MASS CLONAL MULTIPLICATION OF THE THREATENED INDIAN INSECTIVOROUS PLANT (*NEPENTHES KHASIANA* HOOK F.) THROUGH SHOOT BUD CULTURE

P. TANDON AND T. S. RATHORE
Plant Biotechnology Laboratory, Department of Botany,
North–Eastern Hill University, Shillong 793 014

SUMMARY

Multiple shoots were produced in 6–7 weeks from axillary buds of mature plants of *Nepenthes khasiana* Hook f. cultured on modified MS medium (half strength nitrate salts) supplemented with Kn (2·5 mg/*l*). Healthy roots were produced within 5–6 weeks from isolated shoots cultured on 1/2 MS fortified with NAA (1·5 mg/*l*) nutrient medium fortified with NAA (1·5 mg/*l*). The plantlets were strengthened in 1/2 MS nutrient medium, free of growth hormones, but containing reduced amount of sucrose (2%) and agar (0·6%). The hardening of potted plants was accomplished in 2 weeks and about 75% plants survived. They were introduced in their natural habitat and survival under field conditions was about 60%.

KEY WORDS

Clonal multiplication, *Nepenthes khasiana*, shoot bud culture

ABBREVIATIONS

MS, Murashige and Skoog; IAA, indole–3–acetic acid; IBA, indole–3–butyric acid; NAA, α–naphthaleneacetic acid; 2,4–D, 2,4–dichlorophenoxyacetic acid; Kn, kinetin; BAP, 6–benzylaminopurine

INTRODUCTION

Plant tissue culture technique is now being applied for the mass propagation and conservation of threatened/endangered/rare plant species (Alphonso, 1975; Clayton *et al.*, 1986; Cervelli, 1986; Lal *et al.*, 1988). The genetic stability of tissue culture-raised plants is of great significance in germplasm conservation (Vasil and Vasil, 1980), which can be achieved by direct organogenesis in cultured explants without an intervening callus phase (D' Amato, 1978; Bayliss, 1980).

Nepenthes khasiana Hook. f., an endemic pitcher plant of Meghalaya, India, is of great horticultural and ornamental interest. Its leaves modify into showy and variously coloured pitchers used for catching insects. *N. khasiana* grows in nitrogen deficient soil and trapping of insects is a device to meet its nitrogen requirement. The plant has become threatened in its natural habitat due to ruthless exploitation for trade and unplanned human activities (Jain and Shastri, 1980). Unfortunately, the conventional methods of its propagation are quite poor and time consuming. The present communication describes a protocol for rapid multiplication of *N. khasiana* through axillary bud culture and its successful establishment in the natural habitat.

MATERIALS AND METHODS

The axillary buds from mature plants of *Nepenthes khasiana* Hook. f. were surface sterilized with sodium hypochlorite solution (1·5% v/v of available chlorine) for 10–15 min and washed thoroughly with sterile distilled water. These were cultured on modified Murashige and Skoog (1962) medium (full and 1/2 strength nitrate salts) supplemented with 3% sucrose, 0·8% agar–agar and various auxins (IAA, IBA, NAA, 2, 4–D) and cytokinins (Kn, BAP) either alone or in combinations at a range of concentrations (0·1– 10·0 mg/*l*) for preliminary experiments. In addition, activated charcoal

(500 mg/*l*), ascorbic acid (50 mg/*l*) and citric acid (10 mg/l) were incorporated separately in the culture medium. The cultures were incubated initially in dark at 20°C for 72 hr and later in light (2,500 lux for 8 hr) at 25 ± 2°C and 65% relative humidity.

The isolated shoots were subcultured on 1/2 MS nutrient salts medium supplemented with growth regulators mentioned above for rooting. The plantlets were strengthened by growing for 2–3 weeks on 1/2 MS nutrient salts medium (free of growth regulators) but containing reduced amount of sucrose (2%) and agar (0·6%). The plantlets of 4–6 cm size were potted in plastic pots containing different substrata *viz.*, vermiculite and soil mixture (1 : 3), drained soil with fine stone particles, and peat moss with soil (1 : 1). The plantlets were hardened for 2 weeks separately in a glasshouse and a growth chamber under controlled conditions to assess the rate of survival. The hardened plantlets were later transferred to their natural habitat.

RESULTS AND DISCUSSIONS

Amongst the various auxins and cytokinins used, Kn (2·5 mg/*l*) alone in the modified MS medium (half strength nitrate salts) was most effective in multiple shoot induction (Fig. 1A, Table 1). Incorporation of activated charcoal, ascorbic acid and citric acid separately into the medium showed an auxillary effect on shoot proliferation and helped in overcoming the browning of the explants. The excised microshoots were rooted in 1/2 MS nutrient salts medium containing NAA (1·5 mg/*l*) (Fig. 1B, Table 2). Simultaneous formation of root and shoot occurred from the axillary buds cultured in the medium containing NAA (1·5 mg/*l*) and Kn (0·5 mg/*l*) in combination, but the frequency of regeneration was quite poor.

Plantlets of 5–6 cm length were found ideal for potting. Out of the various potting mixtures tested, drained soil with fine stone particles was found to be the best substratum for the establishment of plantlets (Table 3). The precautions suggested by Sommer and Caladas (1981) were found useful for better survival. These were : (a) balance between root and shoot systems, (b) removal of all traces of agar and nutrients to prevent infection by pathogen, and (c) gradual transition from a high to low humidity. Hardening of the potted plants was accomplished within 2 weeks in the growth chamber and about 75% of the plants survived (Fig. 1D, Table 3). On the other hand, plantlets hardened in the glasshouse showed poor survival. The hardened plantlets were transferred and established in their natural habitat.

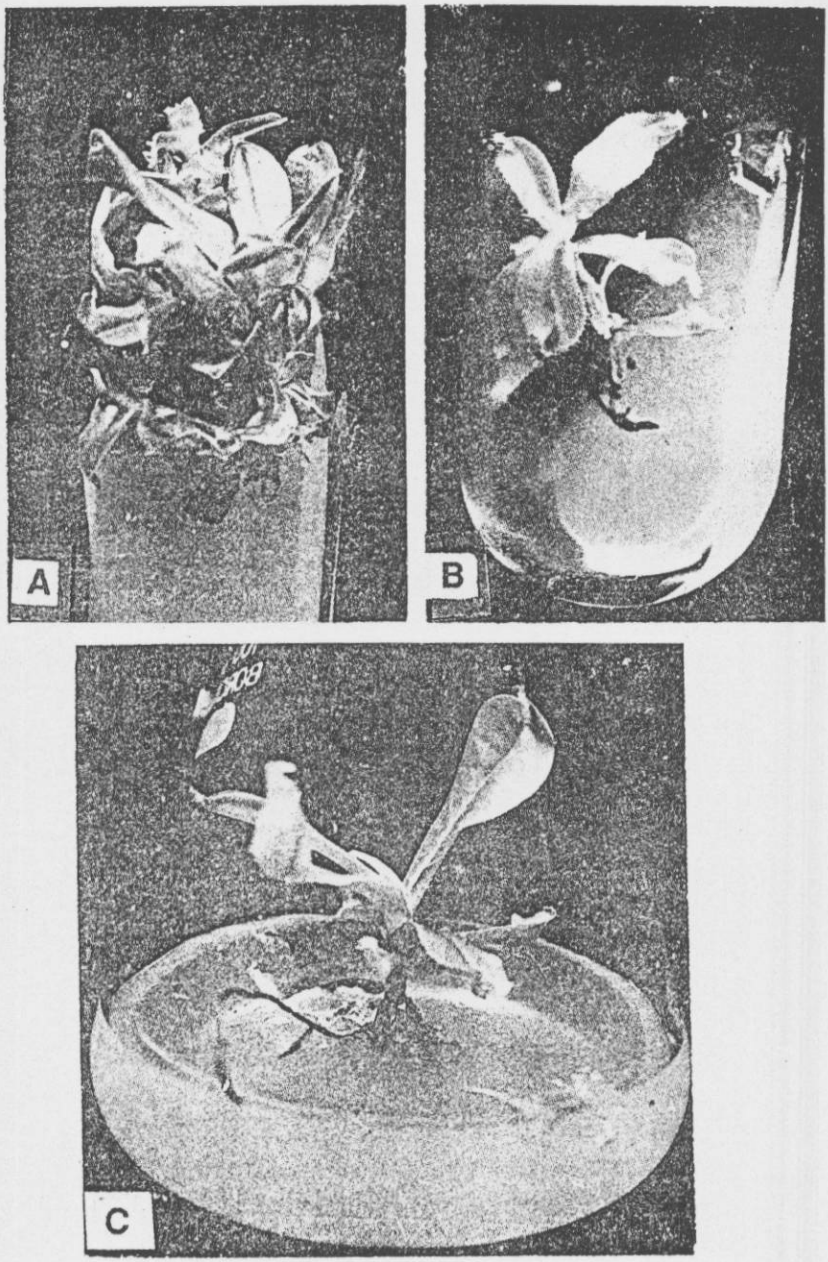

Fig. 1. A–C Clonal propagation of *N. khasiana*. A. Multiple shoot production (6–wk old). B. Rooting from isolated shoots (5–wk old). C. Strengthening of plantlets (showing pitcher) (13–wk old).

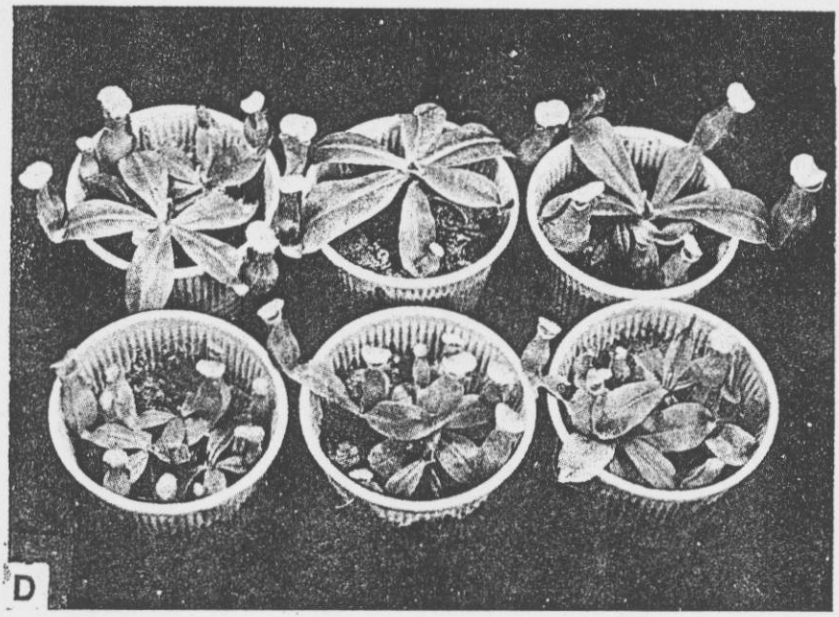

Fig. 1 D–E. Clonal propagation of *N. khasiana*. D. Plantlets transferred into pots containing drained soil (17–wk old). E. Hardened plantlets introduced in the natural habitat (6–mth old). Details in the text.

Table 1. Effect of kinetin on the shoot induction on axillary shoot bud of *N. khasiana* cultured in MS medium*

Kinetin (mg/*l*)	% culture with multiple shoots	No. of shoots/ explant	Remark
Control	–	–	–
0·1	–	1	Dwarf shoot
0·5	30 ± 1·83	2 – 3	Poorly developed shoots
1·0	55 ± 3·15	5 – 6	Multiple shoots
2·5	85 ± 3·27	13 – 15	Healthy multiple shoots
5·0	60 ± 1·60	7 – 8	Poorly developed multiple shoots

Data scored after 6 weeks, 12 replicates for each treatment;
*modified MS medium 1/2 strength nitrate salts;
– no response
± S. E.

Table 2. Effect of NAA on root induction on isolated shoots of *N. khasiana* cultured in MS medium*

NAA (mg/*l*)	% rooting	Roots per shoot
Control	–	–
0·1	–	–
0·5	25·3 ± 1·26	1 – 2
1·0	54·7 ± 1·42	2 – 3
1·5	84·6 ± 1·85	3 – 4
2·0	69·9 ± 1·27	4 – 5
2·5	62·0 ± 0·93	6 – 7
5·0	51·6 ± 0·85	3 – 4

Data scored after 6 weeks, 12 replicates for each treatment;
* MS 1/2 strength nutrient medium;
– no response.
± S. E.

Table 3. Hardening and acclimatization of *in vitro* raised plants of *N. khasiana*

Potting mixture	Environment	Climatic conditions			Plants transferred	Plants survived	Percentage survival
		Temperature °C		Humidity %			
		min	max				
a) Drained soil with fine stone particles	Growth chamber	20	25	80–85	2500	1873	74·9
	Glasshouse	18	32	55–80	200	99	49·5
b) Vermiculite + soil mixture	Growth chamber	20	25	80–85	200	119	59·5
	Glasshouse	18	32	55–80	50	22	44·0
c) Peat moss + soil mixture	Growth chamber	20	25	80–85	150	75	50·0
	Glasshouse	18	32	55–80	100	35	35·0

The survival rate was 60%. About two thousand field-transferred plants, phenotypically normal, are growing luxuriantly with speedy pitcher development and insect trapping (Fig. 1E). The protocol described here is being used for large scale multiplication of *N. khasiana*.

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