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# *In Vitro* Shoot Multiplication of *Flacourtia jangomas* (Lour.) Raeusch

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**Abstract:** Shoot bud multiplication was obtained in *Flacourtia jangomas*, a dioecious woody plant of medicinal as well as pomocultural value, by using nodal segment and shoot apex explants. The basal medium was MS medium supplemented with 3.0% sucrose and 0.8% agar. The optimum PGRs for establishment of culture were 1.0mg/l Kinetin and 0.1mg/l NAA. Maximum shoot buds were noted when transferred in MS medium with 5.0mg/l BAP and 0.1mg/l NAA for shoot multiplication. The cycle was repeated twice and 78-81 shoot buds was obtained per explants. Rooting was noted after 60 days in half strength MS with 3.0mg/l NAA. It was also obtained within 15 days by transferring shoot lets after a week from higher conc. (3.0mg/l) to a lower conc. (1.0mg/l) of NAA. After transferring to pots, about 40% of them survived.

**Keywords:** Micro propagation; Caulogenesis; Rhizogenesis; Shoot let; Propagules

## Introduction

*Flacourtia jangomas* (Lour.) Raeusch, is a woody dioecious plant member of the family Flacourtiaceae possesses some medicinal and economic values. The plant is found frequently in semi wild condition in the Brahmaputra valley and adjoining areas in the North Eastern parts of India, probably migrated from Bangladesh and Upper Myanamar (Mitra, 1993). The plant is cultivated in forests or orchards for its edible fruits and hard wood (Anon, 1956). The dried leaves and barks are used for the treatment of asthma, pre and postnatal blood purification (Jain, 1991). Whereas fresh leaves and young shoots are used as astringent and stomachic. The fruits are used in bilious condition and in diarrhea (Kirtikar and Basu, 1953). The fruit contains tanin and a fixed oil (Anon, 1956) and two limnoids i.e., limolin and jangomolide have been obtained from its stem and bark (Ahmad et.al., 1984). Another species of *Flacourtia* i.e., *F. ramontchi* has anti-inflammatory and antimicrobial properties (Lalsare *et al.,* 2011)

Regarding tissue culture, callus induction and caulogenesis as well as rhizogenesis from callus tissue have been standardized and their process of organogenesis has been investigated through histological and scanning electron microscopical studies (Chandra and Bhanja, 2002) and it has been reported for the first time. After that, tissue culture of this plant has also been reported by Kashyap *et al.,* (2005). Attempts have been made to develop the micro propagation strategies of this valuable plant through nodal segment and shoot tip culture.

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## Materials and Methods

Young shoot tip (0.4-0.5 cm long) and nodal segment (0.5cm long) have been collected from actively growing coppiced shoot of 10-15 years old tree (Fig.1), cultivated in Ramna forest, Burdwan. They were surface sterilized with $HgCl_2$ (0.1% w/v aqueous) for 5-10 min and finally washed thoroughly with sterilized distilled water. Then they were excised and initially transferred into established medium. The basal medium was (Murashige and Skoog, 1962) supplemented with 0.3% sucrose and 0.8% agar. The different plant growth regulators (PGRs) used for establishment medium ($M_1$) were NAA (0.1mg/l), BAP (0.2-5.0mg/l) and KIN (0.2-5.0mg/l). The cultures were incubated at a temperature of 25± $2^0$C and relative humidity of 70 ± 10% under a 16h photoperiod.

For multiplication, the shoot buds were excised and transferred into multiplication medium ($M_2$). The suitable medium for multiplication was MS with 5.0mg/lBAP in combination with 0.1mg/l NAA. For elongation, shoot buds were transferred into elongation medium ($M_3$). The PGRs for elongation medium were 1.0mg/l BAP and 0.05mg/l NAA.

For induction of rooting, shoot lets were transferred into rooting medium. The effective rooting medium was half strength MS supplemented with 3.0% sucrose and 3.0mg/l NAA ($M_4$). For enhancement of rooting shoo lets were transferred from higher conc. of NAA (3.0mg/l) after 7 days to lower conc. of NAA (1.0mg/l) i.e., $M_{5.}$ The rooted plants were transferred into half strength liquid MS medium without vitamins and hormones and

with the support of filter paper bridge for better development of root i.e.,$M_6$.

After development of root, the plants were removed from culture tubes and transferred into pots. Before transferring, hardening was done by placing them into sterilized sand: soil (1:1) mixture.

## Results

The best establishment medium ($M_1$) was MS supplemented with 3.0% sucrose, 1.0mg/l Kin and 0.1mg/l NAA. The maximum number of shoot bud obtained was about 3.0 per explants (3.3±0.16 in nodal segment and 3.0±0.14 in shoot apex, vide Table-1; Fig.2)

**Table.1:** Effect of different Plant Growth Regulators on shoots multiplication from nodal segment and shoot apex of *Flacourtia jangomas*

| Concentration of PGR(s) in MS medium (mg/l) | | | No. of shoot buds regenerated (Mean ± SE) | |
|---|---|---|---|---|
| NAA | BAP | KIN | Nodal Segment | Shoot apex |
| - | - | - | - | - |
| - | 0.5 | - | - | - |
| - | 1.0 | - | 1.5 ± 0.14 | 1.2± 0.10 |
| - | 1.5 | - | 1.8± 0.14 | 1.5±0.12 |
| - | 2.0 | - | 2.6± 0.12 | 2.3±0.14 |
| - | 5.0 | - | 2.1± 0.15 | 1.9±0.10 |
| - | - | 0.5 | - | - |
| - | - | 1.0 | 1.3± 0.10 | 1.1±0.05 |
| - | - | 1.5 | 1.5± 0.09 | 1.2±0.11 |
| - | - | 2.0 | 1.9± 0.13 | 1.7±0.14 |
| - | - | 5.0 | 1.6±0.09 | 1.4±0.12 |
| 0.1 | 0.2 | - | - | - |
| 0.1 | 0.5 | - | 1.7±0.11 | 1.4±0.10 |
| 0.1 | 1.0 | - | 3.1±0.13 | 2.6±0.09 |
| 0.1 | 2.0 | - | 1.9±0.17 | 1.6±0.12 |
| 0.1 | - | 0.2 | - | - |
| 0.1 | - | 0.5 | 2.1±0.17 | 1.9±0.15 |
| 0.1 | - | 1.0 | 3.3±0.16 | 3.0±0.14 |
| 0.1 | - | 2.0 | 2.7±0.14 | 2.2±0.11 |

Data obtained after 28 days on the basis of mean value of 10 replicates each

**Fig.A:** A flowering twig of *Flacourtia jangomas*

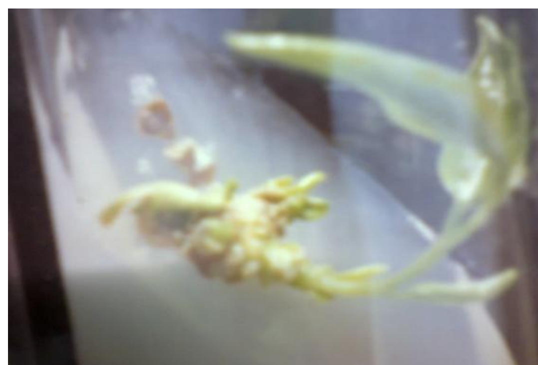

**Fig.B:** Multiple shoots of *Flacourtia jangomas* in MS containing 0.1mg/l NAA and 1.0mg/l Kinetin

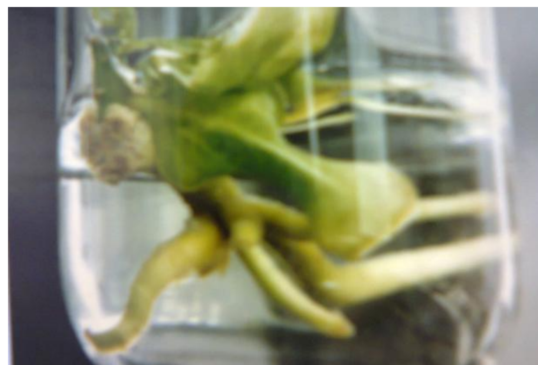

**Fig.C:** Rooted shoot let in half strength MS liquid medium containing 3.0mg/l NAA

**Fig. D:** Hardened Plant in Pot

For further proliferation, the shoot buds or propagules were transferred into $M_2$ medium. At least 16-18 shoot buds were obtained in the first cycle. The cycle was repeated again and about 78-81 shoot buds were produced per explants. For elongation, when the shoot bud were transferred into $M_3$ medium, the maximum length obtained for a shoot was 30 mm and maximum leaf length was 20-25mm.

Induction of rooting was noted after about 60 days when shoot lets were

transferred into $M_4$ medium (Fig.3). But when they were transferred from $M_4$ to $M_5$ medium, rooting was found within 15 days and 80% rooting was obtained. Further growth and proliferation of root was noted when the plantlets were transferred into $M_6$ medium. About 40% plantlets survived after transferring into pots (Fig.4)

## Discussion

During establishment, the number of shoot bud production was low i.e., about 3 per explant. It is possibly due to woody habit of the material. Similar observations have been made earlier by previous workers in some other woody plants viz., in jackfruit,3-4 shoots per explants (Roy et al,1996). However, a combination of auxin and cytokinin showed better efficiency in shoot bud formation than only cytokinin used. Similar result has been found in *Eucalyptus tereticornis* (Das and Mitra, 1990), *Verbascum thapsus* L. (Turker et al., 2001), *Rollinia mucosa* (Jacq.) Baill, (Figueiredo et al., 2001). In order to achieve multiplication, the concentration of cytokinin had to be increased than that of establishment medium with a simultaneous reduction of the concentration of NAA to avoid unwanted callusing. It has been reported that high concentration of auxin not only inhibits bud branching but also promotes callus formation (Hasegawa, 1980). In this material, BAP is the most effective among the known cytokinins for stimulating axillary shoot proliferation, which has also been reported by Bhojwani (1980). However, kinetin in increasing concentration did not show any remarkable response as that of BAP. Similar report has been found earlier (Gupta *et al.,* 2001). In *Swertia*, best shoot multiplication was noted in BAP in combination with IAA than in singly BAP or Kinetin as reported by Pant *et al.,* (2012) which was also observed here. Reduced concentration of BAP stimulated the elongation of sassafras shoot has been reported earlier (Hu and Wang, 1993). In sesame, during elongation of shoot, low concentration of BAP (0.5mg/l) has been used in combination with 0.5mg/l NAA (Gangopadhyay et al., 1998). In this plant, for elongatin of shoot, reduction in concentration of both BAP and NAA (1.0mg/l and 0.05mg/l respectively) has been found to be effective.

Regarding induction of rooting, it was a delayed process, i.e., it took about 60 days, when the high concentration of NAA was maintained throughout Singh et al., (2013) also reported that high concentration of auxin (i.e., 2.5mg/l IBA) was suitable for maximum shoot induction. But when the shoot lets were transferred after a week from the higher concentration to lower concentration of NAA, induction of rooting took place within 15 days. This response was also noted in Maesopsis (Rajsekharan, 1993).

## Conclusion

As it is a woody plant, some problems like low rate of multiplication, delayed rooting, low survival rate are noted. Thus, it can be concluded that in spite of having all these problems, the strategies of micro propagation has been standardized.

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## References


1. Ahmad J, Wijarat K,Shamuddin KM, Zaman A and Connlly JD, Jangomolide, a novel limnoid from *Flacourtia angomas*,Phytochemistry,1984,23(6),1269-1270.

2. Anonymous, *Flacourtia* comm. (Flacourtiaceae) In the Wealth of India: Raw Materials, 1956, 4, CSIR, New Delhi.

3. Bhojwani SS, *In Vitro* propagation of garlic by shoot proliferation, 1980, Sci. Hort., 13, 47-52.

4. Chandra Indrani and Bhanja P, Study of organogenesis *in vitro* from callus tissue of *Flacourtia jangomas* (Lour.) Raeusch,through Scanning electron microscopy, 2002, Curr. Sci.83, 476-479.

5. Das TO and Mitra GC, Micropropagation of *Eucalyptus tereticornis* Smith. Plant Cell Tissue and Organ Cult, 1990, 22, 95-103.

6. Edson JL, Leegl-Brusven AD, Everett RL and Wenny DL, Minimizing growth regulators in shoot culture of an endangered plant, *Hackelia venusta* (Boraginaceae). *In Vitro* Cellular and Developmental Biology-Plant, 1996, 32(4), 267-271.

7. Figueiredo SFL, Albarello N and Viana VRC, Micropropagation of *Rollinia mucosa* (Jacq.) Baill. *In Vitro* Cellular and Developmental Biology-Plant, 2001, 37(4), 471-475.

8. Gangopadhyay G, Poddar R and Gupta S. Micropropagation of Sesame (*Sesamum indicum* L.) by i*n vitro* multiple shoot production from nodal explants. 1998, Phytomorphology, 48(1), 83-90.

9. Gupta SK, Khanuja SPS and Kumar S, *In vitro* micropropagatioon of *Lippia alba*, Curr. Sci., 2001, 81(2), 206-210.

10. Hasegawa PM, Murashige T and Takatori FH, Propagation of *Asparagus* through shoot apex culture. II. Light and temperature requirements, trans-plantability of plants and cytohistological characteristics., 1973, J. Am. Soc. Sci., 98, 143-148.

11. Hu CY and Wang PJ, Meristem shoot tip and bud cultures. In Evans D A, Sharp W R, Ammirato P V and Yamada Y (eds.), 1983, Hand book of Plant cell culture. I (II), 205-210.

12. Jain SK. Dictionary of Indian Folk Medicine and Ethnobotany, Deep Publication, New Delhi, 1991, 91.

13. Kashyap V, Yadav PK, Morya VK, Mishra SK and Yadav D, Plant tissue culture of *Flacourtia jangomas* (Lour.) Raeusch, an endangered plant", National Sensitization Programme on Biotechnology, March 2005, Small Industries Service Institute, Ministry of Small Scale industries, Govt. of India, Allahabad.

14. Kirtikar KR and Basu BD, Indian Medinal Plants (2$^{nd}$ ed.), pub. By L M Basu, Allahabad, 1935, 1, 220.

15. Lalsare S, Verma PK, Khatak M, Ranjan S, Rajurakar S and Gurav SS, Int. J. Of Drug Development and Research, 2011, 3(2).

16. Mitra RL, Flacourtiaceae, In Sharma BD and Balakrishnan (Eds.) Flora of India, BSI, Kolkata, 1993, 2, 403-405.

17. Murashige T and Skoog F, A revised medium for rapid growth and bioassay with tobacco tissue cultures, Physiologia Plant., 1962, 15, 473-479.

18. Pant M, Bisht P and Manju PG, *In Vitro* propagation through root-derived callus culture of *Swertia chirata* Buch. Ham. ex wall, African Journal of Biotechnology, 2012, 11(29), 7408-7416.

19. Rajsekharan P, *In vitro* plantlet regeneration of *Maesopsis emini* Enhl. Indian Journal of Forestry, 1993, 16 (4), 339-346.

20. Singh CK, Raj SR, Patil VR, Jaiswal PS and Subhas N, Plant regeneration from leaf explants of mature sandalwood (*Santalum album* L.) trees under *in vitro* conditions, *In vitro* Cellular and Developmental Biology-Plant, 2013 (online).

21. Turker AU, Camper ND and Gurel E, *In Vitro* culture of common mullein (*Verbascum thapsus* L.), *In Vitro* Cellular and Developmental Biology-Plant, 2001, 37(1), 40-43.

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