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EFFECT OF DIFFERENT PHYTOHORMONES IN CULTURE OF EXPLANTS OF SPONDIAS MANGIFERA (L.)

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ABSTRACT

Spondias mangifera L. (Family: Anacardiaceae) commonly known as *Amra* is an economically important medicinal plant and found all over India. Explants of *S. mangifera* L. were cultured on MS basal media to obtain callus and plantlets. MS media supplemented with different growth regulators like 2, 4-D, IBA, IAA, NAA, BAP, Kinten Zeatin either alone or in combination. Cultures were kept on 25± 5° C and 12 hrs photoperiod. White callus was observed on different concentration of phytohormones. Only callusing and no organogenesis was seen on medium containing 2, 4-D. The best result was observed at 2.5 mgL^{-1} concentration of auxins. Cytokinins also induce callus formation and shoot differentiation. BAP was found to be the most effective cytokinin for shoot differentiation in combination with auxin. Most suitable medium noted for shoot formation from explants (shoot tips or leaves) was MS media supplemented with BAP (2 mgL^{-1}) and IAA (1 mgL^{-1}). Other effective combination for shoot differentiation was with BAP (3 mgL^{-1}) and IAA (2 mgL^{-1}) and other was MS with BAP (1 mgL^{-1}) and NAA (0.5 mgL^{-1}). Shoot developed was rooted the best in MS media with 0.5 mgL^{-1} NAA.

Keywords – *Spondias mangifera* L., MS medium, Explant, Auxin, Cytokinin, Callus.

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Introduction

In recent years, *in vitro* approaches have been used as an efficient tool for micropropagation of trees and it proved that tissue culture technology is suitable for large-scale propagation of trees in short time (Pena and Seguin, 2001). Propagation of woody trees through tissue culture has many advantages over conventional propagation method like fast multiplication of the important genotypes, quick release of improved cultivars, production of disease-free plants, season-independent production of plants, germplasm conservation and facilitating their easy exchange (Asthana *et al*., 2011).

Spondias mangifera L. (Family: Anacardiaceae), locally known as *Amra* or *Jungli-Aam*, is an aromatic, deciduous tree and found all over India. It's every parts are full of medicinal use. It is used in the treatment of dysentery, diarrhoea and vomiting. Fruits barks, leaves and roots are the most important parts of this plant. The roots are useful in regulating menstruation. Leaves are used in dysentery. The bark is aromatic, astringent and refrigerant and is administered in dysentery, diarrhoea, vomiting and muscular rheumatism. The ripe fruits are sweet astringent, cooling, and tonic, constipating. It is used in bilious dyspepsia, diarrhoea and general debility. Saxena and Mukharya (1997) studied the production of Echinocystic acid -3-0-a-D galactopyranosyl (1->5)-0-a-D xylofuranoside from root of *Spondias mangifera* wild. The presence of these

components enhances the medicinal potentiality of the species.

Many workers have studied the effect of auxin and cytokinin in isolation and also in different combinations on tissue culture of different plant and tree species (Mishra et al., 2004; Vidya et al., 2005) and suggested the best suitable combination for better micropropogation of the species they studied. No such reports are available for *Spondias mangifera*. The present investigation is undertaken to evaluate the multiplication and regeneration capacity of this economically important medicinal plant in tissue culture system. It is a rapid method of multiplication and thus saves time, money and space.

Materials and Methods

Explants (leaves, shoot tips, nodes, rachis) of *S. mangifera* L. were collected from garden near the Department of Botany, Ranchi University, Ranchi. The explants were washed with running tap water for 15 minutes and washed with 1-2 drop of Savlon. Explants were surface sterilized in 70% ethanol for 1 minute and immersed in 0.1% $HgCl_2$ for ½ - 1 minute, then rinsed with autoclaved distilled water. Explants were inoculated in test tube (10 X 1.2cm) containing MS basal media (Murashige and Skoog, 1962). Solid MS media containing 3% sucrose with varying concentration of phytohormones and growth adjutants were used for callus formation and root and shoot regeneration. Combination of auxins and cytokinins was also used for plant regeneration. The pH of the media was adjusted to 5.8 before gelling with agar (0.8%W/V). Cultures were maintained at 25±5°C under 12 hours of photoperiod. The morphogenic response of different parts of *S. mangifera* at the end of two to three weeks from the day of cultures was recorded.

Results and Discussion

Explants of *Spondias mangifera L*. cultured in MS medium supplemented with different growth regulators produced callus, root, and shoot differentiation at the end of two to three weeks from the day of cultures (Table-1).

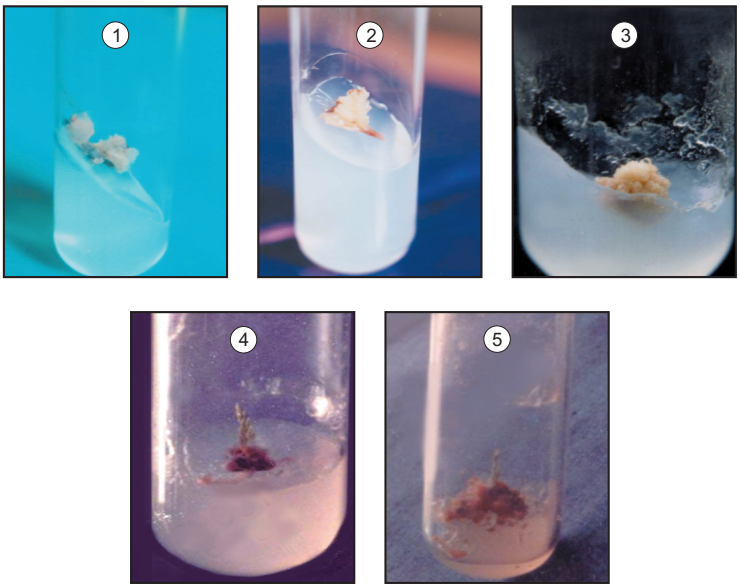

Fig. 1. 14 days old callus from Shoot tip culture on MS medium containing 2, 4D (2.5 mgL^{-1})
Fig. 2. 12 days old callus from leaf on MS medium containing IBA (3 mgL^{-1}).
Fig. 3. 3 week old callus from Shoot tip on MS medium containing NAA (5 mgL^{-1})
Fig.4. A shoot bud differentiated from 3 week old shoot tip derived callus on MS+BAP (2mgL^{-1}) + IAA (1mgL^{-1})
Fig.5. 4 week old culture of shoot tip on MS +BAP (1mgL^{-1}) + NAA (0.5mgL^{-1}) showing initiation of shoot bud at one of the end

Table-1. Effect of different concentration of growth regulators on callus induction and morphogenic response of *S. mangifera L.* grown on MS media.

Growth regulator (mgL^{-1})	Concentration	Leaf		Rachis		Node		Shoot Tip	
		Nature of Response		Nature of Response		Nature of Response		Nature of Response	
		Callus	Organogenesis	Callus	Organogenesis	Callus	Organogenesis	Callus	Organogenesis
2.4-D	0.5	++	-	++	-	++	-	+	-
	1.5	++	-	++	-	++	-	++	-
	2.5	+++	-	+++	-	+++	-	+++	-
	5	++	-	++	-	+	-	++	-
NAA	0.5	+	RT	+	RT		RT	++	-
	1.5	+	RT	++	RT	++	RT	++	-
	2.5	++	-	++	-	++	RT	++	-
	5	+++	-	+++	-	+++	-	+++	-
BAP	0.5	-	-	-	-	-	-	-	-
	1.5	+	-	-	-	-	-	-	-
	2.5	++	SH	++	SH	-	SH	-	SH
	5	-	-	+++	-	-	-	-	-
Zeatin	1.0	++	-	+	-	+	-	++	SH
	2.5	++	SH	++	SH	++	SH	++	SH
BAP+IAA	2+1	-	SH	-	-	++	SH	-	SH
	3+2.5	-	SH	-	-	++	SH	-	SH
BAP+NAA	1+0.5	-	SH	-	-	-	SH+RT	-	SH+RT
	2.5+1	-	-	-	-	-	-	-	SH

= No response, + = Good, ++ = Very Good, +++ = Excellent, SH = Shooting, RT = Rooting.

Effect of Auxins

The effect of auxins on the induction of callusing from explant culture was different. 2, 4-D induces only callus formation. The best result was observed at 2.5 mgL^{-1} 2, 4-D. No organogenesis was seen on medium containing 2, 4-D in tissue culture. 5 mgL^{-1} IAA also induced callus formation in leaf and shoot tip culture. IAA was less effective than other auxin in callus formation Root formation was observed in media containing 0.5 to 2.0 mgL^{-1} of IAA and IBA. Best auxin for callus induction is NAA. The best result was observed at 5 mgL^{-1} NAA (fig- 1, fig- 2 and fig. - 3).

Effect of Cytokinins

When MS medium was supplemented with 5 mgL^{-1} concentration of cytokinin (KN, BAP and Zeatin), callus formation was observed in explant culture. BAP was found to be the most effective cytokinin for shoot differentiation. Differentiation of shoot bud was observed at 2-2.5 mgL^{-1} BAP, also at 1-2 mgL^{-1} of Zeatin in leaf, node and shoot tip culture.

Effect of combination of auxin and cytokinin

BAP was found to be the most effective cytokinin for shoot differentiation in combination with auxin. Most suitable medium for shoot formation from explants-shoot tip or leaves was MS media supplemented with BAP (2 mgL^{-1}) and IAA (1 mgL^{-1}). Other effective combination for shoot differentiation are MS with BAP (3 mgL^{-1}) and IAA (2 mgL^{-1}) and other was MS with BAP (1 mgL^{-1}) and NAA (0.5 mgL^{-1}) (Fig. – 4 and Fig. – 5).

This study clearly demonstrated that rapid *in-vitro* propagation of *Spondias mangifera* can be obtained by

proper manipulation of explant age and nature and concentration of plant growth regulators. Disease free plants of *Spondias mangifera* L. could be produced in large scale in lesser time on the basis of the present study. Thus the plant got the tremendous capacity to establish in tissue culture system which is useful in the future genetic manipulation studies of this species.

References

Asthana, P. Jaiswal, V. S. and Jaiswal, U. 2011. Micropropagation of *Sapindus trifoliatus* L. and assessment of genetic fidelity of micropropagated plants using RAPD analysis. *Acta Physiol Plant.* 33: 1821-1829.

Mishra, P. D., Datta, S. K., Johri, J. K., Singh, H. B. and Srivastava, A. 2004. An improved method for *in vitro* large scale propagation of *Piper betle* L. *J. Plant Biochemistry and Biotechnology*. 13: 161-164.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with *Tobacco* tissue culture *Physiol Plant*. 15: 473.

Pena, L. and Seguin, A. 2001. Recent advances in the genetic transformation of trees. *Trends Biotechnol.* 19: 500-506.

Saxena, V. K. and Mukharya. 1997. Echinocystic acid -3-0-a-D galactopyranosyl (1->5)-0-a-D-xylofuranoside from Root of *Spondias mangifera* wild. *Asian Journal of chemistry.* 9(2): 253-260.

Vidya, S. M., Krishna, V., Manjunatha, B. K. and Shankarmurthy, K. 2005. Micropropagation of *Entada pursaetha* DC- An endangered medicinal plant of Western Ghats.*Indian J.Biotechnology*. 4: 561-564.