Available Online at http://www.recentscientific.com

ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 5, Issue, 11, pp.19-1994, November, 2014

International Journal of Recent Scientific Research

RESEARCH ARTICLE

EFFECT OF DIFFERENT PLANT GROWTH REGULATORS ON CALLUS INDUCTION IN CAESALPINIA SAPPAN L. A MEDICINAL PLANT

Arunkumar Naik B, Fathima Nazneen H and Chandramati Shankar P*

Department of Biotechnology, Yogi Vemana University, Kadapa, Andhra Pradesh, India

ARTICLE INFO

Article History:

Received 2nd, October, 2014
Received in revised form 10th, October, 2014
Accepted 4th, November, 2014
Published online 28th, November, 2014

Key words:

callus, Auxins, cytokinins, Caesalpinia sappan L.

ABSTRACT

Caesalpinia sappan L belongs to family ***Caesalpiniaceae,*** a plant with high medicinal values. The present study evaluated the effect of different combinations of plant growth regulators on callus induction using different vegetative parts in different plant tissue culture media. Maximum callus induction response was observed in nodal explants (93.3%) in MS medium supplemented with NAA (2mg/L), BAP (2mg/L) whereas in medium supplemented with IAA (1mg/L) and KIN (2mg/L) the response was 78%. The callus response was 41% in MS medium supplemented with 2, 4-D (2mg/L) and Kinetin (0.5mg/L) and the callus response was very less(26%) in MS medium with 2,4-D(0.5mg/L). The callus were white compact or friable in nature. A combination of Auxins and cytokinins was found to be effective in induction of callus in Caesalpinia sappan L, when compared to using individual plant growth regulators. In this study callus induction and proliferation protocol has been established.

INTRODUCTION

Caesalpinia sappan L. or East Indian red wood tree (Family *Caesalpiniaceae).* is a medicinal plant having a lot of pharmaceutical and commercial importance. The heartwood of *Caesalpinia sappan* L. is traditionally used in India (Ayurveda), for wound healing, treatment of ulcers, diarrhea, epilepsy, diabetes, etc. Flavonoids (Namikoshi *et al.* 1987, a, b, c) and phenolic (Fuke *et al.,* 1985;Saitoh *et al.,* 1986) such as 4-*O*-methylsappanol, protosappanin A (Nagai *et al.,* 1986), protosappanin (Nagai *et al.,* 1986), protosappanin E, brazilin (Kim *et al.,* 1997), brazilein, caesalpin J (Miyahara *et al.,* 1986), tri terpenoid and steroid (such as camp sterol, stigma sterol, sitosterol)were isolated from the wood**.** The hepatoprotection (Moon *et al.,* 1992), immunomodulation (Choi *et al.,* 1997), hypoglycemia (Kim *et al.,* 1995; Moon *et al.,* 1988), anticomplementary (Oh *et al.,* 1998), anticonvulsant (Baek *et al.,* 2000), anti-inflammatory, antibacterial (Nirajan Reddy *et al.,* 2003), anti oxidation (Badami *et al.,* 2003; Yingming *et al.,* 2004), and other biological activities of sappan have been reported. The tree is also a source of the commercial redwood or Brazil wood. It is used for inlaying work, cabinet making, violin bows and for walking sticks. The stem produces a gum and heartwood yields a valuable red crystalline dye, brazilin, used on cotton, silk and wool fabrics. Bark and pods yield similar dyes; pods contain ca. 40% tannin used for production of light leather goods. Roots give a yellow dye and leaves contain a pleasant smelling volatile oil.

The increased appeal of natural products for medicinal purposes coupled with the low product yields and supply concerns of plant harvesting has renewed interest in large-scale plant cell culture technology (Hall RD 2000). Intact plant based production system have the problem that in many cases the natural product is present at low levels or accumulate only in a specific tissue and at specific vegetative growth stage or upon certain growth or environmental conditions. Furthermore collecting material from a sustainable way; can lead to over collection of endangered species as well as to habitat destruction. The problem with whole plant extraction is that pharmaceutical industry needs homogenous samples with more or less constant levels of active ingredient which cannot be ensured from random wild sampling. Introduction of cell suspension culture offers the opportunity to optimize the yield and achieve a uniform, high quality product. This system could function as an easy, reliable, and well-defined method for the production of secondary metabolite. There are no reports about caulogenesis studies in *Caesalpinia sappan* L., Here we report a established protocol for callus induction using different vegetative explants .The objective of this work was to establish a suitable protocol for callus proliferation for further secondary metabolite production studies.

MATERIALS AND METHODS

Plant material

Explants (leaf, nodal and intermodal segments) were collected from 5 year old plants grown from seeds obtained from a commercial supplier, in the Biotechnology department greenhouse.

Surface Sterilization

All explants were cut into 0.5-1 cm in size, and washed thoroughly under running tap water. Further sterilization was carried out under the Laminar airflow then the explants were pre treated with chilled antioxidants and they were surface sterilized in a sterile Petri dish using 0.1% - 0.5% Mercuric chloride ($HgCl_2$) for 3-5minutes with continuous shaking.

* Corresponding author: **Chandramati Shankar P**
Department of Biotechnology, Yogi Vemana University, Kadapa, Andhra Pradesh, India

Table 1 Effect of different concentrations of Auxins on Nodal explants of ***Caesalpinia sappan L.***

"MS media"	2,4-D	IAA	NAA	IBA	Percentage ± SE
	0.5				26±0.667
	1				7±0.333
	1.5				11±0.000
	2				0±0.000
		0.5			0±0.000
		1			0±0.000
		1.5			0±0.000
		2			0±0.000
			0.5		11±0.000
			1		4±0.333
			1.5		4±0.333
			2		0±0.000
				0.5	0±0.000
				1	0±0.000
				1.5	0±0.000
				2	0±0.000

The values represent the means (Mean±SE). Mean values within column followed by the significantly different by Duncan's multiple range test (P>0.05)

Table 2 Effect of 2, 4-D and Kinetin on leaf explants of ***Caesalpinia sappan L.*** in MS media

2,4-D mg/L	Kinetin mg/L			
	0.5	1	1.5	2
0.1	26±1.202	0±0.000	11±0.577	26±1.202
0.2	26±0.882	4±0.333	15±0.882	18±0.333
0.3	4±0.333	0±0.000	0±0.000	4±0.333
0.4	11±0.577	0±0.000	22±0.000	11±0.577
0.5	30±0.882	15±0.333	0±0.000	19±1.202
1	26±0.882	11±1.000	0±0.000	11±0.577
1.5	23±0.882	22±2.000	0±0.000	26±0.882
2	22±1.528	4±0.333	0±0.000	11±0.000

The values represent the means (Mean±SE). Mean values within column followed by the significantly different by Duncan's multiple range test (P>0.05)

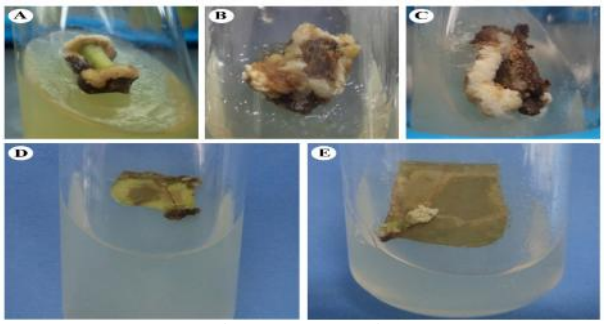

Figure1 Callus induction from nodal & Leaf explants in MS medium supplemented with Auxins: A-B. 0.5mg/L, 2, 4-D (26%) A. Callus induction, B. Proliferation of callus, C. Woody Plant Medium with 0.5mg/L 2, 4-D (74%), D-E. Callus induction in leaf explants D. Swelling of leaf explants, E. Proliferation of callus,

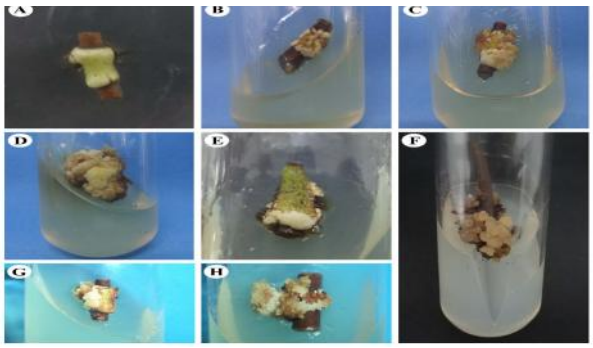

Figure 2 Callus induction from nodal explants in MS medium supplemented with cytokinins in combination with Auxins: **A-D.** Callus response in 2mg/L NAA and 2mg/L BAP (93%) in MS medium **A.** Swollen nodal explants, **B.** initiation of callus after 10days, **C.** Callus after 15days, **D.** Proliferation of callus, **E-F.** callus response in 0.5mg/L 2,4-D and 0.5mg/L Kinetin (41%), **E**· Initiation of callus, **F.** Proliferation of callus, **G-H.** Callus response in NAA (2mg/L) and BAP (0.5mg/L), **G.** Initiation of callus, H. Proliferation of callus

Fig 3 ***Caesalpinia sappan L.*** plant growing in Yogi Vemana University Net house facility

They were repeatedly washed with sterile distilled. Water and then they were inoculated vertically or horizontally on the surface of the medium. The inoculated culture tubes were kept in culture room under controlled conditions.

Callus Induction

Different explants like leaf segments, nodal segments and internodal segments (0.5-1.0 cm) were excised from 5 yr old plant growing in the greenhouse. Explants were cultured on different plant tissue culture media containing different concentrations and combinations of plant growth regulators following standard tissue culture procedures (Table 1,2&3).

The P^H of the medium was adjusted to 5.7± 0.1 with 0.1N NaOH or 0.1N Hcl. Following addition of 0.8% agar. The media was then autoclaved at 121^{o}C, 15psi pressure for 15mins and allowed to cool and solidify under laminar air flow hood. For each treatment and explants the experiment was repeated 3 times. Observations were visually taken and the results were quantified on the basis of the percentage of explants showing callus.

Culture conditions

The cultures were maintained at a temperature of 28±2^{o}C. at a 16-h light/8-h dark cycle

RESULTS

Callus initiation was very poor in MS media containing Auxins (2, 4-D, IAA, NAA, and IBA) (0%) IAA and IBA (2mg/L), to maximum 26%2,4-D (0.5mg/L) (Fig 1, A-B) even after four weeks of culture period (Table 1). Callus response showed an improvement 41% (Fig 2, F) in medium having Auxin (2, 4-D 0.5mg/L) and kinetin (0.5mg/L). Among the different combinations of Auxins and cytokinins tried (Table 3) MS Medium with NAA(2mg/L) and BAP (2mg/L) gave best response i.e. 93% (Fig 2, D) followed by 78% (Fig 2, H) response in medium having 2 mg/L NAA and 0.5mg/L BAP. It was observed that with increase in concentration of cytokinins the callus induction increased. It was observed that incorporation of cytokinins in medium having Auxins improved the callusing response (Table 3). The callus obtained was white, creamy in color and friable in nature. Initially the explants remained green and healthy for 20 days, it was followed by browning. The explants were sub cultured after 2 weeks to fresh medium. The explants swelled after 10 ±2 days in culture medium, followed by slight bursting of tissue, from where white callus (Fig ; 2 A-D) emerged and grew in size. The explants were not very responsive on B5 Medium containing Auxins and cytokinins. Whereas the overall response of explants on WPM with Auxin (2, 4-D 0.5mg/L) was better74%, similar to Auxin and kinetin combinations but

Table 3 Effect of different combinations Of Auxins and Cytokinins on Nodal explants of Caesalpinia sappan L. in MS Media

2,4-D	IAA	NAA	IBA	Kin	BAP	Percentage ± SE
0.5				0.5		41±1.202
1				0.5		33±1.155
1.5				0.5		15±0.882
2				0.5		7±0.333
0.5				2		67±1.155
1				2		52±0.882
1.5				2		19±0.667
2				2		7±0.333
0.5					0.5	7±0.333
1					0.5	11±0
1.5					0.5	41±1.202
2					0.5	15±0.333
0.5					2	0±0
1					2	4±0.333
1.5					2	7±0.667
	0.5			0.5		7±0.333
	1			0.5		33±1.155
	1.5			0.5		59±1.202
	2			0.5		22±0.577
	0.5			2		19±1.202
	1			2		37±0.882
	1.5			2		30±1.202
	2			2		15±0.882
	0.5				0.5	15±0.882
	1				0.5	44±1.155
	1.5				0.5	33±1.155
	2				0.5	63±1.453
	0.5				2	37±1.202
	1				2	44±0.577
	1.5				2	22±0.577
	2				2	11±0.577
		0.5		0.5		22±1
		1		0.5		19±0.667
		1.5		0.5		37±0.667
		2		0.5		22±0.577
		0.5		2		26±1.333
		1		2		26±0.882
		1.5		2		22±0.577
		2		2		15±0.333
		0.5			0.5	22±0.577
		1			0.5	44±2.517
		1.5			0.5	56±1.155
		2			0.5	78±0.577
		0.5			2	30±1.202
		1			2	63±0.882
		1.5			2	78±0.577
		2			2	93±0.333
			0.5	0.5		7±0.333
			1	0.5		15±0.333
			1.5	0.5		30±0.882
			2	0.5		11±0.577
			0.5	2		33±1.155
			1	2		78±1.155
			1.5	2		22±0.577
			2	2		30±1.202
			0.5		0.5	11±0.577
			1		0.5	0±0
			1.5		0.5	30±0.882
			2		0.5	11±0.577
			0.5		2	52±0.882
			1		2	48±1.202
			1.5		2	7±0.667
			2		2	26±0.882

The values represent the means (Mean±SE). Mean values within column followed by the significantly different by Duncan's multiple range test (P>0.05)

explants browned with no further growth. The leaf explants (Table 2, Fig 1 D-E) showed 30% response on MS medium with 2, 4-D (0.5mg/L) and Kin (0.5mg/L).

Callus initiated from the cut ends and centre of the leaf, the callus was white and friable. Among all the explants tried nodal and leaf explants gave significant response but the best response was observed in Nodal explants.

DISCUSSION

The majority of reports so far communicated; report use of intact plant parts to study the pharmacological aspects of secondary product. Lot of studies have been carried out on Photochemistry, biological, pharmaceutical and medicinal properties in *Caesalpinia sappan* L. using the various parts of the plant like heart wood , bark leaf etc. There are no published reports found on research being carried out using biotechnological methods in *Caesalpinia sappan* L. This is the first report being made on studies of callus induction using different vegetative parts with different plant growth regulators in plant tissue culture medium. Development of an efficient callus induction protocol is the first step towards the further study of secondary metabolite production in cell suspension culture which is the future application of this work.

Acknowledgements

The first author is grateful to UGC – Govt. of India for providing Rajiv Gandhi National Fellowship (RGNF). The authors acknowledge Dr. Satish Mohabe, Research Associate Department of Botany helping in photography work. The corresponding author is thankful to Ministry of Industries and Commerce, A.P for the Agri-Science project established at Yogi Vemana University, which financially supported this work.

References

Badami.S.Moorkoth.S.Rai.S.R. Kannan.E...and Bhoojraj.S, (2003). Antioxidant activity of *Caesalpinnia sappan* heart wood. *Biological and Pharmaceutical bulletin*,26.153 1534-1537

Baek. N. I., Jeon, S.G., Ahn. E. M., Hahn .J.T. Bahn , (2000), Anticonvulsant compounds from the wood of *Caesalpinnia sappan* L. archives of *Pharmacal Research*, 23,344-348.

Choi.S.Y.Yang.K.M.Jeon.S.D.Kim J.H. et al., (1997).Brazilin modulates immune function mainly by augmenting T cell activity in halothane administered mice. *Planta medica*. 63.405-408.

Fuke C., Yamahara J., et al. (1985).Two aromatic compounds related to brazilin from *Caesalpinnia sappan* L.*Phytochemistry* 24.2403-2406.

Hall RD (2000) Plant cell culture initiation. Practical tips. Mol. Biotechnol 16:161–173

Kim et al., (1997).NMR assignment of brazilin. *Phytochemistry*, 46,177-178.

Kim You Me et al., (1995); Brazilin stimulates the glucose transport in 3t3-L1 cells.*Planta Medica* 61.297-301.

Miyahara et al., (1986), The X-Ray analysis of *Caesalpinia J* from sappan lignum. *Chemical and Pharmaceutical Bulletin*.34,4166-4169

Moon C.K., Chung J.H.,et al.,(1988), Effects of brazilin on erythrocytes deformability and its related biochemical factors in strptozotocin induced diabetic rats. Archives of *Pharmacal Research*.11. 149-154.

Moon C.K., Park.K.S.Kim, S.G.Won, H.S. and Chung J.H.,(1992), Brazilin protects cultured rat hepatocytes from trichlorobromomethane-induced toxicity. *Drug and Chemical Toxicology* 15. 81-91.

Nagai M., Nagumo.S. Lee.S.M.Eguchi I. and Kkawai.K.I. (1986), Protosappanin A, A biphenyl compound from sappan lignum *Chemical and Pharmaceutical Bulletin*. 34.1-6.

Nagai. M and Nagumo.S. (1986), Protosappanin B, a new dibenzoxocin derivative from sappan lignum (*Caesalpinnia sappan* L.) *Heterocycles*.24.601-606.

Namikoshi M.,Nakata H.Yamada h.Nagai M.and Saitoh, T. (1987); Homoisoflavanoids and related compounds: II .Isolation and absolute configurations of 3,4-dihydroylated homoisoflavans and brazilin from *Caesalpinnia sappan* L. *Chemical and Pharmaceutical Bulletin* 35.3615-3619.

Namikoshi, M. Nakata H. and Saitoh T., (1987) Homoisoflavanoids and related compounds V. A novel dibenzoxocin derivative from *Caesalpinnia sappan* L. *Chemical and Pharmaceutical Bulletin* 35.3615-3619.

Namikoshi.M, and Saitoh,T.(1987); Homoisoflavanoids and related compounds IV.Absolute configuration of Homoisoflavanoids from *Caesalpinnia sappan* L. *Chemical and Pharmaceutical Bulletin*.35 3597-3602.

Nirajan Reddy.V.I.Ravikanth.V .Jhansi Lakshmi et. al (2003),Inhibitory activity of homoisoflavanoids from *Caesalpinia sappan* against Beanveria bassiana *Fitoterpia*.74,600-602.

Oh et al., (1998), Anticomplemantary activity of constituents from the heart wood of *Caesalpinnia sappan*. pp l-87 *Planta medica* 64.456-458.

Saitoh et al., (1986)3-Benzylchroman derivatives related to brazilin from sappan lignum *Chemical and Pharmaceutical Bulletin*. 34.2506-2511.

Yingming et al., (2004),Antioxidant activities of several Chinese medicine herbs. *Food chemistry* 88,347-350.

****