September 2010 ISSN : 0974 - 7435 Volume 4 Issue 3

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 4(3), 2010 [140-144]

In vitro seed culture and induction of enhanced axillary branching in *Pterocarpus marsupium R.*: An endangered leguminous tree

P.Deenathayalan, S.Vijayakumar, K.Kalimuthu*

P.G. & Research Department of Botany, Government Arts College (Autonomous), Coimbatore - 641 018, Tamil Nadu, (INDIA)

E-mail : k_kalimuthu@rediffmail.com

Received: 5th April, 2010 ; Accepted: 15th April, 2010

ABSTRACT

Pterocarpus marsupium. Roxb. has great commercial value as a timber tree in India, An efficient protocol for *in vitro* seed culture and induction of axillary branching has been established. Maximum seed germination (75% to 85%) occurred in control culture (Murashige & Skoog). The result of interest in seed culture was multiple shoot formation and pronounced axillary branches on MS medium supplemented with BAP + NAA (1.5mg/l + 0.3mg/l) and BAP + KIN (1.5mg/l + 0.2mg/l). Shoot proliferation and relatively more number of axillary branches obtained with MS medium containing BAP + KIN + IAA (0.5 + 0.2 + 0.3mg /l) within six weeks from cotyledonary nodal explant. The best callus proliferation and more number of shoot formations from cotyledon explants were observed in the medium containing BAP + KIN + IAA (1.0 + 0.2 + 0.3mg /l). About 85% of shoots were rooted on MS medium with 0.5mg/l of IBA. The well developed plants were hardened and transferred to a mist chamber in a shade house with 84% survival frequency.

KEYWORDS

P.marsupium;
Regeneration;
Bijasal;
Seed culture;
Endangered.

INTRODUCTION

Pterocarpus marsupium. Roxb. (Fabaceae), commonly known as Bijasal, is one of the most important multipurpose forest tree legumes of India, valued greatly for its excellent timber. In addition, the trees are valued for its pharmaceutical properties as the gum kino obtained from the tree is a powerful astringent used for treatments of dysentery, diarrhea, fever, and toothache. An aqueous infusion of the wood is said to be of use in diabetes, and water stored in the vessel made of the wood is reputed to have antidiabetic properties[1]. Two important phenolic constituents, marsupsin and pterostilbene, isolated from the heartwood of *P.marsupium* are reported to possess antihyperglycemic activity[2]. The aqueous extract of stem bark was found to reduce the blood glucose level in alloxan-induced diabetic rats[3].

This species is fast disappearing, though its inherent quality seems to encourage reforestation of these trees. The pod is the only natural propagating material, but the pod germination in field is very poor[4]. It is also known that the regeneration rate of leguminous trees in natural habitats is quite low[5]. Generally the seeds of

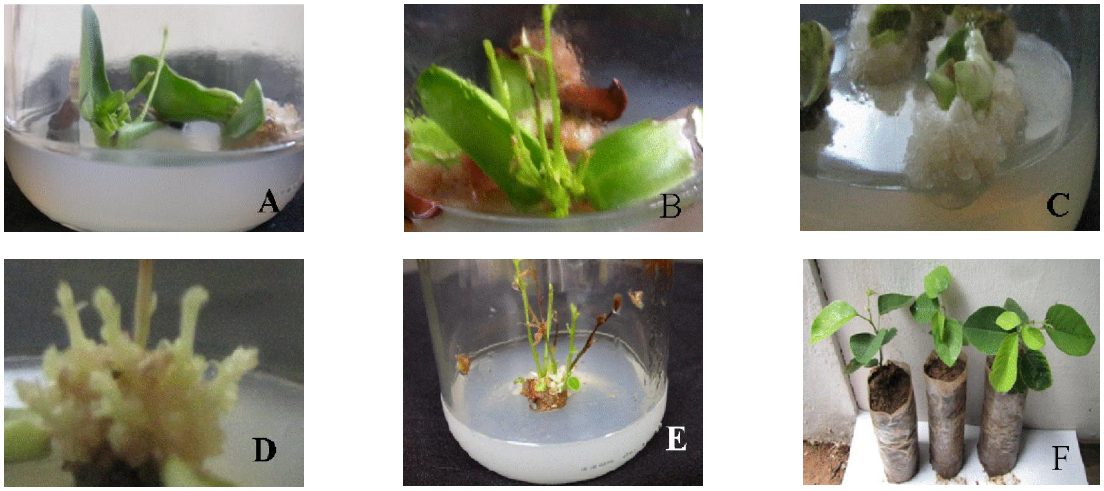

Figure 1 : A & B - Seed germination with multiple shoot formation. C - Callus induction from cotyledonary leaf explant. D - Multiple shoots formation from callus. E - Multiple shoots induction from nodal explant. F - Hardening

the most Leguminous tree species are dormant due to impermeability of fibrous seed coat. Poor pod set, hard fruit coat, less germinability coupled with poor seed viability is responsible for its diminishing population size. The indiscriminate exploitation for its timber and medicinal values of this species together with delay and low percentage of germination has resulted in decrease in size of its natural strand, which ultimately led to its inclusion in the list of depleted plant species[4,6,7].

It is essential for the conservation of *P.marsupium*, to encourage the *ex situ* plantations, which require large-scale planting materials. In view of the problems of conventional propagation and high demand of planting material, the large-scale multiplication of this tree species can only be met efficiently and economically, in a short span of time, by *in vitro* propagation. However, sufficient information has not been available on *in vitro* propagation of *Pterocarpus* species and *in vitro* response of various culture media. Therefore, the present investigation has been carried out to ascertain the most appropriate basal culture media and growth hormones for *in vitro* regeneration of *P.marsupium*.

MATERIALS AND METHODS

Pods of *P.marsupium* were obtained through the courtesy of Tamil Nadu Forest Department, Research division, Coimbatore. After removing the wings and outer covering the pods were treated with 40 percent hydrochloric acid for 10 min. Before excising seeds, the pods were washed in running tap water and soaked for 24 h in the same[4]. Subsequently, the seeds were treated with mild detergent solution followed by 70 percent alcohol for 10 min, before sterilizing with 0.12 percent (w/v) mercuric chloride solution for 7 min. After washing 4-5 times in sterile double distilled water and the seeds were implanted aseptically on MS medium[8] containing 3% sucrose and gelled with 0.8% agar (Himedia) with different concentrations of growth regulators.

The cotyledons, nodal segments, cotyledonary nodes and shoot tips derived form 21-day-old aseptic seedlings were excised and used to initiate culture. The MS medium was variously supplemented with cytokinins (BAP, KIN) or auxins (IAA, NAA) either singly or in combinations. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20 min. All cultures were incubated at 25±2°C in a photoperiod of 16 h for a day under fluorescent light (about 3000lux) and with 60-65% relative humidity.

In vitro differentiated shoots measuring 4-5cm were excised and subjected to *in vitro* rooting. MS medium supplemented with various auxins (IAA, IBA, NAA) were used. All experiments were repeated thrice and three replicates per treatment were taken. The data were analyzed using new Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Germination of seeds began after four days of inoculation and maximum germination attained within

FULL PAPER

TABLE 1 : *In vitro* seed germination of *Pterocarpus marsupium*

Medium	Growth regulator			% of seed germination	No. of shoots per seed
	BAP	KIN	NAA		
MS	0.5	-	-	60-70	1
	1.0	-	-	55-60	1
	1.5	-	-	55-60	1
	2.0	-	-	40-50	1+callus
	2.5	-	-	40-50	1
	3.0	-	-	30-40	1
	0.5	-	0.3	65-70	1[a]
	1.0	-	0.3	60-70	1[a]
	1.5	-	0.3	40-50	2-3[b]
	2.0	-	0.3	40-50	1-2[a]
	2.5	-	0.3	35-40	1[a]
	3.0	-	0.3	30-40	1[a]
	0.5	0.2	-	90-100	1-2[a]
	1.0	0.2	-	90-95	4-5[b]
	1.5	0.2	-	60-65	2-3[c]
	2.0	0.2	-	50-60	1[a]
	2.5	0.2	-	40-50	1[a]
	3.0	0.2	-	10-15	1+callus
	control			75-80	1

Values are mean three replicates, Means followed by common superscript letter are not significant at 5% level, By using DMRT

TABLE 2 : Effect of growth regulators on regeneration from cotyledonary nodal explant of *Pterocarpus marsupium*

Medium	BAP	KIN	IAA	No. of multiple shoots
MS	0.5	-	-	1
	1.0	-	-	1
	1.5	-	-	1
	2.0	-	-	1
	2.5	-	-	1
	0.5	0.2	-	1
	1.0	0.2	-	1
	1.5	0.2	-	1
	2.0	0.2	-	1
	2.5	0.2	-	1
	0.5	0.2	0.3	15-20[a]
	1.0	0.2	0.3	5-6[b]
	1.5	0.2	0.3	2[c]
	2.0	0.2	0.3	2[c]
	2.5	0.2	0.3	2[c]
	0.5	-	0.3	1
	1.0	-	0.3	1
	1.5	-	0.3	1
	2.0	-	0.3	1
	2.5	-	0.3	1
	control			-

Values are mean three replicates, Means followed by common superscript letter are not significant at 5% level, By using DMRT

twenty five days. 75-85% of seeds germinated in control culture (MS). The seedlings possessed only one healthy shoot having broad leaves when the seeds were inoculated as such.

Germination percentage and healthy seedlings increased at 0.5 mg/l BAP+0.2mg/l KIN (TABLE 1). The result of interest in seed cultures was multiple shoot formation and pronounced axillary branches (Figure 1B). The number of axillary branches produced was quite similar on medium with BAP + NAA (1.5mg/l + 0.3mg/l) and BAP + KIN (1.5mg/l + 0.2mg/l), however shoot bud proliferation was totally suppressed at higher concentration. These prolific axillary branching and multiplication of shoots during seed culture occurred without intervention of callus phase. Anuradha and Pullaiah[9] reported prolific shoot formation in *P.marsupium* and *P.santalinus* on B5 medium containing BAP. Malik et al.[10] have also recorded high frequency of direct shoot morphogenesis in seed cultures of Lathyrus on medium with thiadiazuron. Seed cultures have advantages which offer a simple system with one manipulation without wounding and isolation of explant process[9,10].

Cotyeledonary Node showed excellent response among the different explants used. These explants inoculated on MS medium responded to various concentrations of BAP, KIN and IAA (TABLE 2). The recorded results showed that best shoot proliferation and relatively more number of axillary branches obtained with medium containing BAP + KIN + IAA (0.5 + 0.2 + 0.3mg /l) within six weeks. (Figure 1D). MS with BAP (1.0mg/l), KIN (0.2mg/l) and IAA (0.3mg/l) was also effective but not at the level of previous combination. Multiple shoots obtained were divided into 2-3 clumps for further proliferation and to increase the number of shoots. Sub culturing was done every 3-4 weeks on to fresh medium regularly. Increasing the concentration of BAP resulted, decrease in the rate of shoot regeneration ability. This is supported by the results reported in *Albizia chinensis*[11] and *P.santalinus*[12].

Only elongation of single shoot took place and no significant response was observed when BAP was used

TABLE 3 : Effect of growth regulators on regeneration from leaf explants of *Pterocarpus marsupium*

Medium	BAP	KIN	IAA	NAA	Callus	Shoot
	0.5	-	-	-	+	-
	1.0	-	-	-	++	-
	1.5	-	-	-	+++	1
	2.0	-	-	-	+++	1
	2.5	-	-	-	+++	1
	0.5	-	-	0.5	+	-
	1.0	-	-	0.5	++	-
MS	1.5	-	-	0.5	++	-
	2.0	-	-	0.5	+++	1
	2.5	-	-	0.5	++	-
	0.5	0.2	0.3	-	+	2-3[a]
	1.0	0.2	0.3	-	++	6-7[b]
	1.5	0.2	0.3	-	++	3-4[c]
	2.0	0.2	0.3	-	++	2-3[da]
	2.5	0.2	0.3	-	++	1-2[ea]

Values are mean three replicates, Means followed by common superscript letter are not significant at 5% level, By using DMRT

as the sole cytokinins (0.5-1.5mg/l). BAP induced shoot proliferation from CN has also been reported in *Dalbergia sissoo*[13], *Albizia chinensis*[11] and *P.marsupium*[14,15]. Superiority of BA for induced shoot multiplication in Pterocarpus species[9,16,17] has also been reported earlier.

The addition of KIN and IAA with BA significantly increased the frequency of shoot formation compared to BA alone. This is supported by the results reported in *Wrightia tintoria*[18], *Acacia catechu*[19] and *P.marsupium*[14]. Where maximum number of shoot buds were initiated in the BA and NAA combination.

For induction of callus, cotyledon explant excised from aseptic seed germination and cultured on MS medium containing various concentrations of BAP, KIN, IAA and NAA (TABLE 3). Profused callus formation were observed and recorded in the table. The recorded result showed that the best callus proliferation and more number of shoot formation were observed in the medium containing BAP + KIN + IAA (1.0 + 0.2 + 0.3mg/l) (Figure 1C). Healthy and sturdy shoots (5-6cm long) were transferred to rooting medium containing different concentration of MS and auxins.

After 3-4 weeks of incubation, about 85% of shoots were rooted on MS medium with 0.5mg/l of IBA. The incorporation of an auxin in the medium generally promotes rooting. In the present study also auxin alone in the MS medium was found to be effective for rooting. This is in support with the result reported in *P.santalinus* and *P.marsupium*[9].

About eight to ten true to type plantlets of *P.marsupium* were raised *in vitro* from single cotyledonary node explant, transferred to net pot for acclimatization. The maximum survival rate of *P.marsupium* (84%) was obtained in the potting medium containing decomposed coir waste + perlite + compost (Hardening media) followed by Vermiculate + sand + Forest liter (74%). The plantlets establishment was higher in the hardening media. Red soil in combination with sand (1:1) did not improve the plant growth. After acclimatization, the plants were transferred to secondary hardening (Figure 1E).

Our results indicate the direct and indirect regeneration of multiple shoots from cotyledonary node and leaf explants of this endangered legume tree. The present investigation is therefore, of immense scientific utility which presents a dependable system for rapid multiplication in this species.

ACKNOWLEDGEMENT

The author is thankful to the University Grants Commission (UGC), Government of India, New Delhi for the research grant.

REFERENCES

[1] Anonymous, 'The Wealth of India', A Dictionary of Indian Raw Materials and Industrial Products, New Delhi: Publication and Information Directorate, CSIR, **8**, 302-305 **(2003)**.

[2] M.Manickam, M.Ramanathan, Farboodniay A.Jahromi, J.P.N.Chausouria, A.B.Ray; J.Nat. Prod., **60**, 609-610 **(1997)**.

[3] V.Vats, J.K.Grover, S.S.Rathi; J.Ethnopharmacol, **79**, 95-100 **(2002)**.

[4] K.Kalimuthu, K.K.Lakshmanan; Indian Journal of Forestry, **18**, 104-106 **(1995)**.

[5] A.Dewan, K.Nanda, S.C.Gupta; Plant Cell Rep., **12**, 18-21 **(1992)**.

[6] A.B.Chaudhuri, D.D.Sarkar; 'Biodiversity Endangered', India's Threatened Wildlife and Medicinal Plants, Jodhpur: Scientific Puplishers, 169-172

(2002).

[7] S.N.Raj, V.R.Chitrapu, Surendranathan Asari; 'The Key Role of Forestry Sector in Conserving India's Medicinal Plants', Conservation Science Division Ministry of Environment & Forests, Government of India, New Delhi, **(1999)**.

[8] T.Murashige, F.A.Skoog; Physiol.Plant, **15**, 473-487 **(1962)**.

[9] M.Anuradha, T.Pullaiah; Phytomorphology, **49**, 157-163 **(1999)**.

[10] K.A.Malik, S.T.Ali-Khan, P.K.Saxena; Ann.Bot., **72**, 629 **(1993)**.

[11] R.K.Sinha, K.Majumdar, S.Sinha; Dev.Biol.Plant, **36**, 370-373 **(2000)**.

[12] V.Rajeswari, Kailash Paliwal; Indian Journal of Biotechnology, **7**, 541-546 **(2008)**.

[13] C.Pradhan, S.Kar, S.Patnaik, P.K.Chand; Plant Cell Rep., **18**, 122-126 **(1998)**.

[14] K.Kalimuthu, K.K.Lakshmanan; Indian J.of Forestry, **17**, 192-195 **(1994)**.

[15] M.Anis, Mohid Kashif Husain, Anwar Shahzad; Curr.Sci., **88**, 8610-863 **(2005)**.

[16] Sarita Patri, S.P.Bhatnagar, S.S.Bhojwani; Phytomorphology, **38**, 41-45 **(1988)**.

[17] M.K.Husain, M.Anis, A.Shahzad; Dev.Biol.Plant, **43**, 59-64 **(2007)**.

[18] S.D.Purohit, G.Kukda, P.Sharma, K.Tak; Plant Sci., **103**, 67-72 **(1994)**.

[19] K.Kaur, B.Verma, U.Kant; Plant Cell Rep., **17**, 427-429 **(1998)**.