checked by incorporation of 500.0 mg · L^{-1} activated charcoal and 500.0 mg · L^{-1} polyvinylpyrrolidone. About 80% of the *in vitro* produced shoots were rooted on half strength MS medium containing 2.0 mg · L^{-1} NAA and 0.1 mg · L^{-1} Kinetin.

Key words: In vitro, Nepenthes khasiana, Pitcher, plantlets, multiple shoots.

Abbreviations: BAP = 6-benzylaminopurine; 2,4-D = 2,4-dichlorophenoxyacetic acid; GA_3 = gibberellic acid; IAA = indole-3-acetic acid; IBA = indole-3-butyric acid; MS = Murashige and Skoog; NAA = naphthalene acetic acid.

Introduction

Nepenthes khasiana Hook. f., a member of the Nepenthaceae, is an endemic insectivorus plant of Meghalaya (North-East India). This species is of great biological and ecological importance (Jain and Sastry, 1980). Pitcher plant grows in acidic and nitrogen deficient soil experiencing high rainfall and warm climate. A pitcher develops at the top of the leaf and traps a wide group of insects to compensate nitrogen and energy deficiency (Kitching and Schofield, 1986).

The population of *N. khasiana* has dwindled during the last decades due to excessive collection, deforestation, forest fire and jum cultivation (Jain and Sastry, 1980). Natural propagation of this species is mainly through seeds, which show poor germination. *In vitro* techniques have been used in recent years as a non-conventional method of propagation and conservation of germplasm of several plant species (Cervelli, 1987; Upadhyay et al., 1989; Arumugam and Bhojwani, 1990; Tandon and Rathore, 1991). Earlier, we reported *in vitro* propagation of *N. khasiana* from mature field grown plants (Rathore and Tandon, 1989). The present investigation deals with the factors affecting *in vitro* regeneration of *N. khasiana* from stem segments of *in vitro* raised seedlings and the development of pitchers on these plants.

Materials and Methods

Seeds of *Nepenthes khasiana* were collected from Jaraine in Jaintia Hills, Meghalaya (India) and stored at 10 °C. Seeds were washed several times with tap water containing a few drops of tween-80, surface sterilized with 0.1% (W/V) mercuric chloride for 2–3 min and washed with sterilized water. Surface sterilized seeds were soaked in water for 3–4 h, kept in 70% ethanol for 20 seconds and again washed with autoclaved water. Seeds were inoculated on Murashige and Skoog (1962) half strength medium with 0.1 mg · L^{-1} IAA, 0.5 mg · L^{-1} kinetin and 2.0 mg · L^{-1} GA_3, and kept under diffused light at 26 ± 2 °C for germination. *In vitro* raised seedlings, 6–8 weeks old, were used as a source of explants (leaf, nodal shoot, apical shoot and root segment).

Explants of 0.5–2.0 cm size were cultured on full and half strength MS and B_5 (Gamborg et al., 1968) media. The pH of the medium was adjusted to 5.8 before autoclaving. Auxins (IAA, IBA,

NAA and 2,4-D) and cytokinins (Kinetin and BAP) were incorporated in the medium from 0.05–5.0 mg·L^{-1} alone or in combination. Activated charcoal (500.0 mg·L^{-1}), 500.0 mg·L^{-1} polyvinylpyrrolidone (PVP; MW 40,000), 50 mg·L^{-1} ascorbic acid and 25 mg·L^{-1} citric acid were added to the medium to check browning of explants and the medium. Adenine sulphate (10–100 mg·L^{-1}) arginine (10–100 mg·L^{-1}), casein hydrolysate (50–500 mg·L^{-1}), yeast extract (100–1000 mg·L^{-1}), coconut water (5–20 %) and beef extract (100–2500 mg·L^{-1}) were used in the medium to increase the shoot number and subsequent shoot growth. Agar (0.6–0.8 %) was added to the medium as gelling agent. Cultures were kept in dark for 3–4 days and later under 2500 lux light intensity for 12 h per day at 26 ± 2 °C. Each treatment consisted of 10–15 replicates and was repeated 2–3 times.

Differentiated shoot segments were sub-cultured on fresh medium after 4 weeks either for further multiplication or for rooting of individual shoots. Various dilutions of MS medium, viz. full, half and one/forth, supplemented with IBA and NAA (0.1–5.0 mg·L^{-1}) were evaluated for root induction from excised shoots. Cultures for root induction were kept in the dark for 1 week and later under light conditions. For strengthening of plantlets and development of pitchers the plants were cultured on agar gelled (0.6 %) hormone-free ¼ MS salts containing 2 % sucrose. Plantlets of 5–7 cm were washed thoroughly under tap water and finally with distilled water; they were then transferred to pots containing vermiculite and drained soil (3 : 1). Potted plants were hardened in a growth chamber and later transferred under semi-controlled conditions.

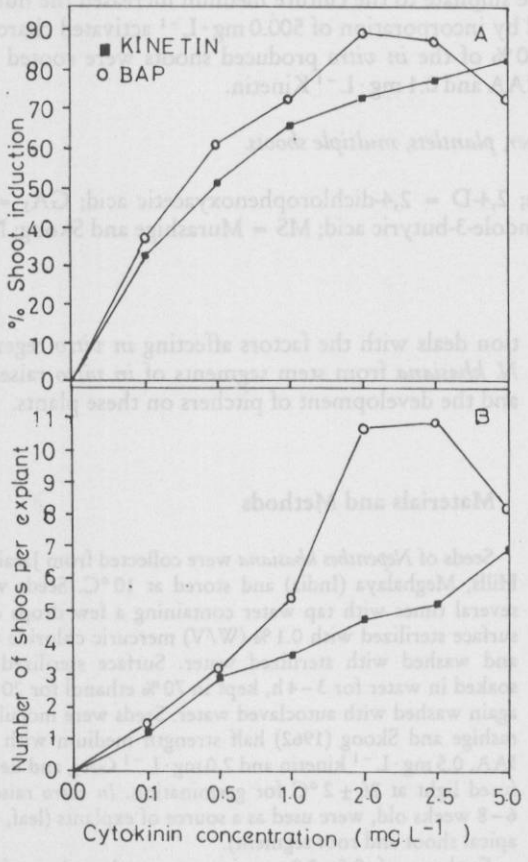

Fig. 1: Effect of cytokinins on multiple shoot induction from nodal shoot segments of *N. khasiana* on MS medium containing 0.1 mg·L^{-1} IAA.

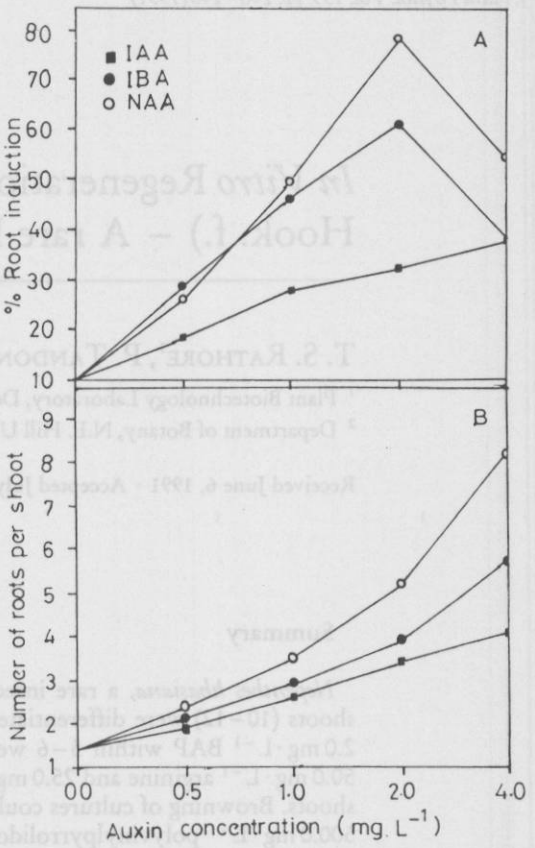

Fig. 2: Effect of auxin concentrations on root induction in shoots of *N. khasiana* on half strength MS medium with kinetin 0.1 mg·L^{-1}.

Results

Nodal shoot segments (1.0–1.5 cm) were found to be the best explants for multiple shoot induction of *N. khasiana*. Leaf and root segments neither produced callus nor shoots on any of the media. About 10–12 shoots were induced from the nodal shoot segment on MS medium supplemented with 0.1 mg·L^{-1} IAA and 2.0 mg·L^{-1} BAP, whereas from the apical shoot segment, only 6–8 shoots were induced on this medium. Addition of ascorbic acid, citric acid, arginine (50.0 mg·L^{-1}) and adenine sulphate (25.0 mg·L^{-1}) to the shoot multiplication medium enhanced the number of shoots to 12–15 per explant. Activated charcoal in the medium reduced leaching from the explants. Higher concentrations of activated charcoal (more than 500.0 mg·L^{-1}) and PVP reduced the number of shoots differentiated from the explant. Out of the other additives used coconut water (5–20 %) showed a moderate auxillary effect. Casein hydrolysate, yeast extract and beef extract were without effect. Out of the various growth regulators tested, the combination of IAA and BAP was found to be the best for shoot induction. Increased concentration of IAA (more than 0.5 mg·L^{-1}) with BAP reduced the number of shoots per explant. Less number of shoots were produced on MS + IAA + Kn. Results obtained with Kinetin and BAP are presented in Fig. 1. No callus induction was obtained on any of the

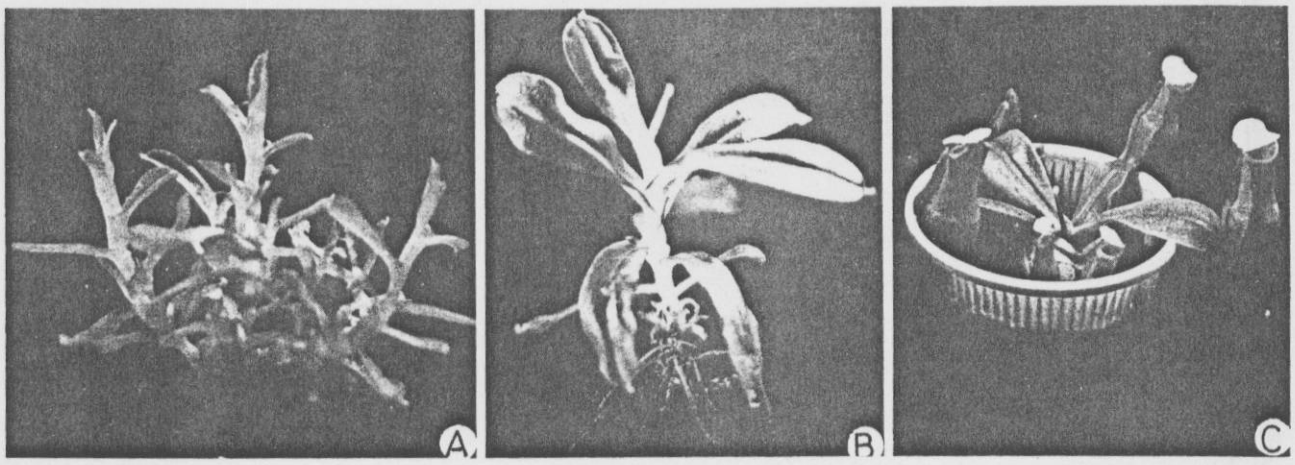

Fig. 3 A–C: *In vitro* regeneration of *N. khasiana* (A) Multiple shoots from differentiated shoot segments on MS + 0.1 mg · L^{-1} IAA + 1.0 mg · L^{-1} BAP (5-weeks-old). (B) Strengthened plantlet on ¼ MS medium devoid of hormones. (C) Potted plantlet developed pitcher with half opened lid (8-week-old plantlet).

media and growth regulators tested. Shoots could be further multiplied on the MS medium with 0.1 mg · L^{-1} IAA and 1.0 mg · L^{-1} BAP, on which 4–6 shoots per shoot segment were differentiated within 5 weeks (Fig. 3 A).

About 80% of isolated shoots rooted on half strength MS medium containing 2.0 mg · L^{-1} NAA and 0.1 mg · L^{-1} Kinetin. Percentage root induction was low on other media. Results obtained with auxins are summarized in Fig. 2. On strengthening medium, plants grew faster and showed pitcher development (Fig. 3 B). Hardening of potted plants before transplantation was found to be essential. Within a 4–5 week period potted plants developed pitchers with normal lids (Fig. 3 C).

Discussion

Results presented prove that *N. khasiana* can be multiplied in culture on MS medium containing BAP and IAA. Addition of ascorbic acid, citric acid, activated charcoal (AC) or PVP to culture medium enhanced shoot differentiation as these prevented browning of cultures. If these were not added to the medium the cultures exudated toxic substances that caused a decline in growth and deterioration of cultures. Ascorbic acid and citric acid are known antioxidants, and AC and PVP remove the toxic effects produced by oxidation of phenolics (Krikorian, 1988). Higher levels of AC and PVP inhibited growth of *N. khasiana* tissues, probably by depleting the growth promoting substances by adsorption from the medium. We suggest that for rapid multiplication and preservation of germplasm of *N. khasiana* this protocol can be used.

Acknowledgement

The authors express their gratefulness to the Meghalaya State Forest Department for permitting collection of *Nepenthes khasiana* seeds.

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