PROTOCOL ESTABLISHMENT FOR MULTIPLICATION AND REGENERATION OF *POLYSCIAS FRUTICOSA* L. Harms. AN IMPORTANT MEDICINAL PLANT IN VIETNAM

Bui Dinh Thach, Le Nguyen Tu Linh, Diep Trung Cang, Trinh Thi Ben, Tran Thi Linh Giang, Nguyen Pham Ai Uyen & Ngo Ke Suong

Institute of Tropical Biology, Vietnam Academy of Science and Technology
VIETNAM

ABSTRACT

Polysicas fruticosa (L) Harm is one of the mo`st common medicinal plants used by diverse cultures and tribal groups. An efficient protocol for rapid propagation of *Polysicas fruticosa*, of the family Araliaceae, was developed using leaf explants culture. The leaf explants cultured on Murashige and Skoog (MS) basal medium were supplemented with various concentrations and combinations of auxins and cytokinins. Callus induction was obtained within 4 weeks, 2,4-D at 3mg/l formed profuse callus and the degree was found to be the highest (++++) among all the treatments. The best response to shoot induction, with maximum shoot number 8.21(mean number of shoot per explant) was obtained using 5.0 mg/l 6-benzyl aminopurine (BA) in combination with 0.1 mg/l Naphthalene Acetic Acid (NAA). *In vitro* shoots were rooted on 0.5mg/l of NAA supplemented medium. The rooted shoots were successfully acclimatized and established under natural conditions, with 74% survival rate.

Keywords: *Polysicas fruticosa*, medicinal plant, micropropagation, callus.

INTRODUCTION

Polysicas fruticosa (L) Harm (Claus, 1956) (Araliaceae), is available throughout the warmer parts of India, Malaysia, Vietnam and Polynesia. This plant has synonyms like *Panax fruticosa*, *Nothopanax fruticosam*, *Parsley panax*, *Ming aralia*, *Dinhlang* or Indian *Polyscias (*Chopra *et al.*, 1956; Nayanar, 1985). The plant grows fairly slowly but can reach up to 1 to 2 meters in height. Leaves alternate, petiolate, irregularly pinnately compound, the leaflets with conspicuous toothed margins, blades often yellowish in color and fragrant if crushed. Flowers relatively small, yellowish-green, borne in umbels. Fruit is a small dry drupe with a single seed. The roots smell and taste like parsley (Trease and Evan, 1983; Nayanar, 1985).

The Plant extract of *Polyscias fruticosa* (L) Harm had showed anti-inflammatory, antitoxin, and an antibacterial (Sakr *et al*., 2014). The root is also used as diuretic, febrifuge, anti-dysentery, and is employed for neuralgia and rheumatic pains, brain performance (Yen, 1990[a]), stimulate sexual performance of aged male rats (Yen, 1990[b]), the activity of the endogenous antioxidant enzyme superoxide dismutase in the striatum and cerebellum, while those of glutathione peroxidase and catalase were unaltered (Yen *et al*., 1990). Alongside with medicinal purposes, *Polyscias fruticosa* (L) Harm is also used as an ornamental plant and a spice.

Research and application of *in vitro* culture *Polyscias fruticosa* (L) Harm plant is recognized by Sakr *et al* (2014) through the young shoots. In this study we present the complete steps in micropropagation *Polyscias fruticosa* (L) Harm plants through leaf tissue.

MATERIALS AND METHODS

Young leaves of *Polyscias fruticosa* (L) Harm were collected from the medicinal plant garden of Institute of Tropical Biology, Vietnamese. Leaves were collected from two years old field grown mature plants (Fig a). Young leaves were rinsed under running tap water for 20 minutes and were surface sterilized separately in an aqueous solution of 0.1% (w/v) mercuric chloride for 3-4 minutes. The sterilized young leaves were cut into 5mm x 5mm squares and these explants were inoculated on Murashige and Skoog's (1962) basal medium (MS medium) supplemented with various concentrations of auxins 2,4 Dichlorophenoxyacetic acid (2,4-D), and NAA (Table 1). The medium was solidified with 0.8 % agar. The pH of the medium was adjusted to 5.8 before autoclaved at 121^0C for 20 minutes. The cultures were maintained in a culture room at 25 ± 2^0C with 16 hrs photoperiod. For each treatment, 3 replicates were taken and each experiment was repeated thrice. Later different concentrations and combinations of BA, kinetin and NAA were added on MS basal medium for differentiation of the callus (Table 2). The excised shoots were transferred to liquid medium for rooting. The *In Vitro* raised shootlets were subcultured on MS medium supplemented with various concentrations of NAA (Table 3). *In vitro* rooted plantlets were transplanted to small earthen pots containing mixture of soil, sand, husk ash, and coir (Table 4) and covered with transparent polyethylene bags to maintain high humidity. They were kept in the green house for further acclimatization and finally transferred to the field.

RESULTS AND DISCUSSION

Induction of Callus and Shoot bud proliferation

The manipulation of plant growth regulators is essential to optimize the induction of callus. After 4 weeks of observation, all the plant growth regulators tested on leaf explants showed 100% of callus formation (Table 1). However the degree of the callus induced from the leaf explants varied from each plant growth regulator. The callus formed was compact, light green in colour with some white callus distributed on the top of the light green callus (Fig 1A).

Among the various treatments to study shoot induction in *Polyscias fruticosa* (L) Harm, the best response with maximum shoot (8.21 mean number of shoot per explant) was obtained after four weeks of subculture, using 5.0 mg/l BA in combination with 0.1 mg/1 NAA (Fig 1B, 1C). This medium was used to culture for high response to shoot bud high (Fig 1D). Response of BA and NAA was found to be the best combination for bud break response as well as shoot bud regeneration in *Polyscias fruticosa* (L) Harm from callus. Similar findings have been reported by various authors (Sahoo *et al*., 1997; Begum *et al*., 2002). Establishment and multiplication of shoot buds in MS nutrient media was achieved with high cytokinin BA (1.0-3.0 mg/l) and lower concentration of auxin IAA or NAA (0.2- 0.5 mg/l). This result is in accordance with Harris and Stevenson, (1982) and Chee and Pool, (1985). In contrast to this, Pattanaik and Chand, (1996) observed that at higher concentrations of BA (2-3 mg/l) (Pattnaik and Chand, 1996).

Table 1: Effects of auxins on callus induction from leaf explant of *Polyscias fruticosa* (L) Harm after 4 weeks of culture in MS medium

Auxin	Concentrations (mg/L)	Callus (%)	Morphology and colour of callus	Degree
2,4-D	0	0	---	---
	1	100	White, light greenish compact callus	++
	3	100	White, light greenish compact callus	++++
	5	100	White, light greenish compact callus	++++
NAA	1	100	White, light greenish compact callus	+
	3	100	White, light greenish compact callus	++
	5	100	White, light greenish compact callus	+++

- = no callus formed, += poor callus, formation, ++=minor callus, formation, +++ average callus formation, ++++=moderate callus formation, +++++= profuse callus formation

Table 2: Effects of growth regulators on MS medium for shoot regeneration of *Polyscias fruticosa* (L) Harm

Growth Regulators (mg/L)			Shooting Response (%)	No. of shoots
BA	Kinetin	NAA		
1.0	0.0	0.1	100	2.35^{e}
2.0	0.0	0.1	100	4.25^{cd}
3.0	0.0	0.1	100	5.32^{c}
4.0	0.0	0.1	100	6.74^{b}
5.0	0.0	0.1	100	8.21^{a}
0.0	0.1	0.1	100	4.05^{d}
0.0	0.5	0.1	100	4.50^{cd}
0.0	1.0	0.1	100	5.01^{c}
0.0	1.5	0.1	100	6.57^{b}
0.0	2.0	0.1	100	6.82^{b}

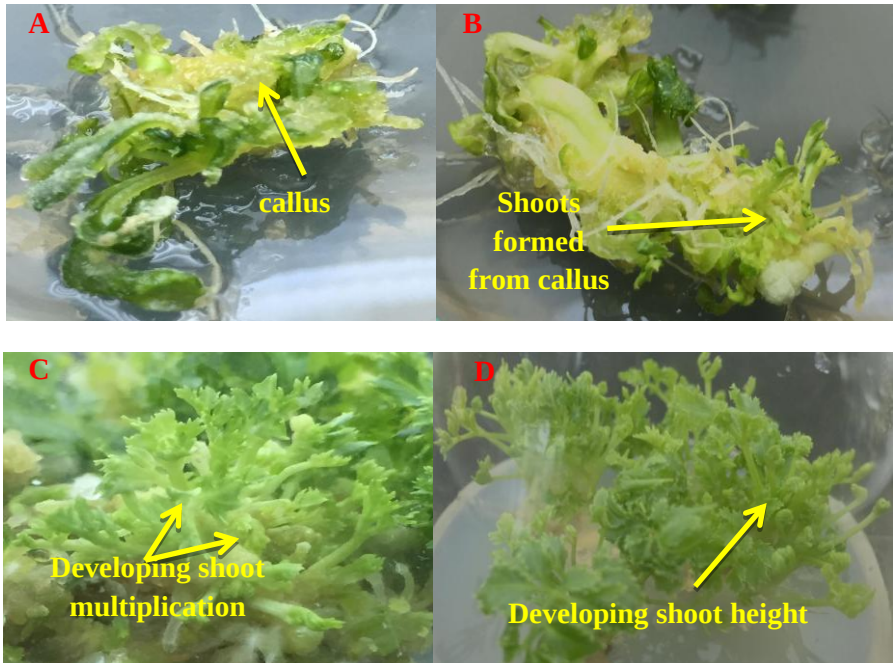

Fig 1. The formation and development of shoots from leaf tissue. A. Callus; B. the formation shoots from callus; C. developing shoot multiplication; D. developing shoot height.

Induction of Root, Hardening and Acclimatization of plantlets

After four weeks, the well developed shoots (2-2.5 cm) were excised and transferred to half strength MS medium supplemented with IBA singly and in combination with NAA (Table 3). In different concentration of NAA tested, 0.5 mg/l of NAA in MS was found to be most suitable for the growthing response (Fig 2A, B; Table 3). The supplementation of auxin either singly or in combination was also reported in many plant species: *Polyscias fruticosa* (L) Harm (Sakr *et al., 2014)*, *Ocimum gratissimum* L. (Gopi *et al*., 2006), *Rauvolfia serpentina* (Linn.) (Baksha *et al*., 2007). However, the addition of NAA also favored rooting in other medicinal plants like *Picrorhia kurrooa* (Chandra *et al*., 2006) and *Sida cordifolia* (Sivanesan and Jeong, 2007).

Data recorded in Table 4 demonstrated that the highest percentage of plant survival (74%) was achieved by transplanting of the plantlets to pots containing soil and husk ash at the ratio of 1:1(w/w), but growth parameters: No of root, root leight, and plant height had the highest value at the ratio of soil and husk ash = 3:1(w/w), (Fig. 3 A, B; Table 4). There were no morphological differences observed, among the *in vivo* and *in vitro* grown plants. These results exhibited that the protocol described for clonal propagation of *Polyscias fruticosa* (L) Harm was very efficient in regeneration of this species.

Table 3: Effect of NAA on the growthing response of *Polyscias fruticosa* (L) Harm

Growth regulators NAA (mg/L)	Rooting response (%)	Plant height (cm)	No. of roots	Root length(cm)
0.0	100	2.67^{c}	2.67^{d}	0.23^{d}
0.1	100	3.07^{bc}	5.00^{cd}	2.17^{c}
0.3	100	3.33^{b}	7.33^{c}	3.33^{b}
0.5	100	4.67^{a}	15.50^{a}	4.17^{a}
0.7	100	4.33^{a}	10.67^{b}	2.83^{bc}
1.0	100	3.40^{b}	10.67^{b}	2.67^{bc}

Fig 2. Effect of NAA on the growthing response of *Polyscias fruticosa* (L) Harm; A: plants in *in vitro*, B. plants *ex vitro*.

Fig 3. Effect of substrate to adapt and grown of *Polyscias fruticosa*(L) Harm: A. No of test 1-8; B. No of test 9-16

Table 4. Effect of substrate to adapt and grown of *Polyscias fruticosa*(L) Harm

					Survival (%)
No of test	Soil (%)	Sand (%)	husk ash (%)	Coir (%)	
1	100	0	0	0	58.33^{ed}
2	0	100	0	0	51.67^{fg}
3	50	50	0	0	64^{bc}
4	50	0	50	0	74.00^{a}

5	50	0	0	50	63.00^{bc}
6	0	50	50	0	63.33^{bc}
7	0	50	0	50	50.00^{gh}
8	25	25	25	25	64.33^{bc}
9	50	25	25	0	71.33^{a}
10	50	25	0	25	70.67^{a}
11	0	50	25	25	55.67^{ef}
12	75	25	0	0	65.33^{b}
13	75	0	25	0	60.00^{cde}
14	75	0	0	25	70.33^{a}
15	0	75	25	0	52.33^{fg}
16	0	75	0	25	46.33^{h}
CV					3.42
$LSD_{0.01}$					4.69

CONCLUSION

2,4-D at 3mg/l concentration was found to formating callus the highest among all the treatments. The highest shoot length, number of shoots was obtained at MS medium supplemented with 5.0 mg/l BA and 0.1 mg/l NAA at multiplication stage. The highest number of roots and root length was obtained on medium supplemented with 0.5 mg/l NAA at rooting stage. The highest percentage of plant survival was achieved by transplanting of the plantlets to pots containing soil and husk ash at the ratio of 1:1(v/v).

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