Botany Research International 6 (1): 13-16, 2013
ISSN 2221-3635

DOI: 10.5829/idosi.bri.2013.6.1.505

# *In-vitro* Flowering in *Vitex trifolia* L.

*C. Nagaveni and L. Rajanna*

Department of Botany, Bangalore University,
Jnanabharathi, Bangalore-560 056, India

**Abstract:** Direct organogenesis and *in vitro* flowering was obtained in *Vitex trifolia* L an endangered medicinal plant. High frequency and maximum number of multiple shoots were obtained from shoot tip explants on MS medium supplemented with BAP (3.96-15.85 µM) and IAA (5.70-22.83 µM). Regenerates, when transferred to rooting medium with IBA at concentration of 2.46-14.76 µM and IAA at concentration of 2.85-17.13 µM initiated flowering along with rooting. *In vitro* flowers set viable seeds. Rooted plantlets were hardened and transferred to green house with 100% survivability. This finding has significant role in Pharmaceutical industries and *in vitro* flowering facilities *in vitro* pollination and fertilization, further it also facilities in advancing the generation at much faster speed under limited progeny size in the segregating generation of *Vitex trifolia* L.

**Abbreviations:** IBA-Indole Butyric Acid · BAP-6-Benzyl adenine · IAA-Indole acetic acid
**Key words:** *In-vitro* · Flowering · Murashige and Skoog's media

## INTRODUCTION

*Vitex trifolia* L is a large coastalshrubor smalltree, less than 5 m in height with the stems covered by soft hairs (tomentose). The leaves are oppositely arranged along the stems and are usually compound, composed of 3 linear leaflets which range between 1-12 cm in length. The upper surfaces of the leaves are green and the lower surface grayish green. The flowers are born inpaniclesor clusters up to 18cm in length. Individual flowers have purple to violet two-lippedcorollasthat are approximately 5mm long. Thestamensare in two pairs and the ovary is superior, or develops above the corolla. The fleshy fruits are about 6mm in diameter and contain 4 small black seeds. *V. trifolia* is naturally found along coastlines from tropical East Africaas far east as French Polynesia. The leaves are used to treat female ailments in theCook Islands and used to relievefeverin Samoa. Additionally in Samoa, the dried leaves are burned to determosquitoes. Chemical constituents of *V. trifolia* are viteosin-A, vitexicarpin and vitetrifolin-E. Some provide valuablelumber. The flexible limbs of some species are used inbasket weaving. Some of thearomaticspecies are used medicinally. Recently simple leaf chaste tree is Red listed by IUCN with Low-Risk status.

*Vitex trifolia* Linn (Simple leaf chaste tree) is widely distributed from the Korean Peninsula to Southeast Asia and Australia. In China, the fruit of simple leaf chaste tree is called *Man Jing Zi* and is used for the treatment of headaches and colds, alleviation of fever, pain relief, sedation, anti-inflammatory treatment, etc. There are reports on use of the plant body or an extract of simple leaf chaste tree as an external medicine to be applied to the scalp as hair tonic [1]. However, the external medicine, such as a hair tonic or hair restorer, does not exhibit satisfactory effects. *Vitex trifolia*results in pregnancy, there is poor evidence based on theoretical and expert opinion and *in vitro* studies state that chaste tree may have estrogenic and progesteronic activity, uterine stimulant activity, emmenagogue activity and prevent miscarriages. In lactation, theoretical and expert opinion conflict as to whether chaste tree increases or decreases lactation [2].

Study of leaves of *V. trifolia* isolated three compounds-viteosin-A, vitexicarpin and vitetrifolin-E. Vitexicarpin was the most active of the three. The mechanism of activity seems to be non-competitive antagonism to histamine and stabilization of mast cells membrane function. Leaves yield an essential oil and resin. Fruit contains an acid resin, an astringent organic

**Corresponding Author:** L. Rajanna, Department of Botany, Bangalore University, Jnanabharathi, Bangalore, 560 056, India.

acid, malic acid, traces of an alkaloid and coloring matter. Chemical studies of leaves and twigs yield an essential oil, 0.11-0.28%. Chief constituents of the oil are l-d-pinene and camphene (55%); terpinyl acetate (10%); and a diterpene alcohol (20%). Study isolated a new benzofuran-type lignan, vitrifol A, from the fruits of *V. trifolia* with three known compounds. In a study investigating the inhibitory effect of vitexicarpin on the proliferation of human cancer cells showed it induces apoptosis in K562 cells via mitochondria-controlled apoptotic pathway.

In a study of four species of Vitex against *Culex quinquefasciatus* larvae, the highest larvicidal activity was found with the extract of *V. trifolia*. The *in vitro* phytochemical and pharmacological investigation of the non-volatile extracts of five South African *Vitex* species (Verbenaceae); *V. obovata* ssp. *obovata*, *V. obovata* ssp. *wilmsii*, *V. pooara*, *V. rehmannii* and *V. zeyheri* are carried out [3]. Many scientific investigations are done in other species of *Vitex* such as *V. rotundifolia, V. cannabifolia*, *V. altissima* and *V. negundo*. A negligible work is carried out in *V. trifolia* which is red-listed by IUCN with risk factor but conservation status near threatened (NT) which inspired us to select the plant.

## MATERIALS AND METHODS

Healthy plants of *V. trifolia* were collected from Botanical garden, Department of Botany, Bangalore University, Jnana Bharathi campus, Bangalore, Karnataka, India and used for experimental purpose. Stem cuttings were grown in pots. Plant is perennial and flowers during the month of August and October after monsoon.

**Plant Material:** Shoot tips were selected as explants for direct regeneration. The explants were thoroughly washed in running tap water for 10 minutes, disinfected by using liquid detergent tween-20 (5%v/v) for 15 min and rinsed with distilled water. The explants were then treated with 0.5% (w/v) Sodium hypochlorite solution (5 min) followed by a rinse in double distilled water. Later the explants were treated with 0.01%(w/v) Mercuric chloride (10 min) and 0.1%(w/v) Mercuric chloride solution (5 min), followed by Bavistin solution (30 min) and washed thoroughly in double distilled water. The surface sterilized explants were transferred aseptically to pre-sterilized culture tubes containing MS media [4] fortified with hormones. Then, the tubes were incubated in culture room under controlled conditions of White fluorescent light (16 hours light-8 hours dark), temperature (24±2°C) and humidity.

**Multiple Shoot Regeneration:** Plant growth regulators *viz.,* BAP (3.96-15.85 µM), IBA (4.92-19.68 µM) and IAA (5.70- 22.83 µM) were tried individually or in combination to obtain the multiple shoot bud induction. All the experiments were repeated three times to confirm the reproducibility.

**Root Induction:** The well-developed shoots were excised and were transferred to half strength MS medium supplemented with IBA (2.46-14.76 µM) and IAA (2.85-17.13 µM).

**Acclimatization:** Rooted plantlets were removed from culture tubes and washed their roots in running tap water and transferred to plastic cups containing 1:1:1 ratio of Soil rite: Coco peat: Vermiculite for about 15days and then transferred to pots containing sand and farm yard mixture (1:1) for a week and subsequently transferred to pots. All the tissue culture raised plantlets need gradual acclimatization for their survival in the field condition from controlled environment. Instead of transferring directly to the pots, plantlets were left for a week in the plastic cups at controlled temperature (25±2°C) with 60% relative humidity. After initiation of new roots, they were kept in the moist chamber and grown till maturity.

**Statistical Analysis:** Experiments were setup in a Randomized Complete Block Design (RCBD) and each experiment was repeated twice. Data were recorded on the percentage of response, number of shoots per explants, shoot length and number of roots per shoot. Means and Standard errors were carried out for each treatment.

## RESULTS AND DISSCUSSION

*Vitex trifolia.* L plants were efficiently regenerated from shoot tips. Explants were capable of directly developing multiple shoots on MS basal medium containing different concentrations of growth regulators. Multiple shoot initiation from shoot tip explants was observed within 10-15 days after inoculation (Fig. 1a). The highest number of shoots (15/ explant) was observed in the medium containing BAP 9.90µM/l followed by IBA 14.76µM/l (11/ explant). Of the two growth regulators (BAP and IBA), BAP was found to be more suitable than IBA for initiation and proliferation of multiple shoot buds (Table 1).

The elongation of shoots and proliferation of nodes were achieved on the same parental medium. In the present study, the relative effectiveness of BAP and IBA varied for *in vitro* multiple shoot regeneration from

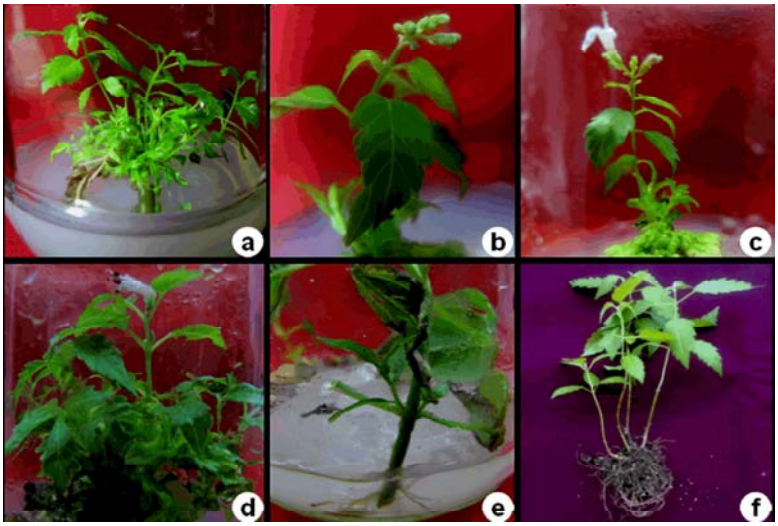


Fig. 1: a. Multiple shoots; b. Flowering bud initiation; c. In-vitro flowering; d. Fertilized flower and multiple shoots; e. Root formation and f. Hardening.

Table 1: Effect of BAP and IBA on shoot Multiplication from shoot tips explants of *Vitex trifolia.* L.

| Growth Regulators µM/l | Percentage of cultures with induced shoots | Number of shoots per explants | Shoot length (cm) |
|---|---|---|---|
| BAP | | | |
| 3.96 | 10.8±1.7 | 4.9±1.4 | 2.0±0.3 |
| 7.92 | 21.4±5.2 | 8.3±1.2 | 4.0±0.1 |
| 5.94 | 30.8±2.0 | 10.9±2.3 | 5.0±0.2 |
| 9.90 | 86.2±3.6 | 18.2±5.1 | 13.4±1.1 |
| 11.88 | 74.5±3.4 | 13.8±3.6 | 11.5±0.9 |
| 13.87 | 62.0±6.0 | 8.8±2.0 | 8.4±0.5 |
| 15.85 | 50.8±2.8 | 4.4±4.2 | 4.2±0.4 |
| IBA | | | |
| 4.92 | 5.1±1.1 | 2.7±1.6 | 3.5±0.3 |
| 7.38 | 16.5±2.4 | 5.7±2.0 | 3.7±0.7 |
| 9.84 | 26.4±2.9 | 7.2±1.5 | 4.7±0.4 |
| 12.30 | 56.5±3.0 | 10.2±2.2 | 7.6±0.3 |
| 14.76 | 91.2±2.7 | 12.6±4.4 | 12.8±0.4 |
| 17.22 | 86.2±4.7 | 9.0±1.5 | 11.7±0.2 |
| 19.68 | 60.7±3.5 | 5.9±2.1 | 8.1±0.1 |

Each value represents the mean ± SD of 10 replicates and each experiment was repeated at least thrice.

Table 2: Effect of different concentrations of IAA and IBA on rooting of *invitro* regenerated shoots of *Vitex trifolia* L.

| Plant Growth Regulators µM/l | Percentage of Root Induction | Number of Roots per Shoot |
|---|---|---|
| IBA | | |
| 2.46 | 10.8±2.0 | 5.7±1.3 |
| 4.92 | 24.5±3.0 | 8.5±1.8 |
| 7.38 | 44.2±6.3 | 11.6±4.7 |
| 9.84 | 95.1±3.9 | 14.3±1.6 |
| 12.30 | 80.8±1.7 | 11.8±1.5 |
| 14.76 | 60.9±1.4 | 7.4±1.0 |
| IAA | | |
| 2.85 | 15.7±4.1 | 3.6±1.6 |
| 5.70 | 20.9±1.9 | 6.4±2.2 |
| 8.56 | 42.9±6.7 | 7.8±1.5 |
| 11.41 | 85.8±2.5 | 11.6±1.3 |
| 14.27 | 70.8±5.0 | 9.7±2.7 |
| 17.13 | 55.2±1.7 | 5.0±0.9 |

Each value represents the mean ± SD of 10 replicates and each experiment was repeated at least thrice.

shoot tip explants. BAP (9.90µM/l) was found to be the best concentration for regeneration of multiple shoots buds (13 - 15). Shoots were harvested every 30-40 days and new shoot lets were harvested periodically. All the plantlets produced roots on the rooting medium (1/2 MS) containing IBA and IAA after 15days of incubation (Fig 1e; Table 2). Highest number of Roots were produced per shoot at 9.84 µ M/l IBA (11-14) and at 11.41 µ M/l IAA (9-12) after a week.

***In vitro* Flowering:** Flowering was considered to be a complex process regulated by both internal and external factors and its induction under *in vitro* culture is extensively rare. Temperature and Photoperiod has a great effect on *In vitro* flowering and was observed within 20 days of culture during our study (Fig. 1b) [5].

For *in vitro* flowering, the response of BAP is better than IBA. The *in vitro* regenerated plantlets were successfully transferred to plastic cups and then to the field (Fig. 1f).

The capacity of shoot bud differentiation and shoot proliferation from shoot tip explants of *Vitex trifolia.* L. depended on hormonal variation. There was a good shoot bud induction and proliferation response only in the presence of auxins and cytokinins and no response in the basal medium. Similar results are well documented in several medicinal plants [6], *Bixa ovellana* L. [7], *Emblila officinale* [8] and *Withania somnifera* [9]. From our study it was clear that BAP 9.90µM/l and IBA 14.76µM/l were significantly more effective for inducing shoot organogenesis. Well-developed shoot lets when transferred to rooting medium containing 9.84 µ M/l IBA induced higher frequency of roots than 11.41 µ M/l IAA (Fig.1e). Similar effect of IBA was reported in *Ocimum americanum*, *O. canum* and *O. sanctum* [6] and also in *Heracleum candicans* [10]. However, BAP was found to be the best *in vitro* flowering hormone than other Auxins and cytokinins, similar results were observed in Sunflower [11], in Mulberry [12] and in Coriander [13].

Hardening of regenerated plants was made on 1:1:1 ratio of Soil rite: Coco peat: Vermiculite and then transferred to pots containing sand and farm yard mixture (1:1) in Green house and subsequently to field. The survival rate was about 90% (Fig. 1 f).

From our experimental data, it is evident that BAP and IBA are the best suited for inducing multiple shoots and *in vitro* flowering and IBA for rooting. In conclusion, this communication describes an efficient rapid propagation system of *Vitex trifolia.* L.

## REFERENCES


1. Chuyaku Jiten, Dictionary of Chinese Medicines; pp: 2456-2458 and Japanese Unexamined Patent Publication Nos. 1984-116211 and 2000-31512.
2. Dugoua, J.J., D. Seely, D. Perri, E. Mills and G. Koren, 2008. Safety and efficacy of chastetree (*Vitex trifolia*) during pregnancy and lactation. Winter; 15(1) Can J. Clin Pharmacol., e80-6. Epub 2008 Jan 18.
3. Nyiligira, E., A.M. Viljoen, Van F.R. Heerden, *et al.,* 2008. Phytochemistry and *in vitro* pharmacological activities of South African Vitex (Verbenaceae) species. J. Ethnopharmacol., 119: 680-685.
4. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.
5. Moss, G.I., 1969. Influence of temperature and photoperiod of flower induction and inflorescence development in sweet orange. (*Citrus sinensis* L. Osbeck). Journal of Horticultural Science, 44: 311-320.
6. Pattnaik, S.K. and P.K. Chand, 1996. *In vitro* propagation of the medicinal herbs *Ocimum americanum* (L.) Sy. *Ocimum canum* Sim (Loary basil) and O. santum (Holly basil) Plant Cell. Rep., 15: 846- 850.
7. Sharon and Marie, 2000. *In vitro* clonal Propagation of Bixa ovellana L. Cur. Sci., 78(12): 1532.
8. Verma, B. and U. Kant, 1996. Micropropagation of Embilica officinale Gaertz through Mature nodel ex plant. J. Playtol. Res., 9(2): 107-109.
9. Deka, A.C., M.C. Kalita and A. Baruah, 1999. Micro propagation of a potent herbal Medicinal Plant, *Withania somnifera*. Environ. Ecol., 17(3): 594-596.
10. Wakhlu, A.K. and R.K. Sharma, 1999. Micropropagation of Heracleum candicans Wall.-a rare medicinal herb. *In vitro* cellular and Developmental Biol. Plant., 35(1): 79-81.
11. Patil, M.S., N.M. Ramaswamy and S.R. Sree Rangasamy, 1993. *In vitro* flowing in sunflower (*H. annuus* L.) Curr. Sc., 65: 565-566.
12. Naik, G.R. and K. Lata, 1996. *In vitro* flowering in *Morus alba* L. (Mulberry) Bull. Of pure and Applied Sci., 15: 129-132.
13. Stephen, R. and N. Jayabalan, 1998. *In vitro* flowering and seed setting formation of coriander (*Coriandrum sativum* L.). Curr. Sci., 74(3): 195-197.