

Original Research Article

In Vitro Plant Regeneration from Nodal Callus of *Taverniera cuneifolia* (Roth) Arn. A Substitute for Commercial Licorice

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ABSTRACT

Keywords

Callus culture, Indian liquorice, Plant regeneration, Growth regulators, *Tavernieracuneifolia*, Silver nitrate

Taverniera cuneifolia (Roth) Arn. of Fabaceae is often referred as Indian licorice as its roots are sweet and taste very similar to that of *Glycyrrhiza glabra*, popularly known as commercial licorice. It is found wild in Marathwada and is used as a substitute for commercial licorice by the local people. It contains a sweetening agent Glycyrrhizin. It has antifungal, antibacterial, antiinflammatory, antimutagenic, antitumor activity and antigerm tube formation in *Candida albicans*. A regeneration protocol from nodal explants of *Tavernieracuneifolia* (Roth) Arn. was developed. The combination of 5.0 mg/l BAP + 1 mg/l IAA added to MS medium resulted in the best callusing as well as shoot induction. However, shoots did not elongate. The supplementation of 5mg/l AgNO₃ to this medium enhanced shoot induction as well as elongation. Rooting of *in vitro* raised shoots was successful in MS + 2 mg/l NAA and 10% CW. The regenerated plantlets were successfully acclimatized.

Introduction

The genus *Taverniera* belongs to family of Fabaceae includes twelve species and is endemic to the Northeast African and Southwestern Asian countries (Stadler *et al.*, 1994). *Taverniera cuneifolia* (Roth) Arn, commonly known as Indian liquorice, is an herb and occurs along the bank of small streams. Roots of this species are sweet in taste and are used by the tribal people as a substitute for the commercial liquorice-*Glycyrrhizaglabra* (Naik 1998). The commercial liquorice has tremendous medicinal properties. The roots of *G. glabra* are widely used in traditional systems of medicines all over the world (Grieve 1992) and are rich in bioactivities like antiulcer, anti-inflammatory, antibacterial, antimalarial,

antithrombic, antidiuretic, antitherosclerotic, antifungal, estrogenic, antiallergic, antidiabetic and antimutagenic (Baltina, 2003; Fukai *et al.*, 2002; Kharazami *et al.*, 1997; Mendes-Silva *et al.*, 2003; Rastogi and Mehrotra, 1993; Sebestain and Thampan, 2003; Shibata, 2000; Takil *et al.*, 2001; Zani *et al.*, 1993). *G. glabra* extract, glycyrrhizin and its derivatives are reported to inhibit growth of viruses like HIV, SARS, Hepatitis B & C, Influenza through the potentiation of immune system, inhibition of reverse transcriptase and induction of interferon production (Cinatl *et al.*, 2003; Sasaki *et al.*, 2003 and Takahara *et al.*, 1994).

In our earlier study, chromatographic and spectral analysis of root extracts of *T. cuneifolia* and *G. glabra* has exhibited similarity in chemoprofile. The similar chromatophores included the sweetening principle, glycyrrhizin. Ethanol and chloroform soluble fractions of both the plant species possessed considerable *in vivo* anti-inflammatory and protective activity from EMS induced toxicity in *Salmonella typhumurium*. *T. cuneifolia* extracts showed inhibition of *Agrobacterium* induced tumors (Zore *et al.*, 2008). *T. cuneifolia* root extract have shown considerable antifungal activity (Zore *et al.*, 2003, 2004).

The commercial liquorice has huge demand in Indian system of medicine Ayurveda and is required to the tune of 5000 tons per year by the Indian pharmaceutical companies. This licuorice is totally imported from Pakistan and Afghanistan spending crores of Rupees (Anonymous, 2000). We report a protocol for regeneration of plant from Nodal explants *in vitro* propagation technique is important method for multiplication of medicinal plants (Lui and Lui, 2001; Wawrosch *et al.*, 2001; Martin 2002, 2003; Azad *et al.*, 2005; Faisal *et al.*, 2003; Hassan and Roy, 2005). In this study, we describe efficient method for *in vitro* propagation and mass multiplication of *Taverniera cuneifolia*. This protocol may be useful in conservation of this plant species which is totally exploited from the nature as a substitute for the commercial liquorice.

Materials and Methods

Mature fruits of *Taverniera cuneifolia* were collected from "Bavee" village in Bhoom Taluka of Osmanabad District, India. Voucher specimens have been deposited in the Herbarium Centre of our school (T.C.2). The shade dried seeds, collected during December to January were used for seed germination. The seeds were thoroughly

washed under running tap water in tissue culture bottle for about 30 minutes. Seeds disinfected in 70 % v/v ethanol for 4 min. were surface sterilized in 1 % HgCl₂ for 8 min. Thereafter, seeds were washed thrice with sterile distilled water to remove the traces of mercuric chloride prior to placing onto tissue culture medium (pH 5.8) with half strength of Murashige and Skoog (MS) (1962) solidified with agar (0.9 %) (w/v) (HiMedia, Bangalore, India). Sterilized seeds were germinated on 1/2 MS containing 3 % sucrose and solidified with 9 g l⁻¹ agar. Since seeds show seed coat dormancy, seeds were mechanically scarified with blade on opposite side of the embryo to facilitate the intake of water and nutrients from the surrounding medium. Five seeds were kept in a single bottle (350 ml Kasablanka tissue culture bottles with 50 ml medium in each) these *in vitro* raised and a month old seedlings were used as a source of explants. Nodal explants were prepared by cutting the parts in aseptic conditions.

Callus induction, culture media and condition

The nodal explants (1cm).were cultured for callus induction and shoots Regeneration in 15x 140mm culture tubes containing Murashige and Skoogs medium supplemented with 0.9% (w/v) Agar and 3% (w/v) sucrose and various concentrations of cytokines 2mg/l, 5mg/l, 8mg/l BAP and 2mg/l, 5mg/l, 8mg/l Kinetin and Auxin 0.1mg/l, 0.5mg/l, 1.0 mg/l such as naphthalene acetic acid (NAA), Indole 3-acetic acid (IAA), Indole 3-butyric acid (IBA) either alone or combination described table 1 and 2. The pH of the medium was adjusted to pH 5.8 with 1 N HCl and 1 N NaOH before addition of agar. The melted agar (Approximate 10 ml/test tube) dispersed into culture tubes (15 x 140 mm) was autoclaved at pressure of 1.5 kPa at 121⁰C for 20 min. All cultures were

maintained in culture room at $25 \pm 2^{\circ}\text{C}$ temperature under a 16 hr photoperiod at intensity of $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by cool daylight florescent lamps (Philips, India). Humidity maintained in culture room was 70-90%. The calli were isolated from explants and transferred on fresh medium of the same compositions. The explants responding for callus and shoot regeneration, no of shoots producing per explants and length of shoots was measured. Explants were sub-cultured after 2 weeks for each treatment 25 replicates were used.

Rooting and acclimatization

The shoots (5-6 cm; 4-5 week old) were cultured on MS medium supplemented with different concentrations of NAA or IAA alone (Himedia, Bangalore, India). Cultures were maintained at $25 \pm 2^{\circ}\text{C}$ under 16 hr photoperiod at an intensity of $30 \mu\text{M m}^{-2} \text{s}^{-1}$ provided by cool daylight fluorescent tube lights. All media were supplemented with 3 % (w/v) sucrose and 0.9 % (w/v) Agar-agar.

For acclimatization, 7-8 week old rooted shoots were removed from culture tubes. After washing away the adherent medium with distilled water, plantlets were transferred to plastic pots containing soil: sand (1:1). Humidity was maintained initially by covering the pots with polythene bags. Polythene bags were progressively removed to reduce the humidity. After acclimatization for 15 days, plantlets were transferred to shade house for one month with an average temperature at 25°C .

Statistical analysis

The culture responses were expressed in terms of percentage responding explants, number of regenerants (shoot or root) per explant and average length of shoots or roots. A completely randomized block design with 3 replications was used. All data

were subjected to one way analysis of variance (ANOVA) and comparisons of means were made with least significant difference (LSD) at the 5 % level. For each treatment of a replicate experiment, 25 explants were used.

Results and Discussion

Nodal explants were cultured on different conc. of BA and KN alone within four to 25 days of culture callus formed at cut surface of explants. The nodal explants have high morphogenic efficiency (Martin, 2002). In direct organogenesis was reported in many medicinal plants species. *Aparagus cooperi* (Ghosh and Sen, 1980), *Plumbagozeylanica* (Das and Rout 2002), *Holostemaada-kodien* (Martin, 2002) *Rotula aquatica* (Martin 2003), *Gloriosa superba* (Sivakumar *et al.*, 2004), *Phellodendron amurense* (Azad *et al.*, 2005), *Momordica cymbalaria* (Nikam *et al.*,)

The ability of callus formation and shoot regeneration depend on type of growth regulator and their conc. in the nutrient medium (Bhojwani and Razdan, 1996; Thorpe, 2007). The best response of 12 shoots was recorded in 5mg/l BAP and 1mg/ IAA with 90% response (Fig. 1).

Root induction and acclimatization

On basal medium alone development of roots took place at the cut ends of *in vitro* regenerated shoots with little callusing phase 2-3 weeks after shoot transfer. However, the growth of roots was slow. To promote the development of the root 5-6 cm long shoots were placed on MS medium supplemented with different concentrations of NAA and IAA. On IAA supplemented medium, the growth of roots was stunted. The incorporation of NAA enhanced rooting significantly. The best rhizogenic response was observed with 2 mg l^{-1} NAA, where 80

% of the shoots rooted (Table 2). The number of roots per shoots was 7.16 ± 0.18 (Fig. 2). NAA is widely used for induction of roots on regenerated shoots in medicinal plants. *Chlorophytum borivilianum* (Purohit *et al.*, 1994) and *Curculagoorchoides* (Bhavisha and Joshi, 2003).

The plantlets were transferred in plastic cups. The humidity was maintained covering cups with the polythene bags. After the development of new leaves, the plants were moved to shade house. The survival was 60 %. The survived plantlets grew in without any obvious morphological aberrations.

Regeneration protocols have been standardized for many medicinal plants for the continuous supply of many medicinal plants. As a result of the overexploitation of plant material from the natural stands for traditional medicinal purpose, the standardization of the regeneration protocol for such plants is becoming important. The protocol will facilitate the conservation of the species and could serve as an alternative source of materials for use. The regeneration protocol described herein would benefit the conservation of *T. cuneifolia*, which is extensively used in traditional medicines by the local people.

Table.1 Effect of different concentration and combination of growth regulators on MS for callus/shoots induction in *Taverniera cuneifolia* (Roth) Arn.

Growth regulators(mg/l)						% explants producing callus/shoots	Mean No. of shoots	Length(cm) of shoots
BAP	KN	NAA	IAA	IBA	AgNO ₃			
2						90	1	2
5						100	1	2
8						70	1	2
	2					70	1	2
	5					30	1	2
	8					50	1	2
		0.1			1.7	50	4	2
		0.5			3.4	70	3	2
		1.0			5.1	80	4	2
			0.1		1.7	80	4	3
			0.5		3.4	90	5	3
			1.0		5.1	100	14	5
				0.1	1.7	50	2	3
				0.5	3.4	40	3	3
				1.0	5.1	70	2	2

Table.2 Effect of NAA on Induction of roots.

Medium/ Hormones used	Conc. mg/l	Coconut milk % (v/v)	No. of roots/shoots	% of response	Average root length in cm
MS + NAA	1		3	60	1.5
MS + NAA	2		4	60	2.0
MS + NAA	3		3	60	1.5
MS + NAA	1	5	5	60	2.0
MS + NAA	2	10	9	70	3.0
MS + NAA	3	15	6	60	2.5

Fig.1 Shoots induction in *Taverniera cuneifolia*



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