**Publications:**

# Phytotechnology : Emerging Trends

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Chapter - 34

# *In-vitro* Clonal Propagation of Fig (*Ficus carica* L.) through Axillary Bud Culture

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## ABSTRACT

The fig tree *Ficus carica* L. (Moraceae), is deciduous and subtropical tree, well known for its nutritive value. The fruit, morphologically called as 'Syconium" is a vegetative fleshy tissue, with tiny true fruits enclosed inside. Fig and is consumed fresh or as dried fruit worldwide and has a high nutritive index. The fruit development is parthenocarpic and seeds are sterile and hence propagation is done through vegetative methods.

*In vitro* micro propagation technique allows production of disease free, uniform plants during a short span, and large quantities can be cloned from a selected original elite plant. However, there is no indication if the field trials have been carried out and whether the protocol is commercially viable. Moreover, such a protocol is no available for varieties grown in India.

Present paper reports a complete protocol for micro propagation of Fig from mature tree along with field trials. Nodal segments containing Axillary buds from a mature Fig tree were induced to produce large number of multiple shoots by culturing on MS medium (Murashige & Skoog, 1962) with different concentrations of 6-benzyl amino purine (BAP) in combination with naphthalene acetic acid (NAA). Excised shoots from this culture were rooted on ½ MS medium with 0.5 mg/l Indole butyric acid and activated Charcoal.

During Primary hardening, regenerated plantlets were successfully established in pro-trays with 78% success on a mixture of soil: coco peat: sand (1:1:1). Secondary hardening was done in 50% shade net nursery by transferring plants in poly bags filled with soil : farmyard manure mixture (2 : 1) and fully hardened plants were ready for field planting after two months. Comparisons were made on growth and fruiting of the tissue cultured plants with control plants.

**Keywords:** Fig, Axillary buds, *Ficus carica*, Primary and Secondary hardening.

*Ficus carica* L. (Moraceae), commonly known as fig or anjeer, provides the succulent fruit which is technically a Syconium, that is, fleshy, pear shaped hollow receptacle with a small opening at the apex partly closed by small scales. Anjeer is a well known for its nutritive value, both when dried and fresh. As a natural sweetener it is now commonly used in preparation of sweets. Its mild laxative action and high alkalinity finds its use in various drug preparations (Kirtikar and Basu, 1985).

## 34.1 WHY TISSUE CULTURE APPROACH IS REQUIRED FOR FIG?

Fig being a parthenocarpic fruit, does not have viable seeds, hence it is propagated through conventional methods such as; cutting, grafting and air layering. Out of these conventional methods only 20-30% cuttings survive due to poor rooting. Over the last few years, micro propagation techniques have been used for rapid large scale propagation of a number of fruit trees (Bajaj, 1986, Zimmerman, 1986). *In-vitro* work with figs has been restricted to production of fig-mosaic-virus free plants by single shoot tip elongation (Murithi *et al.*,1982; Pontikis and Melas, 1986). This communication provides the first protocols for multiple shoots induction from axillary buds and a complete protocol with field results for an Indian variety of fig.

## 34.2 MATERIALS AND METHODS

One or two days prior to collection of explants, selected mother plants were sprayed with fungicide solution (1 g/l of Carbendazim). Collected explants (axillary buds) were washed thoroughly under running tap water for 30 minutes, followed by immersion in 1% sodium hypochlorite solution containing APSA 2-3 drops (Amway product, USA) for 20 min. After rinsing with water, treatment with a mixture of fungicide and bactericide, i.e. Bavistin 1 mg /l and Streptomycin sulphate 40 mg/l was given for 45 min. Explants were surface sterilized with 0.1% $HgCL_2$ for 5 min. and washed several times with sterile water. Final immersion was done in solution containing 150 mg /l L-ascorbic acid and 100 mg /l citric acid.

The surface sterilized explants were inoculated vertically in different combination nutrient media of MS medium (Murashige and Skoog, 1962). The pH of different media used in the present study was adjusted to 5.7 to 5.8 before gelling with gelrite 2gm/l, dispensed into glass culture tubes (25 x 150 mm) stoppered with non-absorbent cotton and autoclaved at 121°C and 15 PSI for 15 min.

For induction of multiple shoots, explants were implanted on MS medium supplemented with BAP (1-5 mg/l) and NAA (0.1 to 0.5 mg/l). Cultures were incubated at 25± 1°C under 14 h photoperiod by cool white

fluorescent tubes. Then *in-vitro* regenerated shoots were subcultured every 2 weeks on fresh medium. Individual elongated shoots were used for the induction of roots on ½ MS strength supplemented with various concentrations of IBA, NAA, and IAA individually. Then *in-vitro* regenerated plantlets with well developed shoots and root systems were removed from rooting medium without breaking any roots), and transferred to cell tray containing Co opeat : Sand : Soil (1:1:1) and incubated in greenhouse where high humidity (95-80% RH) was maintained around the plantlets. During the 4 weeks of primary hardening, humidity was gradually reduced from 95 to 80% RH. Secondary hardening was done in a 50% shade nursery in polybags filled with mixture of Farm yard manure: Soil (1:2), for 1to 2 months. Finally hardened plants were field planted along with conventionally propagated plants of similar size.

## 34.3 RESULTS AND DISCUSSION

### 34.3.1 Culture Medium and Multiple shoot induction

On medium supplemented with 2 mg/l BAP with 0.2 mg/l NAA, axillary buds started sprouting from second week onwards. An average of 4-5 shoots developed from each axillary bud. This concentration of BAP was optimal. Lower or higher levels 1 or 3 to 5 mg/l of BA, the rate of multiplication (number of shoots /bud) does not increase.

### 34.3.2 Root Initiation and Elongation

After 5 subculture of multiple shoot cultures, all cultures were transferred in rooting medium for initiation of roots. In this rooting experiments different combination of IAA, IBA were used with ½ MS strength medium. The initiation of root development in *in vitro* regenerated individual shoots was achieved with 85% success after 4 weeks on ½ MS medium with 2 mg/l IBA and with activated charcoal (0.25 mg/l) (Table 34.1). Roots showed rapid elongation, when transferred to ½ MS medium with 2 mg/l IBA and with activated charcoal. In 4 weeks of subculture, roots induction and elongation were observed, average root induction occurred in 3 weeks and root length was 0.5 to 1.5 cm (Table 34.1). Similar results were observed in Jack fruit, where application of auxin was essential for adventitious root formation.

Repeated sub culturing may change the physiological state and gradually regenerate the shoots, which in turn promotes better rooting.

### 34.3.3 Transfer of Plantlets to the Green house

Plantlets with well developed root systems, transferred for primary hardening in different combination of Cocopeat, Soil, Vermiculate and Sand. Primary hardening experiment occurred in plastic poly tray and

maintained humidity 80% by covering plastic sheet on the tray. In this experiment got best result by using

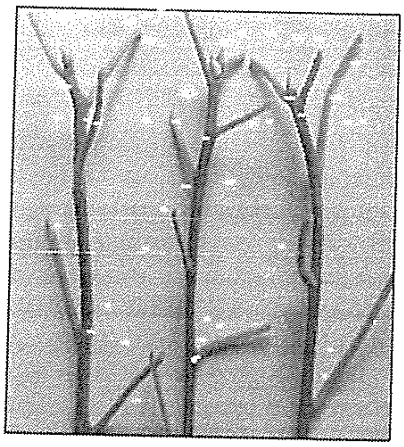
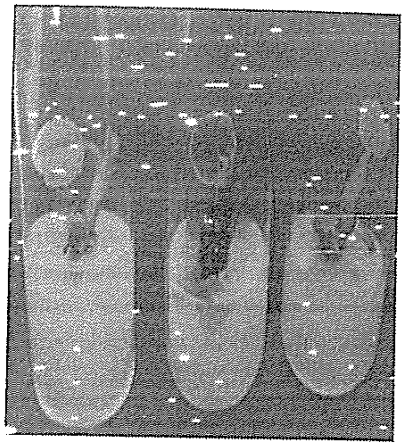
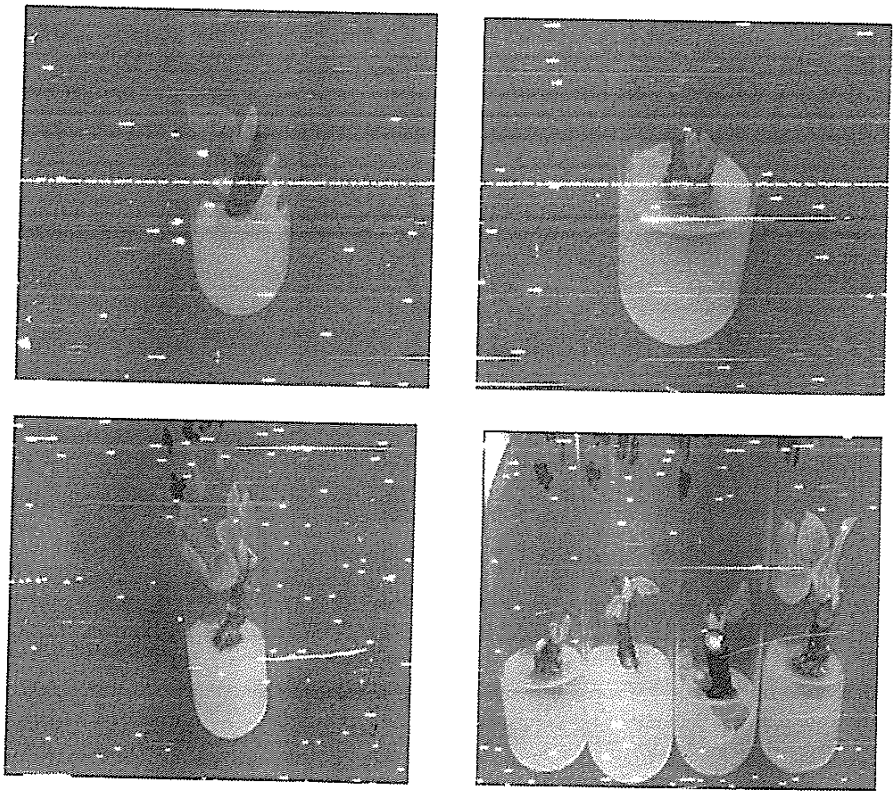


Fig explants

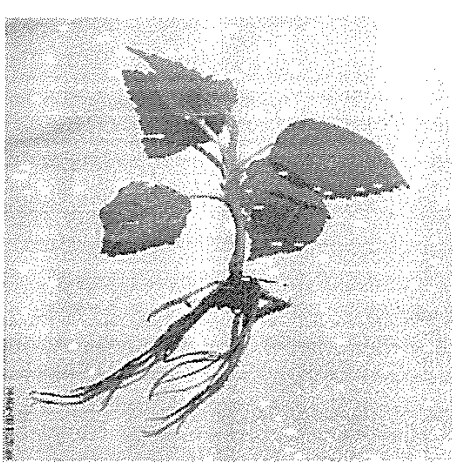


Sprouted cultures

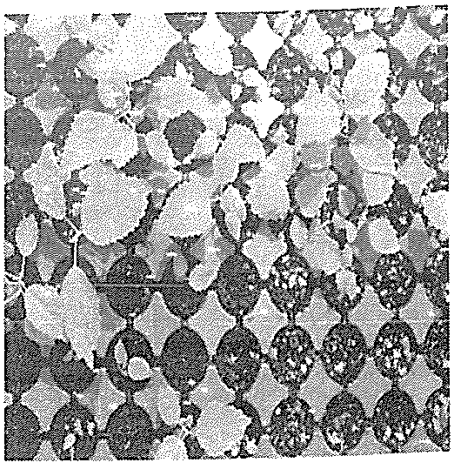
**Fig (Sprouting of Axillary buds)**



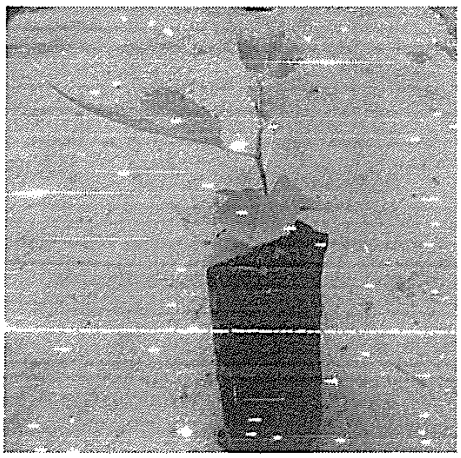
Photographs of Fig in Different stages



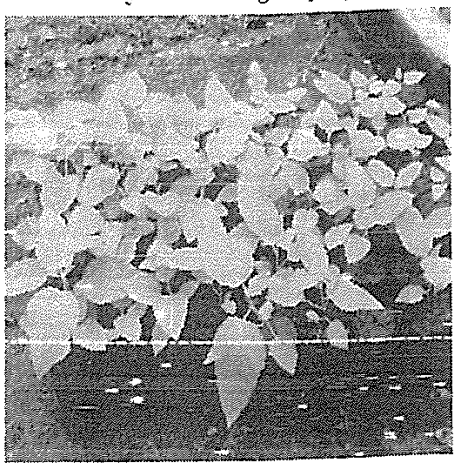
Rooted Plantlet



Primary Hardening in poly tray



Secondary hardening



Plant in polybag ready for field planting

**Table 34.1.** Morphogenesis response (sprouting and percentage survival rate) of *Ficus carica* grown in MS media

| S.No. | BAP (mg/l) | NAA (mg/l) | Bud Break after 15 days interval (% Survival) | Average shoots / ex-plant | Average shoot length (cm) |
|---|---|---|---|---|---|
| 1 | 1.0 | 0.1 | 45 | 1-3 | 0.5 |
| 2 | 2.0 | 0.1 | 75 | 4-5 | 0.8 |
| 3 | 3.0 | 0.1 | 55 | 3-4 | 1.3 |
| 4 | 4.0 | 0.1 | 45 | 3 | 1.2 |
| 5 | 5.0 | 0.1 | 35 | 2-3 | 0.9 |

*Note:* Values determined after four weeks from three individual experiments.

**Table 34.2.** Effect of Basal salt concentration

| MS Salt Concentration | Average shoot/culture | Shoot Length (cm) | % Plant showing Roots | Average roots/plant | Leaves |
|---|---|---|---|---|---|
| ½ MS Salt | 1.6 | 0.8 | 66 | 1.8 | Normal |
| Full MS Salt | 1.66 | 0.75 | 46 | 1.2 | Normal |

*Note:* All media contain 2 mg/l IBA
Data based on 250 cultures inoculated.

**Table 34.3.** Effect of Auxin (IAA, IBA, NAA)

| | Auxin Concentration | No. of roots/ culture | Root length (cm) | % rooting | Leaves |
|---|---|---|---|---|---|
| IAA | 5 µM | 3 | 1.5 | 15.62 | Normal |
| | 10 µM | 2 | 1.0 | 6.85 | |
| | 15 µM | 1 | 0.8 | 7.81 | |
| IBA | 5 µM | 5 | 0.7 | 21.4 | Normal |
| | 10 µM | 8 | 1.3 | 9.84 | |
| | 15 µM | 4 | 1.0 | 5.07 | |
| NAA | 5 µM | 2 | 0.5 | 16.66 | Normal |
| | 10 µM | 2 | 0.7 | 4.03 | |
| | 15 µM | 1 | 0.9 | 1.56 | |

Cocopeat, soil and sand (1:1:1) with 78.57% success. (Table 34.4). After 4 weeks interval healthy primary hardened plantlets (plugs), transferred for secondary hardening in 50% shade net house. In secondary hardening used potting mixture of Soil and Farm yard manure (2:1). In this experiments got 82.08% survival rate of primary hardened plants. Fully hardened plants were ready for field planting after two months. The regenerated plants did not show any detectable variation in growth as morphological characteristics of leaves and fruits when compared with donor plants. Due to constraints in conventional propagation methods in *Ficus carica*, there is a shortage of planting stock for commercial propagation. This could be overcome by following the protocol developed in the present study.

**Table 34.4.** Primary Hardening Experiments

| S.N. | Potting Mixture | Inoculate plantlets | After 15 days | After 21 days | After 29 days | Percentage Survival |
|---|---|---|---|---|---|---|
| 1 | Coco peat | 28 | 7 | 3 | 18 | 64.28 |
| 2 | Coco peat + 25% Vermiculate | 28 | 8 | 4 | 16 | 57.14 |
| 3 | Coco peat + 25% Vermicompost | 28 | 12 | 5 | 11 | 39.28 |
| 4 | Coco peat + 25% Sand | 28 | 4 | 2 | 22 | 78.57 |

### 34.3.4 Secondary Hardening:

- In sec. Hardening used potting Mixture: (1:1:1), Soil: Cocopeat: sand.
- Total transferred plants in sec. Hardening : 67
- Mortality occurred in the end of April and may : 12 plants
- Currently total survive of sec. hardened plant : 55

Survival rate in sec. hardened plants: **82.08 %.**

## REFERENCES

Bajaj, Y.P.S. (1986) *Biotechnology in agriculture and forestry*, vol 1 Trees I. Springer, Berlin Heiderberg Newyork.

Hepaksoy, S. and Aksoy, U. (2006). Propagation of *Ficus carica* L. clones by *in-vitro* culture *Biologia Plantarum* 50 (3): 433-436.

Kirtikar, K.R. and Basu, B.D. (1986) In Blatter E. Caius IF (eds.) Indian Medicinal Plant, Vol. III 2nd ed. International Book Distributor, Dehradun, pp 2329-2331

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*15: 473-497.

Murithi, M., Rangan, T.S. and Waite, B.H. (1982) *In vitro* propagation fig through shoot tip culture. *Hort Sci* 17:86-87

Pasqual, M. and Ferreira, E.A. (2007) Micro propagation of Fig tree, Protocols of Micro propagation for Woody Trees and Fruits. 409-416.

Pontikis, C.A. and Melas, P. (1986) Micro propagation of *Ficus carica* L. *Hort. Sci* 21:153S.

# COMMERCIAL MICROPROPAGATION OF *Citrus aurantifolia*

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**Abstract** *Citrus aurantifolia* Swingle L occupies third place among Indian fruits covering 0.23 million ha. area with production of about 7.52 tons /ha fruits. There is a great requirement of quality planting material of this fruit crop as it is best suited in the semi arid climate of North Gujarat and Rajasthan giving handsome revenue of Rs. 3 to 4 lac per acre in orchard with quality planting material.

This paper describes a commercial micro propagation technology using ex-plants taken from elite, mature trees of **Kagzi** lime. Elite characters considered are:

1. Large size of fruit
2. Thin exocarp of fruit
3. High quantity of juice
4. Disease free plant
5. High yield

Apical buds and Axillary buds explants from fresh sprouts of mature trees were used for culture initiation on Murashige and Skoog (1962) medium containing combinations of NAA (0.1 mg/l) with 6-benzyl aminopurine (1.0 mg/l).

Optimum multiplication of shoots (4-6 shoots/culture) was obtained with (1.0 mg/l BAP and 0.1 mg/l NAA). Transfer of shoots to a rooting medium induced the highest percentage of rooting (70%) on media containing IBA & BAP (1.0mg/l & 0.1 mg/l respectively). Primary hardening was done in pro-trays in a mixture of soil: vermiculite mixture (1:1) in poly tunnel kept in the

green house. Secondary hardening was done in 50% shade net nursery by transferring plants in poly bags filled with soil: farmyard manure mixture ( 2: 1 ). Total hardening cycle was 3 months - one month of primary hardening and two months of secondary hardening.

Several thousand plants of 6 month age were planted in field with no mortality. These plants flowered after three years of vegetative growth and produced normal fruits. Thus the micro propagation technology produced true to type plants.

**Key words:** - In vitro micro propagation, *Citrus aurantifolia* Swingle L., Kagzi lime.

**Abbreviations**:-

MS medium :- ( Murashige and Skoog medium ),

Cytokinin (6- BAP: – 6-Benzyl aminopurine).

Auxin (NAA:-1- Naphthalene acetic acid), (IBA: - Indole-3-Butyric acid).

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# COMPARATIVE ANALYSIS OF BT AND NON BT HYBRID COTTON WITH SPECIAL REFERENCE TO GROWTH, BIOCHEMICAL CHARACTERS AND RHIZOSPHERIC MICROBES

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## Abstract

Transgenic technology has been used to develop cotton with the beneficial trait of insect resistance by inserting a *Cry 1 Ac* gene, from the naturally occurring soil bacterium *Bacillus thuringiensis* sub sp. *Kurstaki*. We investigated the efficacy of Bt and conventional non Bt cotton in terms of growth parameters such as shoot and root length, fresh and dry weight and leaf area, biochemical parameters *viz.*, photosynthetic pigments, protein, glucose, leaf nitrate, nitrate reductase activity and total free amino acid, enumerating rhizosphere soil microflora and germination of seed under three different temperatures. Results of basic growth parameters of Bt cotton were found to be higher than non Bt cotton plants, which could be due to insecticidal protein secretion by Bt. Photosynthetic pigments of Bt cotton were higher than that of non Bt cotton, which indicates the mobilization of resources for synthesis of pigments in Bt cotton. From day 50 of seedling development there was trend for a decreasing levels in Bt cotton plants, which was in contrast to the glucose content that increased in Bt and non Bt cotton due to maturity and health of the plants. Rhizosphere soil microbial population of Bt cotton were found to be low compared to non Bt cotton, which we hypothesized as indicating an effect of *Cry 1 Ac* (endotoxin) protein in the soil. Ambient temperature (21-30°C) is suitable for the germination of Bt and non Bt cotton seeds. Finally, we conclude that further studies at molecular level are needed to prove the reduction of microbial population in Bt soil for biosafety assessment.

***Key words :*** Growth, biochemical, nitrate reductase activity, chlorophyll, rhizospheric, soil microflora.

## Introduction

Scientific advances in cell and molecular biology are now gaining interest in genetic engineering for enhancing beneficial traits of plants by introducing desired genes. Such plants carrying the alien gene(s) are called transgenic plants. Transgenic technology can be utilized to develop plants with the beneficial traits of insect resistance, salt stress tolerance and cold tolerance etc. Cotton is a commercial crop and cultivated in all over the world for its valuable fiber. However, it is highly susceptible to insects, especially to the larvae of Lepidopteron pests such as *Helicoverpa armigera* commonly referred to as the bollworm. For controlling the bollworm, farmers are applying huge amount of insecticides to cotton plants. 12 billion Rupees worth of pesticides are used in India to control the bollworm of cotton (Barwale *et al.*, 2004). In many developing countries, like India, small scale farmers suffer pest related yield losses because of technical and economic constraints. In this case, insecticidal proteins present in the soil borne bacterium, *Bacillus thuringiensis*.(Bt), which has been demonstrated its efficacy as a spray formulation in agriculture over the past five decades, have been expressed in many crop species including cotton with positive results (Kumar *et al.*, 1996).

Bt cotton, a transgenic plant, produces an insect controlling protein *Cry 1 Ac* (which provides resistance to American and spotted bollworm), the gene for which has been derived from the naturally occurring bacterium,

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*Bacillus thuringiensis* sub sp. *Kurstaki* (Anandakumar, 2007). The cotton containing Bt gene produces its own toxin for bollworm attack, thus, significantly reducing the chemical insecticide use and providing a major benefit to cotton growers and the environment (Velkov *et al.*, 2005; Qaim and Zilberaman, 2003). The first transgenic Bt crops *viz.*, cotton, corn and potato were commercialized in USA in 1995 and 1996. Currently more than a dozen countries cultivate Bt crops. Bt cotton was permitted for commercial cultivation in India in 2002. Bt transgenic crop species such as corn, rice, tomato and potato have been commercialized with substantial benefits to the farmers (Kumar, 2003). Bt crops were cultivated in an area of 32.1 million hectares out of the global transgenic area of 102 million hectares in 2006 (James, 2006).

Introduction of commercial Bt cotton varieties producing the insecticidal proteins is expected to improve growth profitability, reduce environmental pollution from synthetic insecticides (Gould, 1988; Gasser and Fraley, 1989). One million acres of insect tolerant cotton were used in commercial planting in China in 1997, where the technology had reduced both the cost of pesticide applications and exposure to pesticides (Guo *et al.*, 1999; Zhao *et al.*, 2000; Pray *et al.*, 2002). However, the introduction of GM plants into agricultural ecosystems has raised the ecological impact of these plants in soil ecosystem. GM plants are capable of causing damage not only to the insect pests, but also to the environment (Genetic Pollution) and the consumer human beings (allergic reaction) (Zhang *et al.*, 2002). In fact, the soil organisms may be affected on being exposed to *Cry* proteins which are leached from roots of Bt cotton or from incorporation of above ground plant tissues into the soil after harvest (Icoz and Stotzky, 2008; Conner *et al.*, 2003). The paucity of information about the growth, biochemical characters and the rhizosphere soil microflora of transgenic and non transgenic cotton plants have led us to carried out this work. So, the main objective of our study was to compare the efficacy in terms of growth and biochemical characters of Bt cotton and non Bt cotton, enumerating the soil microflora for their biosafety assessment.

## Materials and Methods

BT cotton (RCH–20, Rasi Seeds, Salem) and non-transgenic cotton (Supriya, Agnar Efforts, Srivilliputtur) were raised in managed land located near the Botany Department, Ayya Nadar Janaki Ammal College, Sivakasi. Soil at this land is brown to black in colour and loamy. We measured the growth parameters, photo synthetic pigments and biochemical characters of Bt cotton and non Bt cotton at the regular 10 day interval from 20 to 60 days. We measured the following growth parameters such as Shoot and root length, fresh and dry weight and leaf area of the plants by using the standard methods. Photosynthetic pigments *viz.*, chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids were determined using the ELICO SL 171 spectrophotometer at 662 nm, 645 nm and 470 nm respectively (Wellburn and Lichtenthaler, 1984). Biochemical characters such as protein, glucose, leaf nitrate, nitrate reductase activity and total free amino acid (Lowry, 1951; Jayaraman, 1981; Cataldo *et al.*, 1978; Jaworski, 1971; Jayaraman, 1981) were assayed in the fresh leaf samples at the end of 20, 30, 40, 50 and 60 days of seedlings. We conducted a germination experiment of seed (Bt and non Bt cotton) under three different temperatures; high (31-40°C), ambient (21-30°C) and low (10-20°C) for ascertaining how the different temperatures affect the germination of seeds. Microbial population of the rhizosphere soil was analyzed at the end of 50$^{th}$ day of seedlings using a plate technique (Gunasekaran, 1996). Briefly, totally three replicates with four different concentration of dilutions ($10^{-1}$–$10^{-4}$) were used to identify and count the colonies. Microbial colonies were enumerated with the help of colony counter and the morphological characters of colonies were identified based on their appearance.

## Results and Discussion

Results of the growth parameters are mentioned in table 1. Shoot length of Bt cotton was found to be high when compared to that of non Bt cotton at the end of 20$^{th}$ day. Likewise, in 30 day old seedlings also, the shoot length was increased slightly in Bt cotton and the trend was same in 40, 50, 60$^{th}$ day old seedlings of Bt cotton. Bt cotton root length was also slightly higher at 20, 30, 40, 50 and 60$^{th}$ days when compared to that of its respective non-Bt cotton seedlings.

Fresh weight of Bt cotton was also higher than that of non-Bt cotton. Slight variations are observed in the 20, 30, 40, 50 and 60$^{th}$ day plant of Bt cotton. It was found that the dry weight of Bt cotton also slightly higher than that of non-Bt cotton at the end of 20, 30, 40, 50 and 60$^{th}$ day of seedlings. Estimation of leaf area is considered to be an important parameter in assessing the plant growth. At the end of the analysis, leaf area of Bt cotton was higher than that of non-Bt cotton. The growth parameters such as, shoot length, root length, fresh weight, dry weight and leaf area of Bt cotton was found to be higher than the non Bt cotton, which might be due to the insecticidal protein (*Cry 1 Ac* protein), secreted by the Bt cotton plants. It was earlier reported that *Cry 1 Ac* protein may

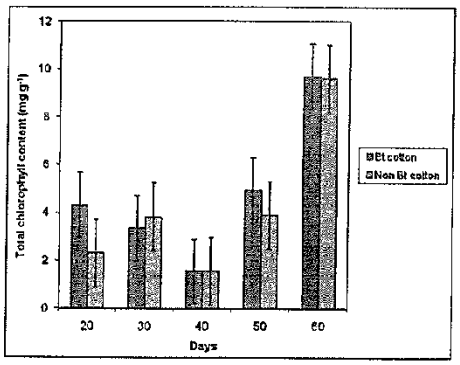


Fig. 1 : Comparative analysis of Bt and non Bt cotton in relation to their total chlorophyll content (mg/g).

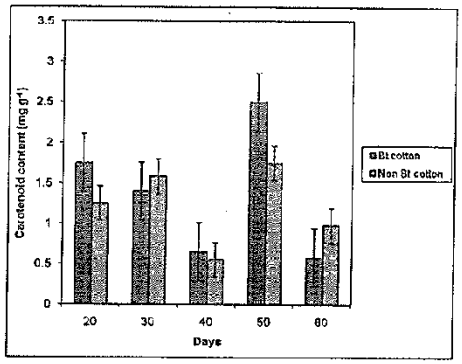


Fig. 2 : Comparative analysis of Bt and non Bt cotton in relation to their carotenoid content (mg/g).

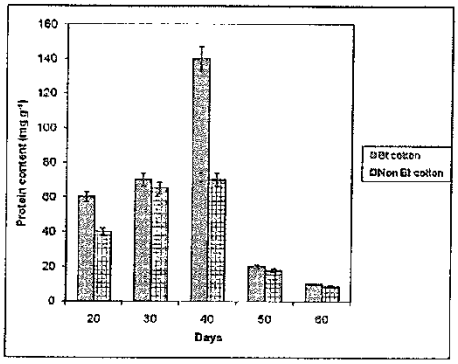


Fig. 3 : Comparative analysis of Bt and non Bt cotton in relation to their protein content (mg/g).

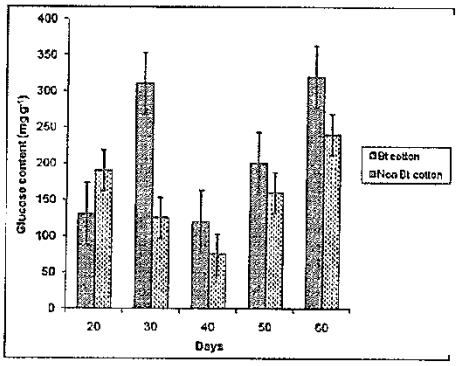


Fig. 4 : Comparative analysis of Bt and non Bt cotton in relation to their glucose content (mg/g).

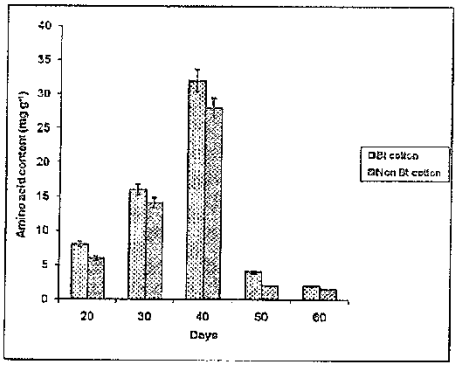


Fig. 5 : Comparative analysis of Bt and non Bt cotton in relation to their amino acid content (mg/g).

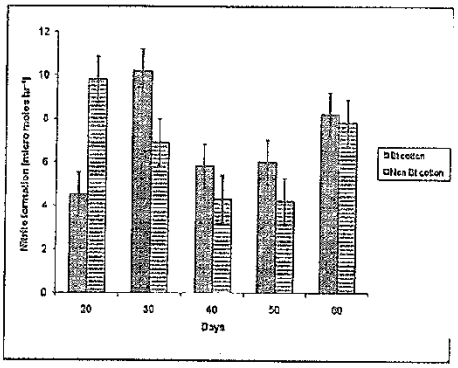


Fig. 6 : Comparative analysis of Bt and non Bt cotton in relation to their NR activity in terms of nitrite formed (µ moles/hr).

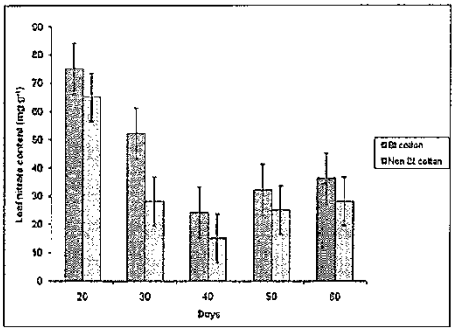


Fig. 7 : Comparative analysis of Bt and non Bt cotton in relation to their leaf nitrate content (mg/g).

and 60th day compared to non-Bt cotton (fig. 2), this might be due to the mobilization of resources for the synthesis of pigments. This was in confirmation with the earlier report (Gould, 1988).

Results of the biochemical studies are mentioned in figs. 1-7. Under normal growth conditions, the biochemical constituents were studied in terms of protein, free amino-acid, glucose content, leaf nitrate content and nitrate reductase activity. The level of protein content in Bt cotton was high, when compared to non-Bt cotton. The level of protein content was high only in trace amount and no appreciable changes were found between Bt cotton and non-Bt cotton (fig. 3). It was contrast to the earlier finding (Hosagoudar *et al.*, 2008) where they reported that the protein content was reduced from healthy to infected

Table 1 : Growth characters of Bt and Non Bt cotton at different days of seedlings.

| S.No. | Characters | Days | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 20 | | 30 | | 40 | | 50 | | 60 | |
| | | Bt cotton | Non Bt cotton | Bt cotton | Non Bt cotton | Bt cotton | Non Bt cotton | Bt cotton | Non Bt cotton | Bt cotton | Non Bt cotton |
| 1. | Shoot length (cm) | 13.32 (±1.04) | 13.0 (±0.39) | 14.36 (±0.52) | 14.06 (±0.82) | 14.62 (±0.28) | 14.26 (±0.14) | 26.2 (±0.52) | 18.06 (±0.29) | 27.58 (±0.26) | 20.16 (±0.34) |
| 2. | Root length (cm) | 10.25 (±0.77) | 10.25 (±0.13) | 12.43 (±1.43) | 11.60 (±0.40) | 12.95 (±0.34) | 11.96 (±0.33) | 22.03 (±1.13) | 17.50 (±0.87) | 22.44 (±1.12) | 18.17 (±0.77) |
| 3. | Fresh weight (gm) | 1.43 (±0.04) | 1.30 (±0.05) | 1.51 (±0.01) | 1.42 (±0.05) | 1.21 (±0.03) | 1.15 (±0.04) | 2.03 (±0.57) | 1.98 (±0.11) | 3.08 (±0.04) | 2.96 (±1.21) |
| 4. | Dry weight (gm) | 0.81 (±0.01) | 0.78 (±0.05) | 0.98 (±0.31) | 0.96 (±0.30) | 1.0 (±0.21) | 0.91 (±0.01) | 0.68 (±0.04) | 0.62 (±0.03) | 0.98 (±0.32) | 0.72 (±0.11) |
| 5. | Leaf area ($cm^2$) | 5.38 (±0.07) | 4.85 (±0.21) | 7.12 (±0.05) | 7.02 (±0.05) | 7.22 (±0.01) | 7.18 (±0.31) | 11.64 (±0.52) | 10.24 (±0.27) | 14.17 (±0.41) | 12.25 (±0.28) |

± Values indicate standard error, $n = 5$.

Table 2 : Morphological characters of Bt cotton rhizosphere soil microbes.

| S.No. | Growth characters | Moderate yellow colour colony | Golden yellow colour colony | Abundant white colour colony | Abundant thick white glistering colony | White moist green colony | Pink colour colony |
|---|---|---|---|---|---|---|---|
| 1. | Size | Small | Small | Small | Moderate | Small | Small |
| 2. | Pigmentation | Yellow | Yellow | White | Watery white | Green | Pink |
| 3. | Form | Irregular | Circular | Circular | Irregular | Circular | Circular |
| 4. | Margin | Undulated | Entire | Entire | Undulated | Entire | Entire |
| 5. | Elevation | Flat | Flat | Raised | Raised | Raised | Raised |

influence the growth of Bt cotton plants when compared to other plants (Gould, 1988). Accumulation of chlorophyll pigment was higher in Bt cotton, compared to non-Bt cotton plants (fig. 1). The level of carotenoid was also high in Bt cotton at 20, 30, 40th day plant, but low in 50 conditions and also the protein content was little higher in non Bt cotton genotype than the Bt cottons. However, there is a decreasing trend as far as the total protein content of leaves is concerned as the plant gets maturity. In contrast to this, it was observed that the sugar content

Table 3 : Morphological characters of non Bt cotton rhizosphere soil microbes.

| S.No. | Growth characters | Moderate yellow colour colony | Golden yellow colour colony | Abundant white colour colony | Abundant thick white glistering colony | White moist green colony | Pink colour colony | Brown colour colony |
|---|---|---|---|---|---|---|---|---|
| 1. | Size | Small | Small | Moderate | Moderate | Small | Small | Moderate |
| 2. | Pigmentation | Yellow | Yellow | White | Watery white | Green | Pink | Brownish white |
| 3. | Form | Irregular | Circular | Circular | Irregular | Circular | Circular | Circular |
| 4. | Margin | Undulated | Entire | Entire | Undulated | Entire | Entire | Entire |
| 5. | Elevation | Flat | Flat | Raised | Raised | Raised | Flat | Raised |

Table 4 : Germination of BT cotton and non Bt cotton of seeds under three different temperatures ranges.

| S. No. | Temperature | Bt cotton (percentage of seed germination) | Non Bt cotton (percentage of seed germination) |
|---|---|---|---|
| 1. | 10–20°C | 20% | 40% |
| 2. | 21–30°C | 40% | 60% |
| 3. | 31–40°C | 20% | 20% |

was high in Bt cotton than the non Bt cotton, and there was reduction from healthy to infected conditions (Hosagoudar *et al.*, 2008). Results of our study are in the same line, in which the glucose content of the leaf also increased as the plant gets maturity. Accumulation of glucose content was also varied at the end of 30, 40, 50 and 60th day plant. Glucose content of non-Bt cotton was high at the end of 20th day when compared to Bt cotton (fig. 4). Amino acid content was also slightly higher in Bt cotton than non-Bt cotton (fig. 5). Nitrate reductase activity of Bt cotton was high in 30, 40, 50 and 60th day plant. At 20th day, the NR activity was increased in non-Bt cotton compared to Bt cotton (fig. 6). Overall, NR activity was increased when compared to non Bt cotton. Recent study (Sarkar *et al.*, 2008) found that NR activity in the soil of Bt cotton was increased while it was low in non Bt cotton soil. Leaf nitrate content in leaves were high in Bt cotton when compared to that of non-Bt cotton. Because of the availability of nutrient in the soil, leaf nitrate content was slightly higher in Bt cotton (fig. 7). The NR activity and leaf nitrate was found to be affected a lot which might be due to the impairment of nitrogen metabolism by the action of Bt toxin protein which was secreted by the Bt cotton (Chen *et al.*, 2003).

**Rhizosphere soil microbes of Bt cotton and non Bt cotton**

Soil microflora of Bt cotton and non Bt cotton were analyzed at the end of 50th day. Bt cotton rhizosphere soil had a low number of microbes and compared to that of non-Bt cotton (tables 2-3). This was totally in line with the earlier views Giovannetti *et al.* (2005), where they argued that the risks associated with the transgenic plants because of the Bt protein effects in natural agro ecosystems, out weighing harmfulness against costs.

**Germination of Bt and non Bt cotton under three different temperatures**

In the present study, the effect of three different temperatures on germination of Bt cotton and non Bt cotton were observed and found that high temperature and low temperature affected the germination of seed (table 4). Ambient temperature (21-30°C) is better for the growth of both the cotton plants and percentage of seed germination at this temperature was higher in Bt cotton than non Bt cotton seeds. This was in confirmation with the earlier report (Chen *et al.*, 2004) which reported that different temperatures would affect the germination of Bt and non Bt cotton seeds.

## Conclusion

The present study aimed at measuring the efficacy of Bt cotton and non Bt cotton vis-à-vis various growth and biochemical characters have concluded that the foreign gene (*Cry 1 Ac*) has a impact on the various growth (Shoot and root length, fresh and dry weight and leaf area) and biochemical characters (Photo synthetic pigments, protein, glucose, leaf nitrate, nitrate reductase activity and total free amino acid). Low protein and high glucose content of Bt and non Bt cotton gave the maturity and healthiness to infected plants. Less number of rhizosphere microbial colonies of Bt cotton compared to non Bt cotton indicate that the effect of Bt toxin protein. It may leach from the Bt cotton roots. Results of the germination study confirm that the ambient temperature is suitable for the growth and the germination of Bt and non Bt cotton seeds.

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## References

Barwale, R. B., V. R. Gadwal, U. Zehr and B. Zehr (2004). Prospects for Bt cotton technology in India. *Ag. Bioforum*, **7** : 23-26.

Kumar, P. A., R. P. Sharma and V. S. Malik (1996). Insecticidal proteins of *Bacillus thuringiensis*. *Advances in Applied Microbiology*, **42** : 1-43.

Anandakumar, P. (2007). Biosafety of Bt crops: safe transition. *Biotech News*, Dept of Gov. Ind, **2** : 1-3.

Velkov, V. V., A. B. Medvinsky, M. S. Sokolov and A. I. Morchenko (2005). Will transgenic plants adversely affect the environment? *J. Bio Sci.*, **30(4)** : 515-548.

Qaim, M. and D. Zilberman (2003). Yield effects of genetically modified crops in developing countries, *Science*, **299** : 900–902.

Kumar, A. P. (2003). Insect pest–resistant transgenic crops. In R. K. Upadhyay (Ed), Advances in microbial control pf insect pests, Kluwer academic, 2003, 71-82.

James, C. (2006). Global status of commercialized botech/GM crops. ISAAA Brief No. **35**, 2006, ISAAA, Ithaca.

Gould, F. (1988). Evolutionary biology and genetically engineered Crops. *Bio. Sci.*, **38** : 26-33.

Gasser, C. S. and R. T. Fraley (1989). Genetically Engineered plants for Crop Improvement, *Science*, 1993-1299.

Guo, X. M., H. T. Liu, Y. S. Zhang and A. D. Qiang (1999). The technique and achievement of Bt transgenic Cotton breeding China. *Chin. Cottons*, **7** : 2-5.

Zhao, J. Z., K. J. Zhao, M. G. Lu, X. L. Fan and S. D. (2000). Guo, Interactions between *Helicoverpa armigera* and transgenic Bt Cotton in North China. *Chin. Agric. Sci.*, **6** : 1-7.

Pray, C. E., J. K. Huang, R. F. Hu and S. Rozlle (2002). Five years of Bt Cotton in China. The benefits Continue. *Plant J.*, 4 : 423-430.

Zhang, B. H., Q. L. Wang, K. B. Weng, D. Zhar and F. Liu (2002). Bt cotton in India. *Curr. Sci.*, **86(6)** : 25.

Icoz, I. and G. Stotzky (2008). Fate and effects of insect–resistant Bt cropsin soi ecosystems. *Soil Bio. Biochem.*, **40** : 559-586.

Conner, A. J., T. R. Glare and J. P. Nap (2003). The release of genetically modified crops in the environment. *The Plant J.*, **33** : 19-46.

Wellburn, A. R. and A. Lichtenthaler (1984). Formulae and programme to determine total carotenoids and chlorophyll *a* and *b* of leaf extracts in different solvents. *In* : Sybesma (Ed), Advances in Photosynthesis Research, Martinus Nisjhoff / Dr. W. Junk. *The Hague*. **2** : 9–12.

Lowry, O. H., N. J. Rosenburg, A. L. Fair, R. J. Randall. Protein measurement with folin phenol reagent. *J. Biochem.*, **193** : 265–275.

Jayaraman, J. (1951). Estimation of Glucose. *In*: Laboratory Manual in Biochemistry, Willey – Eastern Limited, Madras. 1981, 1–65.

Cataldo, D. A., M. Haroon, L. E. Schroader and V. C. Yonger (1978). Rapid Colorimetric determination of nitrate in plant by nitrate salicylic acid. *Commun Soil Sci., Plant. Anal.*, **6** : 71-80.

Jaworski, E. G. (1971). Nirtrate reductace assay in nitrate plant tissues. *Biochem. Biophy. Res. Commun.*, **43** : 1274–1279.

Gunasekaran, P. (1996). Enumeration of rhizospheric microbial population. *In*: Laboratory Manual in Microbiology. New Age International (P) Limited, New Delhi, 101–102.

Govindappa, N., S. Hosagoudar, N. Chattannavar and S. Kulkarni (2008). Biochemical Studies in Bt and Non-Bt Cotton Genotypes Against Alternaria Blight Disease( *Alternaria macrospora* Zimm.). *Karnataka J. Agric. Sci.*, **21(1)** : 70-73.

Sarkar, B., A. K. Patra, T. J. Purakayastha and M. Megharaj (2008). Assessment of biological and biochemical indicators in soil under transgenic Bt and non Bt cotton cropin a sub–tropical environment, Environ Monit Assess.

Zhen, D. H., C. Q. Yang, Y. Chen and Y. K. Wu (2003). The studies on the expression characteristics of the insecticidal protein and relationship with nitrogen metabolism in Bt Cotton. *Chin. Cotton.*, **7** : 10-12.

Gupta, V. V. S. R. and S. Watson (2004). Ecological impacts of GM cotton on soil biodiversity: Below ground production of Bt by Gm cotton and Bt cotton impacts on soil biological processes. Australian government department of the environment and heritage, CSIRO land use and water, 1-72.

Turrini, A., C. Sbrana, M. P. Nuti, B. Pietrangli and M. Giovannetti (2004). Development of model system toassess the impact of genetically modified corn and aubergine plantson arbuscular mycorrhizal fungi. *Plant and Soil*, 266 : 69-75.

Xue, K., H. F. Luo, H. Y. Qi and H. X. Zhang (2005). Changes in soil microbial community structure associated with two types of genetically engineered plants analyzing by PLFA. *J. Environ. Sci.(China)*, **17** : 130-134.

Giovannetti, M., C. Sbrana and A. Turrini (2005). The impact of genetically modified crops of soil microbial communities. *Rivista di Biologia/ Biology forum*, **98** : 393-418.

Chen, D., G. Ye, C. Yang, Y. Chen and Y. Wu (2004). The effect of high temperature on the insecticidal properties of Bt cotton. *Env. Exp. Bot.*, **53** : 333-342.

***Abbreviation:*** **Bt-** *Bacillus thuringiensis.*