Research Article

In vitro multiplication of *Podocarpus macrophyllus* D. Don var. maki

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Abstract

Podocarpus macrophyllus var. maki is evergreen shrub or tree usually. It is drought tolerant; used as specimen, hedge or screen; 'Maki' is a slower growing version of the species, reaching only 20-35 ft. in height, 3-5 ft. in width. Some species of *Podocarpus* are used in systems of traditional medicine for conditions such as fevers, coughs, arthritis, sexually transmitted diseases, and canine distemper. Recently new compounds have been extracted from the leaves of *Podocarpus*. An efficient protocol for multiplication has been developed for this medicinal plant. Regeneration was achieved from nodal explants raised on Murashige and Skoog medium (MS) containing BAP (0.5-3.0mg/l), 3% sucrose and 2.8 gm/l Clarigar along with IAA (0.2mg/l). The regeneration protocol developed during this piece of work provides an important method for micropropagation of this plant *in vitro*.
Keywords: *In vitro, Podocarpusmacrophyllus*, callus, multiplication.

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INTRODUCTION

Podocarpus (from the Greek, podos, meaning "foot", and karpos, meaning "fruit") is a genus of conifers, the most numerous and widely distributed of the podocarp family, Podocarpaceae. The plant is drought tolerant; use as specimen, hedge or screen; 'Maki' is a slower growing version of the species, reaching 20-35 feet in height, 3-5 feet wide. It is evergreen shrub or tree; Branches are numerous, crowded and upright. Leaves are alternate, simple, dark green with lighter midrib, narrow-linear to lanceolate, stiff with an acute tip and obtuse base. Blade is dark green above and pale green below, with entire margins. Male and female cones are in separate plants. Pollen cones are yellowish and elongated; seed light blue, globose-ovoid, fleshy, with red, enlarged stalk. Some species of *Podocarpus* are used in systems of traditional medicine for conditions such as fevers, coughs, arthritis, sexually transmitted diseases, and canine distemper. Recently Podolactone D[1], a new norditerpenedilactone having a methylsulfoxide moiety, was isolated from the leaves of *P.macrophyllus* D. Don var. maki along with known podolactone D (2, S (S)()-podolactone D) (Park *et al*,2003). Secondly some activities like Anti-pigmentation (Kur-Ta Cheng *et al*, 2007), Antimicrobial or antifungal (Abdillahi *et al*, 2008), Ponasterone or Mammary Gland Disease (Albanese *et al*, 2000), Cytotoxic activity (Kimihiko Sato *et al*, 2009) have been reported from *Podocarpus*. Looking towards the importance it was decided to carry out in vitro studies on *P.macrophyllus var.* maki.

MATERIALS AND METHODS

Explants were collected from naturally growing plant in botanical garden, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Shoot tips, Axillary buds, leaves and herbaceous stem were taken as an explant. Explants were rinsed with running tap water followed by treatment of 70% Ethanol for a minute and surface sterilized with 0.3% (w/v) $HgCl_2$ (RFCL Ltd, India) for four - six minutes followed by rinsing with sterile distilled water 4-

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5 times. Sterilized explants were cut with the help of scalpel and cultured on MS-media (Murashige and Skoog 1962).Culture medium was fortified with 0.3% (w/v) clerigar (Himedia Pvt. Ltd., India) as a solidifying agent and growth regulators like BAP, KIN, IAA and IBA were tried at different combinations and concentrations.

Culture Condition

The present research work was carried out under controlled conditions. MS medium was fortified with 3% sucrose. After the addition of phytohormones at different combinations and concentrations the pH was adjusted to 5.8. Medium was sterilized in an autoclave under 15 psi pressures and 121° C temperature for 20 minutes. Sterilized media was transferred into laminar air flow for inoculation. After inoculation culture vessels were transferred into culture room 25± 2°C temperatures with 8/16 hours of photoperiod and 70% relative humidity. Results were recorded after every week and analyzed by five replicated with mean ± SE.

RESULT AND DISCUSSION

During the present studies effect of various growth regulators on multiplication was recorded by taking various combinations and concentrations of plant growth hormones. The MS medium supplemented with BAP (0.5-3.0 mg/l) combined with IAA (0.2 mg/l) was significantly affected on shoot proliferation. Whereas increased concentration of BAP (above 2.0 mg/l) results relevant that, initiation of shoot along with callus. MS-medium incorporated with BAP (0.5-2.0 mg/l) in combination with 2, 4-D (0.2 mg/l) induce callus formation from axillary node and shoot tip explants. Medium supplemented with Kin (0.5-3.0 mg/l) in combination with IAA/2, 4-D (0.2 mg/l) results relevant to MS + BAP + IAA/2,4-D. Data was recorded for percent of callus growth, response of callus, dry weight of callus and number of shoot per culture, summarized in table 1 and 2.

Table 1: Effect of different growth regulators on shoot regeneration from nodal segment

MS + Growth Hormones (mg/L)	Explant responding (%)	Response Callus/Shoot	Number of shoots/explants (mean ± SE)
BAP+IAA			
0.5+0.2	10	CS	1.0±0.16
1.0+0.2	50	CS	2.1±0.15
1.5+0.2	80	S	4.2±0.15
2.0+0.2	90	S	8.6±0.16
2.5+0.2	60	S	3.7±0.14
3.0+0.2	40	CS	2.1±0.15
KIN+IAA			
0.5+0.2	-	-	-
1.0+0.2	-	C	-
1.5+0.2	20	CS	3.2±0.17
2.0+0.2	30	CS	5.8±0.15
2.5+0.2	-	C	-
3.0+0.2	-	C	-

Table 2: Effect of Plant growth regulator on induction of callus

Source of Explants	MS + Growth Hormones (mg/L)	Frequency of induction of callus	Response Callus/Shoot	Dry weight of callus (mean ± SE)
	BAP+2, 4-D			
	0.5+0.2	-	-	-
Leaf, Axillary Shoot	1.0+0.2	+	CS	0.5 ± 0.25
	1.5+0.2	++	C	1.2 ± 0.20
	2.0+0.2	++	C	1.4 ± 0.19
	2.5+0.2	++++	C	1.9 ± 0.14
	3.0+0.2	+++	C	1.5 ± 0.18
	KIN+2, 4-D			
	0.5+0.2	-	-	-
Leaf, Axillary Shoot	1.0+0.2	+	C	0.4 ± 0.27
	1.5+0.2	+	C	0.6 ± 0.23
	2.0+0.2	++	CS	0.7 ± 0.24
	2.5+0.2	++	C	1.2 ± 0.20
	3.0+0.2	++	C	1.5 ± 0.18

*Mean ± standard error, percent response of five replicate, C- Callus induction, and CS- callus mediated shoots

Among all the concentrations of BAP and IAA, concentration use di. e 2.0+0.2mg/l showed better shoot induction. The maximum number of shoots recorded8.6±0.16which was followed by4.2±0.15 with BAP and IAA at 1.5+0.2 mg/l. Callus obtained from leaf as an explant was compact, greenish and cream colored which was inoculated with 0.5–0.2 mg/l BAP in combination with 0.2 mg/1 IAA (Table-1). Growth hormones viz. KIN 0.5 to 3.0 mg/l and IAA 0.2 mg/l along with MS were tested separately during the piece of work. Induction of callus was noticed with KIN and IAA at the level of 0.1-3.0 and 0.2 mg/l respectively. No callus or shoot induction was noticed with KIN and IAA at the level of 0.5+0.2 mg/l. Among all the concentrations of KIN and IAA concentrations used 2.0+0.2mg/l showed better regeneration of shoots along with callus. Maximum number of multiple shoots recorded were5.8±0.15. Minimum no. of shoots were recorded 3.2±0.17 with KIN and IAA at 1.5+0.2 mg/l. All the possible combinations of KIN and IAA used in the present investigation showed better callus induction (Table-1). The *in vitro* regeneration protocol has not been recorded previously with any species of *Podocarpus*. In situ fertility decline and provenance differences in the East African Yellow Wood (*Podocarpusfalcatus*) measured through in vitro seed germination were reported by Legesse Negash in 2003. In vitro methods for the rapid germination of seeds of *Podocarpusfalcatus* was also reported by Legesse Negash (1992). There were significant differences in regeneration frequencies, number of shoots/culture within four weeks. Similar findings for multiplication of various plant species were achieved by several workers from axillary shoot explants in *Terminaliaarjuna* (Arumugam and Gopinath, 2011) and Ananas*comosus* (Amin *et al*., 2005). During present studies leaf, nodal segments as explants were tried but only nodal segments responded well with BAP and IAA in MS media ranging from 3.0 to 0.5 mg/l. The combination of BAP and IAA promotes multiplication rate satisfactorily. Rhizogenesis experiments yet to be conducted. Highest frequency of callus induction was obtained on MS-media supplemented with BAP and2, 4-D at 2.5+0.2 mg/l , whereas low frequency of callus induction was observed on MS-media along with KIN and 2,4-D. Callus obtained from leaf axillary shoot was green and compact (Table-2).

Figure a: Shoots proliferation from nodal segment as an explants

Figure b: Multiple shoots on MS-media supplemented with BAP+IA**A**

CONCLUSION

Looking towards the importance of *Podocarpusin vitro* studies will be useful for multiplication. The present results represents a standardized protocol for efficient plant regeneration system through direct organogenesis from nodal explants. The most potent PGRs for multiplication were BAP and IAA as compare to KIN and 2, 4-D, while for callus induction BAP and 2,4-D were more suitable.

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