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Short communication

In vitro propagation of *Mitragyna parvifolia* Korth.

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Abstract. Multiple shoot formation and their elongation from excised apical vegetative shoots of a 40-year old-tree of *Mitragyna parvifolia* Korth. was achieved in Murashige and Skoog's medium supplemented with 4.44 μM benzyl adenine. The in vitro regenerated shoots rooted when cultured on modified Murashige and Skoog's medium containing low inorganic salts and the three auxins. Regeneration by this method was suitable for mass propagation of the plant.

Introduction

Mitragyna parvifolia Korth. syn. *Stephegyne parvifolia* Korth. (Rubiaceae) is a tall forest tree, a popular avenue tree, a useful fuel and timber yielder, and a medicinal plant [1]. The plant is found growing in West and East Africa, India, South East Asia, and often in Australasia and Pacific islands [4]. Propagation is chiefly from minute seeds (10,000 g^{-1}). Reports regarding its propagation by cutting, layering or grafting are few, fragmentary and of unsuccessful trials where coppice is a better choice [10]. Seedlings are minute, delicate and liable to be beaten down or washed away by rain. Micropropagation has gained considerable importance as a tool for clonal propagation of timber trees [2, 3, 5, 6, 9, 11]. Our objective was to develop a micropropagation system for *M. parvifolia*.

Materials and methods

Selection of a regeneration medium

Excised shoot tips (axillary and apical vegetative buds along with a portion of stem, total length being 1 cm) from a 40-year-old *M. parvifolia* tree grown at Calcutta University Botanic Garden were used as explants. The explants

were washed several times by distilled water, soaked in 0.5% aqueous polyvinyl pyrrolidone (PVP) (MW 40000) for 45 minutes and then surface sterilized with 0.5 percent mercuric chloride. The sterilant was removed after 4–5 minutes by sterile water. Explants were cultured in Murashige and Skoog medium (MS) [8] containing a range of concentrations of auxins: indole-3-acetic acid (IAA) (2.85, 5.7, 11.4 μM), indole 3-butyric acid (IBA) (2.45, 4.9, 9.8 μM), indole propionic acid (IPA) (2.64, 5.28, 10.57 μM), α-naphthalene-acetic acid (NAA) (2.68, 5.37, 10.74 μM) or 2,4-dichlorophenoxy acetic acid (2,4-D) (2.26, 4.52, 9.04 μM) and cytokinins: 6-benzyladenine (BA) (2.22, 4.44, 8.88 μM) and kinetin (2.32, 4.64, 9.29 μM) added either individually or in various combinations.

All experiments had 15 replicates and were repeated thrice. The pH of all media was adjusted to 5.8 using NaOH and HCl before sterilization and solidified by 0.8% Difco bacto-agar. Media were sterilized in an autoclave at 120 °C for 20 min. All chemicals were of analytical grade (BDH, E. Merck, Difco or Sigma) and glassware from Corning or Sigcol.

Rhizogenesis

For root induction on the in vitro shoots, we cultured the shoots on $\frac{1}{2}$ MS with NAA, IAA, IBA, IPA and 2,4-D supplements, individually and in possible combinations (at concentrations stated above) and with 1.5% sucrose. The incubation period was 20–30 days, of which the first 15 days were in total darkness and the remainder on 16 hr light per day. The temperature and the irradiance were 26 $\pm$ 4 °C and 30 μmol s^{-1} m^{-2} respectively.

Acclimatization

Rooted shoots of different ages were transferred to liquid MS with filter paper platform where they grew for different periods at 25 °C and 30 μmol $m^{-2}s^{-1}$ illumination and 70–80 relative humidity. Later, they were transplanted to pots containing a mixture of soil, vermiculite and sand (2:1:1) and maintained in the same environmental condition. Daily watering was needed. High humidity was maintained by covering the potted plants with glass jars. Plants when attained 5–6 cm were transferred to glass house and later to the field.

Results and discussion

Initially the explants turned black and died within a few days. This was overcome by suspending the explants in a 0.5% aqueous polyvinyl pyrrolidone.

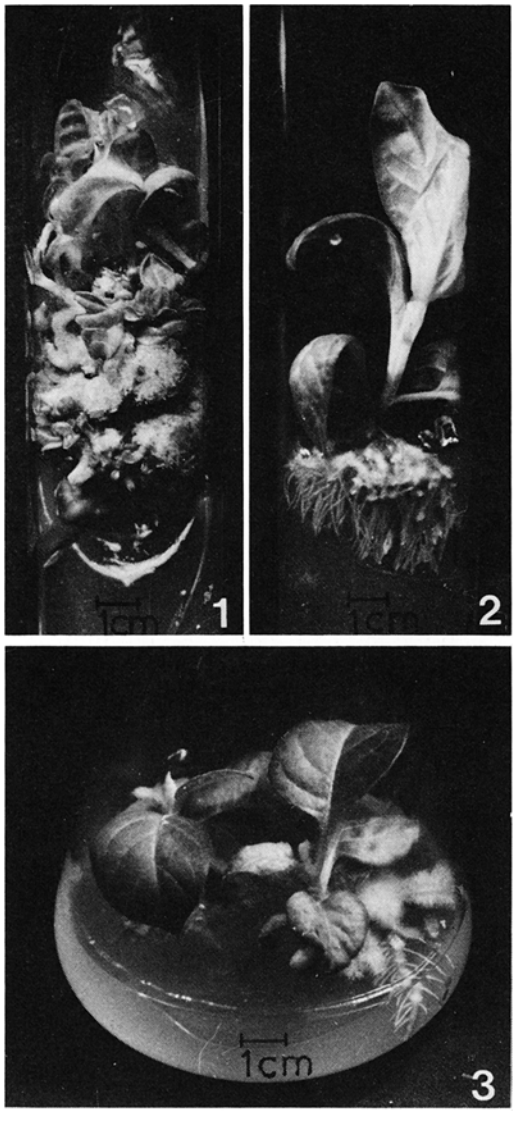

Fig. 1. Shoot development from apical bud explant on MS medium with BA, 4.4 μM (30 days culture).

Fig. 2. Fibrous root development in the shoot on MS medium with IAA + IBA + IPA (5.7 μM) + (4.9 μM) + (5.28 μM) (30 days culture).

Fig. 3. Stout tap root development in the shoots on MS medium with NAA + IBA + IAA (5.37 μM) + (4.9 μM) + (5.7 μM) (30 days culture).

Table 1. Response of apical bud explants of *Mitragyna parvifolia* to various hormones in MS medium.

Growth regulator (μM)		Explant shooted %		Axillary shoot bud number explant		Explants rooted %		Explant forming callus	Nature of response
		20 days (±S.E.)	30 days (±S.E.)	20 days (±S.E.)	30 days (±S.E.)	20 days (±S.E.)	30 days (±S.E.)	30 days (±S.E.)	
BA	4.44	80 (1.4)	90 (1.2)	8 (0.9)	10 (1.4)	–	–	–	Healthy long shoots
Kn	4.64	20 (1.4)	35 (2.0)	4 (0.9)	5 (0.4)	–	–	–	Moderate shoots
IBA	4.9	–	–	–	–	10 (3.0)	20 (2.4)	–	Small roots
IAA	5.7	–	–	–	–	10 (3.2)	20 (2.9)	–	Moderate roots
IPA	5.28	–	–	–	–	5 (0.8)	10 (1.2)	–	Small roots
NAA	5.37	–	–	–	–	60 (1.8)	70 (1.8)	–	Long roots
2,4-D	4.52	–	–	–	–	–	–	80 (0.8)	White friable callus
BA	2.22	5	8	4	4	–	–	–	Small shoots
NAA	2.68	(1.2)	(1.6)	(0.8)	(0.8)				
BA	2.22	5	10	3	4	–	–	–	Small shoots
IAA	2.85	(1.6)	(2.0)	(0.8)	(0.4)				
BA	2.22	–	10	–	2	–	–	–	Small shoots
IBA	2.45		(0.9)		(0.4)				
BA	2.22	10	20	–	2	–	–	–	Small shoots
IPA	2.64	(2.3)	(3.2)		(0.4)				

Among the hormones added singly to the medium, BA produced shoots (8–10 per explant) only (on 80–90% explants) after 20–30 days (Fig. 1), NAA roots only (on 60–70% explants) after 20–30 days and 2,4-D a fine, friable white callus (on 80% explants) after 30 days (Table 1). In MS medium supplemented with IAA (5.7 μM) + IBA (4.9 μM) + IPA (5.28 μM), 90% of the shoots produced fibrous roots (Fig. 2; Table 2). IAA (5.7 μM) + IBA (4.9 μM) + NAA (5.37 μM) produced stout tap roots (Fig. 3) in 99% shoots.

60 percent plants survived in the pots. 90 percent field transplanted plants survived and were healthy.

The present result illustrated the potentiality of shoot tip (with a portion of stem) to form plants in two stages: Initiation of shoots on the cytokinin medium and later culturing on auxins for initiation and development of a root system (Table 2).

Table 2. Response of root formation of the shoots derived from apical bud explants of *M. parvifolia* cultured on modified MS medium containing different auxins.

Growth regulator[y] (μM/l)		Callus after 30 days	Rooting percentage	
			20 days (±S.E.)	30 days (±S.E.)
IAA	2.85	–	20 (2.8)	40 (2.1)
	5.70	–	30 (2.0)	50 (1.6)
	11.40	–	20 (2.0)	30 (1.6)
IBA	2.45	–	10 (0.9)	40 (1.5)
	4.90	–	30 (1.4)	60 (1.6)
	9.80	–	20 (2.0)	30 (1.6)
IPA	2.64	–	10 (1.2)	40 (0.9)
	5.28	–	40 (0.8)	70 (1.6)
	10.57	–	10 (0.8)	30 (1.2)
NAA	2.68	–	40 (0.9)	70 (1.2)
	5.37	–	60 (1.2)	80 (0.9)
	10.74	–	50 (0.4)	70 (1.2)
2,4-D	2.26	+	–	–
	4.52	+	–	–
	9.04	+	–	–
NAA + IBA	5.37 + 4.90	–	30 (1.4)	60 (1.2)
NAA + IPA	5.37 + 5.28	–	30 (2.8)	60 (2.4)
NAA + IAA	5.37 + 5.70	–	40 (1.8)	60 (1.6)
NAA + IBA + IPA	5.37 + 4.90 + 5.28	–	60 (4.2)	70 (2.9)
NAA + IBA + IAA	5.37 + 4.90 + 5.70	–	80 (0.4)	99 (0.2)
IAA + IBA + IPA	5.70 + 4.90 + 5.28	–	60 (2.3)	90 (1.8)

[x] = The + signs indicate an increasing degree of good callusing and – signs means no response.

[y] = 1/2 MS medium of inorganic salts. For other details see Materials and Methods.

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