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Callus Induction of Michelia champaca L. through petiole - An aromatic tree of high economic value

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Abstract: The different parts of Michelia champaca L belonging to the family Magnoliaceae has many medicinal, cosmetic and economic uses. The flowers from this tree has high aromatic odour and used to make the world's most expensive perfume (Warren, 1998). M. champaca is being propagated only by the means of seed. Low seed germination rate and quick loss of viability of Michelia champaca has been reported earlier (Zabala, 1990). Moreover in spite of being the native of India, but due to poor distribution of the plant species limited to only certain part of the country. Keeping view the high economic value and demand the present study was investigated. Petiole was used as explants for callus induction. Maximum and rapid callus formation was observed in B_5 medium supplemented with 8.0mg/l of 2,4-D.

Key words: Michelia champaca, callus induction, germination rate, 2,4-D, B_5 medium.

Introduction

Michelia champaca are commonly known for their fragrant flowers and as ornamentals. The flowers of Michelia champaca are marketed for their scent, used in making garlands, placed between stored clothes, sprinkled in bridal beds and used in the preparation of scented hair lotions. It is also highly used in incense sticks' industry. Michelia champaca also has medicinal properties. Michelia champaca leaf extracts have been tested for its anti diabetic property on pancreas morphology of diabetic rat model (Gupta S et al., 2011). Both aqueous and alcoholic extract of its leaf and flower have been tested and found to have anti ulcerogenic property (Kumar S et.al, 2011).The bark of Michelia champaca contains sesquiterpene lactones of the guiane type which are of interest in treatment of cancer. Its different extracts has been reported to possess certain pharmacological properties like antipyretic, anti-inflammatory (Vimala R el.al., 1998), insecticidal (Ulla J et.al., 1995), anti oxidant, antimicrobial (Khan MR et.al., 2002) and antifungal activities (lee seeong et.al., 2011). The bark is used as a stimulant, expectorant, astringent and also posses febrifugal properties (Chopra RN et.al., 1986). The flowers and fruits in combination with other drugs are recommended as an anti-dote to snake and scorpion venoms (Kirtikar KR and Basu BD, 1984). Some fractions of the leaves stem and root bark demonstrated antifungal activity against some of the tested moulds. Liriodenine was the active constituent of the root bark, with a broader spectrum of antifungal activity (Khan MR et.al., 2002) .Traditionally, it is being used in treatment of fever, colic, leprosy, post partum protection (Khan MR et.al., 2002) and in eye disorders (Sobhagini N et.al., 2004).

The methanolic extract of Michelia champaca flower is being reported to show burn wound healing property as per stated by Shanbhag T et.al.,(2011). Kumar S et.al.,(2011) have stated that on aspirin both the aqueous and alcoholic extract of Michelia champaca L. flower and leaves possess anti ulcer property. Atjana suppata et.al., (2009) stated that M. Champaca leaf extract shows the inhibitory activity against C32 cells and Michelia champaca flower and seed extract is also found to show positive response against cancer cell lines of MCF-7 cells as reported by Seong L, et.al (2011). Khan M.R et.al., (2002) have also reported extracts of Michelia champaca to possess certain pharmacological properties also like antipyretic, anti-inflammatory , insecticidal , anti oxidant, antimicrobial and antifungal activities Armiyanti et al., (2004) have provided information about the successful somatic embryogenesis from immature seeds of M. champaca by using different concentrations and auxin types (2,4-D and NAA). The individual work done by Amriyanti have also provided information about the Plant regeneration system via direct organogenesis using different concentration of auxin and cytokinin (BAP and NAA) for shoot induction from shoot tip derived from seedling explants. Among other species of this family, the flower especially gynaecium and stamens of Michelia alba were reported to be cultured for callus formation by Shah N, et.al., (2009) in WPM medium supplemented with 4mg/l NAA and 2mg/l Kn. The germination of Michelia champaca L. seeds is being reported to be hindered due to the presence of inhibiting substances in the aril (Candiani G et al., 2004). With the expansion in the champaca industry there is also increase demand for the plant. Moreover in spite of being the native of India, but due to poor distribution of the plant species to only certain part of the country, the work on it is being restricted to many extend because of the difficulties faced in propagation of Michelia champaca through conventional methods (Rugini and Guitierrez-Pesce, 2003), micro propagation is being used as an alternative method by many researchers so as to increase its number (Kozai et.al., 1997). Considering the potential economic importance of this species, the present work of callus induction Michelia champaca L. was investigated.

Materials and Method

Apical shoot tip, nodal segment and petioles of Michelia champaca were taken as explants. Apical shoot tip were incised from the field grown plantlets and petioles were incised from the mature leaves of Michelia champaca with the help of sharp and sterilized scalpel. The plantlets of Michelia champaca were collected from Assam Agricultural University, Assam. Explants (apical shoot tip, nodal segment and petiole) were taken and treated with labolene (detergent) for 10 minutes and rinsed properly with distilled water for 2-3 times. After that treated with 1% sodium hypochlorite for 5 minutes followed by rinsing with distilled water for 2-3 times. Final treatment of the explants material was carried out inside the LAF with 0.1% $HgCl_2$ for 2-3 minutes.The explants were inoculated in MS, SH and B_5 medium. The effect of all three medium, rate of callusing was observed and studied. After selection of best suitable explant and medium the explants were inoculated in medium fortified with different concentrations of auxins, cytokinins and adjuvants.

Result

The effect of different medium (MS, SH and B_5) was studied on callus induction from petiole of Michelia champaca. Basal B_5 medium have shown best result with maximum and fast callusing rate as compared with basal MS and SH medium(Table 1,Fig 3). However, experiments were carried out with both B_5 and MS medium**.** The study of effect of B_5 medium supplemented with different concentration of 2,4-D (2.0mg/l, 4.0mg/l, 6.0mg/l and 8.0mg/l) was found best suitable result for callus induction from petiole of Michelia champaca. The concentration of 6.0mg/l and 8.0mg/l 2,4-D in B_5 medium have shown best result with 60% and 80% respectively of intense, continuous and fast callusing rate (Table 2).

Discussion

Petiole of Michelia champaca were successfully induced to produce callus using different medium (MS and B_5) supplemented with different concentration and combination of auxin and cytokinin. B_5 medium was found to give better result with 100% callusing as compared basal MS medium with 66.66 % callusing (Fig 1). B_5 medium supplemented with 8.0mg/l 2,4-D supported the maximum and fast formation of callus after only a week of inoculation (Fig 4). This result is in match with those of Rodriguez and Wetzstein (1994) reported that 2,4-D treatment produce more callus compared with NAA treatment in their experiment on induction of somatic embryo of pecan (caryail linoinensis) and reported the same in their experiment on induction of somatic embryo from immature seed of Michelia champaca Though both the MS and B_5 medium used were found to induce callus formation from different explants of Michelia champaca but duration of callus initiation was found to differ from 1 week in B_5 medium to 3 weeks in MS medium. Hence B_5 medium supplemented with 8.0mg/l 2,4-D was found to show best result for inducing callusing in Michelia champaca with both higher rate of callusing and less duration of callus initiation.

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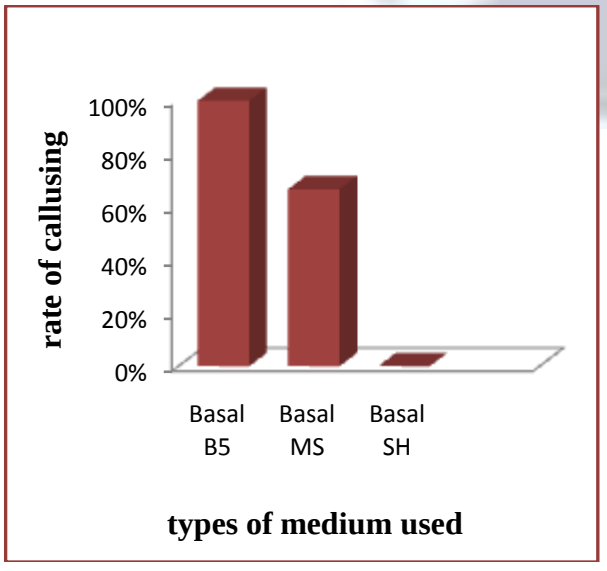

Fig 1: graph showing rate of callusing in different medium (MS, SH and B_5)

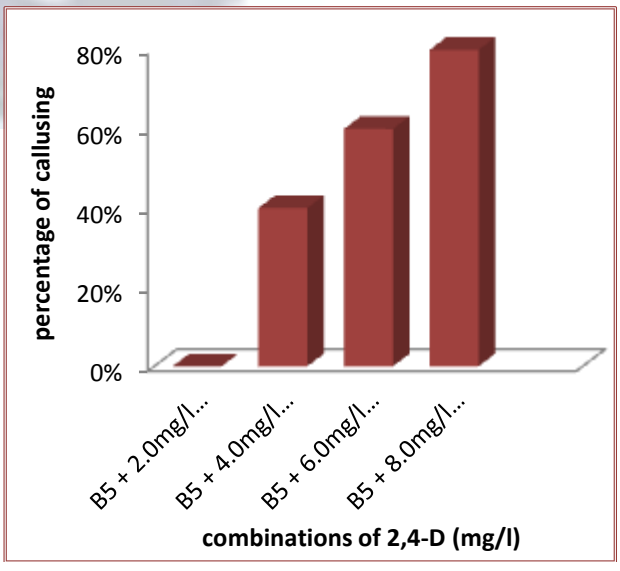

Fig 2: graph showing rate of callusing in B_5 medium supplemented with different concentration of 2,4-D

Fig 3: Callus formation of Michelia champaca in basal B_5 medium

Fig 4: Callus formation in B_5 medium supplemented with 8.0mg/l 2,4-D

Table 1: Study of effect of different media on callus induction in Michelia champaca from different explants on different medium.

Media used : MS , SH and B_5 medium **Explants : petiole** **Incubation condition : 16 hrs of light and 8 hrs of dark**						
No of explants	**Media used**	**Response**				Remrks
		Week1	**Week2**	**Week3**	**Week4**	
1. 2. 3.	MS basal	-- -- --	-- -- --	++ + --	++ + --	Little and delayed callusing observed after 3 weeks of inoculation.
4. 5. 6.	SH basal	-- -- --	-- -- --	-- -- --	-- -- --	No callusing observed.
7. 8. 9.	B_5 basal	++ + +	+++ ++ +	+++ ++ ++	+++ ++ ++	Profuse callusing observed after 1 week of inoculation.

+: good, ++: very good, +++: excellent, ++++: profuse

Table 2: Study of effect of different concentration of auxins (2,4-D) on callus induction in Michelia champaca from different explants in B_5 medium.

Media : B5 + Sucrose (3%) + auxin (2,4-D)
Explants : petiole
Incubation condition :

No of explants	Concentration of auxin(mg/l)	Response				Remarks
		Week1	Week2	Week3	Week4	
1. 2. 3. 4. 5.	B_5 + 2.0mg/l 2,4-D	-- -- -- -- --	-- -- -- -- --	-- -- -- -- --	-- -- -- -- --	No callusing observed
6. 7. 8. 9. 10.	B_5 + 4.0mg/l 2,4-D	+ + + -- --	+ + + -- --	+ + + -- --	+ ++ ++ -- --	Swelling observed after 1 week of inoculation. But no further growth/ callusing observed
11. 12. 13. 14. 15.	B_5 + 6.0mg/l 2,4-D	-- -- -- -- --	+ + + -- --	++ + + -- --	+++ ++ + -- --	Softening of outer wall observed after 1 week. Yellowish green colour callusing seen after 2nd week of inoculation.
16. 17. 18. 19. 20.	B_5 + 8.0mg/l 2,4-D	++ + -- -- --	++ ++ ++ + --	+++ +++ +++ ++ +	++++ +++ +++ ++ ++	Rupturing of outer cell wall after 1 week. Fresh callusing observed. yellowish green in colour after 1 week of inoculation. Fast growing with high regenerative potential.

+: good, ++: very good, +++: excellent, ++++: profuse

References

[1]. Armiyanti[1], Kadir A[1*], Kadzimin S[2], Panjaitan B.S[3] (2010); Plant regeneration of Michelia champaca L., through somatic embryogenesis; African Journal of Biotechnology; 9(18): 2640-2647.
[2]. Atjanasuppata K, Wongkhama W, Meepowpan P, Kittakoop P, Sobhon P, Bartlett A, Whitfield PJ. (2009); In vitro screening for anthelmintic and antitumour activity of ethnomedicinal plants from Thailand; Ethnopharmacol. 123: 475–482.
[3]. Candiani G[I], Galetti M[II], Victor Jose Mendes Cardoso[I](2004) Seed germination and removal of Michelia champaca L. (Magnoliaceae) in eucalypt stands: the influence of the aril; Revista arvore; 28(3): 327-332
[4]. Chopra RN, Nayar SL, Chopra IC (1996); Glossary of Indian Medicinal plants; Council of Scientific and Industrial Research,New Delhi; 66.
[5]. Dasuki U.A and Kuswanto MS (1999) Michelia Linn; Plant resources of South East Asia; 19: 135-140.
[6]. GuptaS[*], Mehla K, Chauhan D, Kumar S, Nair A (2011); Morphological changes and antihyperglycemic effect of M.champaca leaves extract on Beta cell in Alloxen induced diabetic rats; Recent research in Science and Technology; 3(1) : 81-87
[7]. Khan MR, Kihara M, Omoloso AD. (2002); Antimicrobial activity of Michelia champaca; Fitoterapia; 73: 744-748.
[8]. Kirtikar KR, Basu BD (1984) Magnoliaceae in medicinal plants (editor - Bishan Singh Mahender Pal Singh), Dehradun, India: 55.
[9]. Kozai T, Kubota C, Jeong BR (1997). Environmental control for the large scale production of plants through in vitro technique; Plant Cell, Tissue Organ Cult; 51: 49-56.
[10]. Kumar S[1*], Aparna P[1], Poojitha K[1], S. K. Karishma [1] and Astalakshmi N[2] (2011) ; A comparative study of Michelia champaca Linn flower and leaves for anti – ulcer activity; International Journal of Pharmaceutical Sciences and Research; 2(6) : 1554-1558.
[11]. Negi R[1] , Sharma K[2], Sharma M[3](2011); Micropropagation and Anatomical Comparision of In Vivo and In Vitro Developed Shoot and Root in Cassia auriculata L. - A Medicinally Important Plant; Indian journal of fundamental and Applied life sciences; 1(1):21-29.

[12]. Rodriguez APM, Wetzstein HY (1998); A morphological and histological comparison of the initiation and development of pecan (Carya illinoinensis) somatic embryogenic cultures induced with NAA or 2, 4-D; Protoplasma 204: 71-83.
[13]. Rugini E, Gutierrez-Pesce P (2003). Micropropgation of kiwi fruit (Actinidia sp). In Micropropagation of Woody Trees and Fruits, editors : Mohan Jain S, Ishii K, The Netherlands: Kluwer Academic Publishers; 75: 647-66.
[14]. *Seong L[1], Wendy Wee[2], Julius Yong Fu Siong[3], Desy Fitrya Syamsumir[3](2011); Characterization of Antimicrobial, Antioxidant, Anticancer Property and Chemical Composition of Michelia champaca Seed and Flower Extracts; Stamford journal of Pharmaceutical Sciences; 4(1): 19-24.
[15]. Shah N[1], Muse R[2], Sukari A[3] (2009); The Fragrance Production from Callus culture of Gynoecium and Stamen of Michelia alba Flower; http://www.ipicex.com/docs/posters/Nor'aishah%20et% 20al.pdf.
[16]. Sobhagini N, Soumit KB (2004); Malaya KM. Ethnomedico-botanical survey of Kalahandi district of Orissa; Indian Journal of Traditional Knowledge; 3:72-79.
[17]. Shanbhag T[1] , Kodidela S[2*], Shenoy S[1], Amuthan A[2], Kurra S[3] (2011); Effect of Michelia champaca Linn flowers on burn wound healing in wistar rats; International Journal of Pharmaceutical Sciences Review and Research; 7(2) : 112-115.
[18]. Ulla J, Vijaya K, Shantini S (1995) Sesquiterpene lactones from Michelia champaca; Fitoterpia; 39(4): 839-843.
[19]. Vimala R, Nagarajan S, Alam M, Susan T, Joy S. (1997); Antiinflammatory and antipyretic activity of Michelia champaca Linn., (white variety), Ixora brachiata Roxb. and Rhynchosia cana (Willd.) D.C. flower extract; Indian ournal of. Experimantal Biology; 35:1310.
[20]. Warren W (1998); Tropical Flowers of Malaysia and Singapore; Periplus Edition, Hongkong.
[21]. Zabala NQ (1990); Silviculture of Michelia champaca In: Silviculture of species. Chittagong, Bangladesh: Chittagong University, Institute of Forestry; Food and Agriculture Organization, Rome, Italy : 68-70.