

EFFECT OF GROWTH HORMONES ON SHOOT INITIATION OF *MICHELIA CHAMPACA* L.

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Abstract: *Michelia champaca* L. belongs to family Magnoliaceae commonly known as Son Champa. It has various uses in traditional medicine, perfumery etc. The essential oil obtained from the flowers has useful application in perfumery and pharmaceutical industries. Traditionally it is being used in fever, colic, leprosy, after delivery protection and in eye disorders. There are several problems related to propagation of this plant such as low percentage of seed germination caused by alkaloids content in seeds that inhibit seed germination. The present study was conducted to develop a protocol for the shoot initiation of *Michelia champaca* L. The best result obtained from MS media supplemented with 2.5mg/l BAP, 1.0mg/l KN and 0.5mg/l IAA plus activated charcoal, which plays the significant role.

Keywords: *Michelia champaca* L.; Shoot initiation; Growth hormones; Phenolics; Activated charcoal.

I. INTRODUCTION

Michelia champaca L. is a woody tree species belongs to family Magnoliaceae. It has high medicinal and commercial value. Its common name is son champa, golden champa, kanak champa etc. *Michelia champaca* L. is an evergreen or semi-deciduous, graceful, small to medium sized tree up to 50 m tall. It has commercial value especially of flower whereby the essential oil could be extracted from the flowers and used for production of perfumes, cosmetics and hair oil. (Barlow et al., 1991). The plant is traditionally used for the treatment of fever, colic, leprosy and cure for coughs and rheumatism and for remedies of various disorders (Perry, 1980; Vimala et al., 1997; Hasan et al., 2009; Armiyanti et al., 2010). A decoction of the bark and leaves is given after childbirth; the bark is used as a febrifuge. According to Indian Medicinal Plants, root and root bark of *Michelia champaca* L. are purgative and emmenagogue and are useful in the treatment of abscesses, inflammation, constipation, amenorrhoea and dysmenorrhoea. The stem bark is astringent, febrifuge, diuretic, stimulant and expectorant and is useful in chronic gastritis, fever, strangury, cough, bronchitis and cardiac debility. Flower, flower buds and fruits are bitter, astringent, acrid, refrigerant, haemostatic, digestive, carminative, depurative, digestive, anthelmintic, diuretic, expectorant, cardiotoxic, stimulant, stomachic and antipyretic. They are useful in nausea, burning sensation, haemolysis, skin diseases, leprosy, ulcers, gout, cough, bronchitis, dysmenorrhoea, strangury, and malarial fever. The fruits are used in dyspepsia and renal diseases (Chatterjee et al., 2005) and are applied for healing cracked feet.

Few works have been done on tissue culture of this plant in India. However, some of the authors have provided information about the micropropagation of this plant outside India. Armiyanti et al., 2010 provided information about the somatic embryogenesis of *Michelia champaca* L. They used MS medium supplemented with 2.0mg/l IAA for highest percentage of embryonic callus. Armiyanti et al., 2012 established culture through cell suspension technique. High frequency somatic embryos was obtained by using MS media supplemented with 2.0mg/l IAA. Abdelmageed et al., 2012

induced callus from axillary bud explant. The combination of 2.0mg/l BAP with 0.5 mg/l IAA and all the combinations of BAP with 2, 4-D showed the highest rate of callus proliferation.

There are several problems associated to propagation of *M. champaca* L. such as low percentage of seeds germination caused by alkaloids content in seeds that inhibit seed germination (Zabala, 1990; Wan et al; 1990). The other problem is its vegetative propagation which is very slow to meet the needs of elite varieties in time. Also, very less vegetative methods are successful in multiplying new plants. Therefore, to overcome these problem tissue culture methods is applicable for shoot initiation and new plant generation. Present work was conducted to understand the effect of different growth hormones on the shoot initiation of *Michelia champaca* L.

II. MATERIAL AND METHODS

A. Explant and surface sterilization:

Explants were collected from the *Michelia champaca* L. tree situated in the Rishabh Dev Udyan, Shahpura, Bhopal. Axillary meristem and apical meristem was taken as explant. Explants were washed with Clorox for 3 min, then were kept under running tap water for 1 h, and then rinsed in 6% NaOCl for 35 min, followed by 2% NaOCl for 15 min in which 0.01% Tween 20 were added as surfactants. After this explants were rinsed with tap water 3-4 times. Final treatment of the explants was carried out inside laminar air flow (LAF) with 0.1% HgCl₂ for 2-3 minutes.

B. Basal Medium and Incubation conditions:

Murashige and Skoog (1962) basal medium (MS) supplemented with 30g/l sucrose was used in this study. All media prepared were adjusted to pH 5.8 before solidified with 5g/l agar and autoclaved at 121°C, 15 psi for 20 min. Explants were cultured on agar-solidified MS media supplemented with different concentrations of cytokinin i.e. 6-benzylaminopurine(BAP), kinetin (KN) and auxin i.e. indole-3-acetic acid (IAA) for shoot initiation. The range of concentrations is in between 0.0mg/l to 2.5mg/l for different growth hormones.

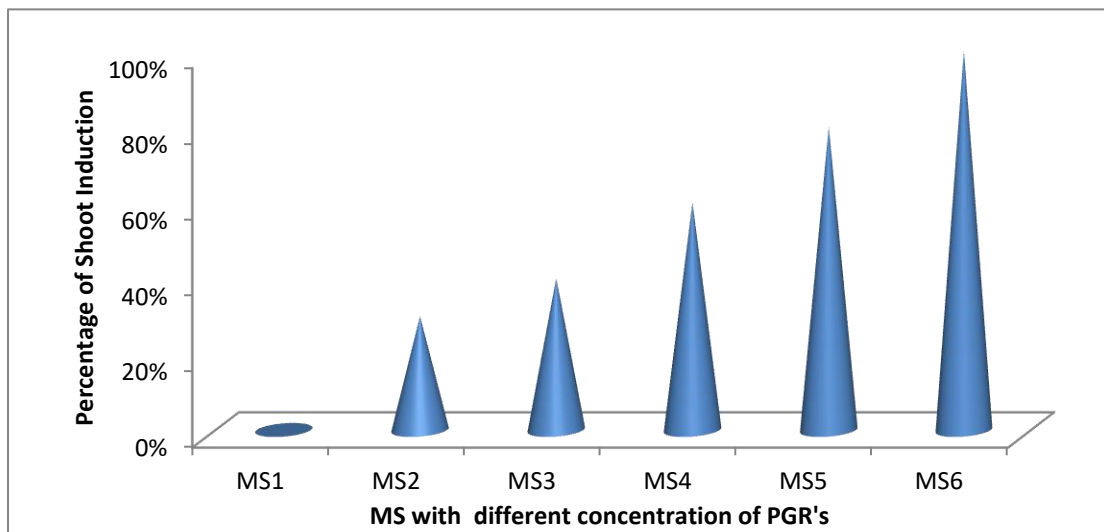
III. RESULT AND DISCUSSION

The effect of different concentrations of plant growth regulators on shoot initiation of *Michelia champaca* L. was studied and depicted in table I. and graph 1. They are successfully induced to produce shoot using MS media with different combinations of cytokinins and auxin. Some good results obtained with the combination of BAP, KN and IAA, but culture does not exist for long time and slowly dehydrate due to phenolics, it is shown as MS5 media in Graph 1. Very good result is obtained by combining 2.5mg/l BAP, 1.0mg/l KN, 0.5mg/l IAA and activated charcoal, it is shown as MS6 media in Graph 1. In this culture very good initiation occurs and growth is continuous, after few weeks leaves can be clearly seen. Activated charcoal plays a very significant role in the treatment of phenolic. Phenolic compounds are responsible for dehydrating cultures. Fig.1. showing initiation of shoot from the meristem and in Fig.2. stage after initiation is shown, in which leaf can be seen very clearly. Presence of activated charcoal can be seen as black coloured medium. From the results it is observed that in the combination of hormones, quantity of BAP is needed in high amount as compared to others. This result is in support of the result by Abdelmageed et al., (2012) who reported that BAP showed the highest percentage of shoot initiation as compared to other hormones.

Table I. Shoot initiation of *Michelia champaca* L. on MS basal media supplemented with different concentrations of growth regulators.

S.N.	Plant Growth Regulator (mg/l)	Media	Response of culture					Result
			Week 1	Week 2	Week 3	Week 4	Week 5	
1.	0.1 BAP	MS1	----	----	----	-----	----	No response
2.	0.1 BAP+ 0.1 KN	MS2	++	++	++	++	++	Initiation appears but no further growth

3.	0.5 BAP+ 0.5 KN	MS3	+++	+++	+++	++	+	Initiated good but after some weeks brown coloured phenolics appeared
4	1 BAP+ 0.5 KN + 0.1 IAA	MS4	+++	+++	++	+	+	Good initiation seen, but very soon culture affected by phenolics.
5.	2 BAP+ 0.5KN + 0.5 IAA + Activated Charcoal	MS5	+++	+++	++++	+++	+++	Initiated very good, culture is green and growing.
6.	2.5 BAP + 1KN + 0.5 IAA + Activated Charcoal	MS6	+++	+++	++++	++++	++++	Very good culture, leaves are clearly seen in 5 th week.



Graph 1. Showing shoot initiation of *Michelia champaca* L. on MS basal media supplemented with different concentrations of growth regulators.



Fig.1. Showing shoot initiation from meristem of *Michelia champaca* L.



Fig.2. Showing origin of leaf from the shoot of *Michelia champaca* L.

IV. CONCLUSION

From the above results, it is concluded that combination of different hormones does effect the shoot initiation of *Michelia champaca* L. Cytokinin (2.5mg/l BAP and 1.0mg/l kinetin) in combination of auxin (0.5mg/l IAA) with activated charcoal gives best result in MS media. These results will be beneficial for increasing the number of this plant, as seed germination and vegetative methods are not very effective in this tree. There are several problems related to propagation of this plant such as low percentage of seed germination caused by alkaloids content in seeds that inhibit seed germination. As far as our knowledge is concerned this is the first work to develop a protocol for the shoot initiation of *Michelia champaca* L. No such study has been undertaken so far.

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