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In vitro Multiple Shoot Regeneration from Nodal Explants of *Zehneria scabra* (L.f.) Sonder - An Important Medicinal Climber

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Abstract

A protocol was developed for *in vitro* propagation by multiple shoot induction of *Zehneria scabra* (L.f.) Sonder, a medicinal climber having high medicinal values, belonging to Cucurbitaceae family. High frequencies of multiple shoot regeneration were achieved from nodal explants on MS fortified with 5 mg/l BAP and 0.5 mg/l IAA. Eight to ten shoots per explant were obtained. The elongated shoots were subcultured for rooting on MS supplemented with 2 mg/l NAA. The *in vitro* raised plantlets were acclimatized in green house and successfully transplanted to natural condition with 70% survival.

Introduction

Tissue culture techniques are being increasingly exploited for clonal multiplication and *in vitro* conservation of valuable indigenous germplasm threatened with extinction. Greater demand for these plants especially for the purpose of food and medicine is one of the causes of their rapid depletion from primary habitats. Micropropagation offers a great potential for large scale multiplication of such useful species and subsequent exploitation (Boro et al. 1998).

Zehneria scabra, a climber belongs to the Cucurbitaceae**.** It is rarely distributed in the hill region of Eastern Ghats. In Tamil it is called Akazakarudan kodi (Matthew 1982) and it is used to treat fever and stomach pain in the form of root and leaf extracts by tribals. The ethanolic root extracts had significant antibacterial activity against *E. coli* and *Pseudomonas aeroginosa* (Anand and Jeyachandran 2003). The root extract of the plant is used with milk to treat fever and diarrhoea. The leaf extract is used to treat

skin rashes (Kirtikar and Basu 1975). This plant is dioecious in nature, limiting pollination and fertilization, thereby, reducing its availability. Hence, the present study was undertaken to develop a suitable micropropagation technique to counteract the natural check in their population.

There has been progress in tissue culture studies in many Cucurbitaceae members such as *Momordica dioica* (Shiragave and Chavan 2001), *Coccinia indica* (Venkateshwaralu 2001), *Citrullus vulgaris* (Dong and Jia 1991), *Cucumis melo* (Mackay et al. 1989) and *Coccinia grandis* (Anugulati 1988). But no such *in vitro* culture studies have been carried out in this valuable medicinal climber.

The present investigation elucidates *in vitro* multiple shoot regeneration through nodal segments of *Zehneria scabra* for better exploitation and also preservation of this valuable germplasm which has already undergone a severe biotic pressure.

Materials and Methods

Zehneria scabra (L.f.) Sonder plants were collected from Bodamalai, Namakkal District of Tamilnadu. Its identity was confirmed in the Rabinat Herbarium, St. Joseph's College, Tiruchirappalli, South India. The collected plants were maintained at the college garden. Nodal segments were excised from the garden-grown plants and washed thoroughly in running tap water for 10 min, then in 1% teepol solution for 5 min and washed five times with sterile distilled water under aseptic condition. For surface sterilization, explants were rinsed by 0.1% aqueous $HgCl_2$ solution for 3 min and rinsed with sterile distilled water five times to remove traces of $HgCl_2$. Nodal segments were further trimmed to remove excess tissues. The explants were cultured on MS fortified with different concentrations of plant growth hormones along with 3% sucrose (w/v) and 0.8% (w/v) of agar. The pH of the medium was adjusted to 5.7 using 0.1 N NaOH or 0.1 N HCl before autoclaving and adding agar. About 10 ml of the medium were dispensed in each culture tube and sealed with non-absorbent cotton plugs prior to autoclaving at 121_C for 15 min. All cultures were maintained at 16 hr photoperiod with 3000 lux light intensity at 25 ± 2_C. Results were observed at regular intervals and tabulated. For each treatment 15 replicates were used and all experiments were conducted thrice.

Results and Disscussion

In a significant development it was observed that multiple shoot buds originated from nodal explants, when MS was supplemented with different

concentrations (1.0 - 7.0 mg/l) of BAP along with IAA (0.5 mg/l). The nodal explants showed slight swelling prior to the emergence of shoot buds developing from the pre-existing material 15 days after inoculation. Initially two to four shoot buds per explant emerged 25 days after inoculation and

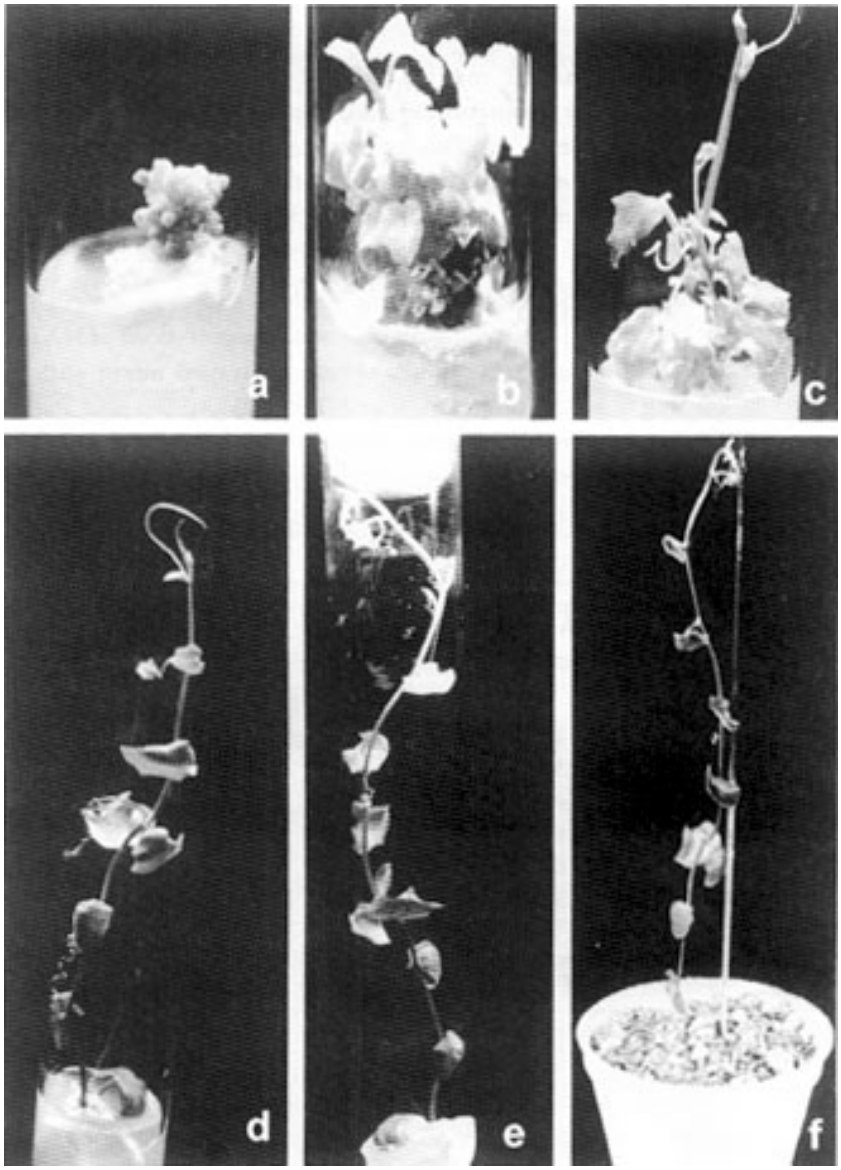

Plate 1. *In vitro* regeneration of *Zehneria scabra* from nodal explant. a. Initiation of multiple shoots. b. Multiple shoots. c. Shoot elongation. d. Root growth. e. Root growth after 25 days. f. Acclamatized plantlet.

gradually the number of shoot buds per explant increased up to 8 - 10 (Plate 1a and Table 1) on MS fortified with 5.0 mg/l BAP along with combination of 0.5 mg/l IAA. But a very low number of buds developed in the combination of 1.0 - 4.0 mg/l BAP along with 0.5 mg/l IAA; similar results were observed with BAP at a concentration of 6.0 and 7.0 mg/l.

It can be concluded that 5.0 mg/l BAP and 0.5 mg/l IAA are suitable phytohormones for shoot proliferation and shoot elongation from nodal explants of *Z. scabra* (Plate 1b, c). This is in accordance with the results as reported earlier (Kulkarni et al. 2002; Yokoya and Handro 2002; Nishikoshta and Bansal 2002; John Britto et al. 2001; Deora and Shekhawat 1995; Dong and Jia 1991; Reddy et al. 1998).

Following multiple shoot elongation, the healthy shoots (5 - 6 cm long) were transferred on MS supplemented with different concentrations of NAA (0.5 - 3.0 mg/l). Shoot elongation was simultaneously observed along with root induction in 2.0 mg/l NAA (Plate 1d, e and Table 2). Anitha and Pullaiah (2002) in *Decalepis hamiltonii* and Latha et al. (1998) in *Porteresia coarctata* also demonstrated similar results.

Table 1. Effect of BAP and IAA on multiple shoot proliferation from nodal explants of *Z. scabra*.

Hormone conc. (mg/l)		Multiple shoot induction (%)	Shoot length (cm)	No. of shoots/explant
BAP	IAA			
1	0.5	-	-	-
2	0.5	24	1.6 ± 0.20	3.3 ± 0.47
3	0.5	52	1.9 ± 0.08	4.0 ± 0.81
4	0.5	65	3.3 ± 0.16	5.6 ± 0.47
5	**0.5**	**87**	**5.6 ± 0.47**	**9.0 ± 0.81**
6	0.5	64	2.7 ± 0.08	5.3 ± 0.94
7	0.5	57	1.9 ± 0.12	4.0 ± 0.81

Table 2. Rooting response of excised shoots of *Zehneria scabra*.

NAA (mg/l)	% of root induction	Root length (cm)	No. of roots/shoot
1.0	-	-	-
1.5	24	1.6 0.20	3.30.47
2.0	**70**	**2.2 0.04**	**5.60.47**
2.5	59	1.9 0.08	4.0 0.81
3.0	51	1.8 0.06	3.90.81

After 30 days, well developed shoots (15 cm) and roots were observed. Subsequently, cultures were removed from agar medium, washed thoroughly and placed in pots containing a mixture of sterilized vermiculite and sterilized soil (1 : 1) (Plate 1f). *In vitro* raised plants were acclimatized in greenhouse and successfully transplanted into field with 70% survival.

In the present investigation, a high frequency of multiple shoot induction was achieved in *Z. scabra* through nodal explants with BAP (5.0 mg/l) and combination of IAA (0.5 mg/l). Further, an increase or decrease of this hormone level showed a negative trend in multiple shoot formation. NAA (2 mg/l) was found to be an ideal growth regulator for root induction as well as shoot elongation. The method developed for rapid micropropagation of *Z. scabra* is reliable and definitely a promising one for this valuable folklore medicine.

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