

## Impact of cefotaxime on *in vitro* shoot elongation and regeneration in banana (*Musa acuminata*)

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### Abstract

This is the first report of antibacterial and growth promoting effectiveness of cefotaxime in banana tissue culture. Young suckers (3-13 cm diameter) of commercially important banana varieties *viz.*, 'Grand Naine' and 'Robusta' were regenerated into shoots on MS-medium supplemented with 6-benzylaminopurine (BA, 5 mgL<sup>-1</sup>) and multiplied on Banana Multiplication Medium (BMM) for 7 cycles of 3 weeks each. Shoots were separated and cultured on BMM supplemented with IBA (1.0 mgL<sup>-1</sup>) and cefotaxime at various concentrations *viz.*, 0, 100, 200, 300, 400, 500, 600 and 700 mgL<sup>-1</sup>. Maximum shoot multiplication and elongation with respect to number of shoots, shoot length and plantlet fresh weight in both the genotypes was obtained with cefotaxime used at the rate of 400 and 500 mgL<sup>-1</sup> in the medium. Among the different varieties, on the basis of mean of 50 shoot cultures, number of shoots per culture and shoot length was highest in *cv.* Robusta *i.e.* 7.20 (41.6% increase over control) and 8.50 cm (30.5% increase over control), respectively. Whereas, plantlet fresh weight was highest in *cv.* Grand Naine (494.8 mg, 38.9% increase over control) after 3 weeks of culturing with cefotaxime used at the rate of 500 mgL<sup>-1</sup>. Results revealed significant differences among varieties and media for different shoot multiplication and elongation parameters. Therefore, use of cefotaxime during tissue culture and genetic transformation of banana can improve frequency of shoot multiplication and transformation, respectively.

**Key words:** Suckers, tissue culture, transformation, shoot length, fresh weight, antibiotic

### Introduction

Banana (*Musa spp.*, 2n = 22 - 44) is the largest monocotyledonous, herbaceous and evergreen perennial fruit plant, which belongs to family Musaceae and is a member of order Zingiberales. It is the fourth most important food commodity, after wheat, rice and maize, that grows throughout the humid tropics and subtropics with an annual production of 97.5 million tonnes (Ganapathi *et al.*, 2008). In banana (*Musa acuminata*), shoots can be easily regenerated through various cultured tissues, such as adventitious buds (Ma and Shii, 1972), shoot apices (Doreswamy *et al.*, 1983), meristem tips (Alvard *et al.*, 1993), floral apices (Alloufa *et al.*, 2002), inflorescence apices (Resmi and Nair, 2007) and male inflorescences (Darvari *et al.*, 2010). However, many times due to excessive formation of shoot buds *in vitro*, there is a problem of crowding, which inhibits subsequent normal shoot elongation. This lowers the rate of multiplication through micropropagation in banana. Besides this, due to endogenous bacterial contamination, there are less chances of establishment of cultures. Also, during plant regeneration from long-term maintained cultures, there is a problem of albinism in the regenerated shoots. Further, during *Agrobacterium*-mediated genetic transformation, use of antibiotics such as carbenicillin, hygromycin and kanamycin also results into regeneration of weaker shoots and sometimes, albino shoots, ultimately, ending into a very low frequency of transformation (Arencibia *et al.*, 1998; Bower and Birch, 1992). In this regard, use of cefotaxime (C<sub>16</sub>H<sub>16</sub>O<sub>5</sub>S<sub>2</sub>Na), a semi-synthetic, broad-spectrum and cephalosporin antibiotic, instead of carbenicillin can improve genetic transformation frequency by killing the adhering *Agrobacteria* after co-cultivation and improving the growth of regenerated shoots. Cefotaxime is a

β-lactam antibiotic that inhibits cell wall synthesis in dividing bacterial cells and results in cell lysis (Selwyn, 1980). Cefotaxime can be added into the culture medium to overcome endogenous bacterial contamination, which is a major hurdle during *in vitro* success of plant cultures, as microbial contamination in cultured plantlets can reduce multiplication or rooting rates or even induce plant death (Cassells, 1991; Leifert *et al.*, 1991).

After explant establishment and shoot regeneration, cultures are expected to be free from contamination. But, even after these stages, contamination due to endogenous microorganisms in tissue culture system has been reported (Reed *et al.*, 1997; Tanprasert and Reed, 1997). Plant tissue cultures harbor latent bacteria (Leifert and Waites, 1990), which affect multiplication of plant cultures when these are transferred to a medium containing reduced concentration of salt and sucrose. The antibacterial property of cefotaxime has been harnessed to enhance frequency of plant regeneration in barley (Mithias and Mukasa, 1987), finger millet (Eapen and George, 1990), wheat (Borrelli *et al.*, 1992), *Dianthus* (Nakano and Mii, 1993), pearl-millet (Pius *et al.*, 1993), apple (Yepes and Aldwinckle, 1994), sorghum (Rao *et al.*, 1995), *Vitis vinifera* (Torregorsa *et al.*, 2000), papaya (Yu *et al.*, 2001), maize (Danilova and Yu, 2004), tea (Aoshima, 2005) and rice (Grewal *et al.*, 2006). Recently, it has been reported that cefotaxime also improved the elongation and multiplication rate in sugarcane (Kaur *et al.*, 2008; Mittal *et al.*, 2008). However, in banana, as such there is no report regarding the influence of cefotaxime on shoot multiplication and elongation. Therefore, the present study was carried out with the objective to investigate the effect of cefotaxime on shoot multiplication and elongation in banana tissue culture.

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## Materials and methods

**Plant material:** Young suckers (3-13 cm diameter) of economically important banana (*Musa acuminata* L.) cultivars, 'Grand Naine' and 'Robusta' were excised from field-grown (2-year old) healthy plants.

**Preparation of explants:** Shoot-tip explants (4.5-5.5 cm in length) were isolated from suckers by removing extraneous corm tissue, surface-sterilized with 0.1% carbendazim (Bavistin™ from BASF India Ltd, Mumbai) for 45 min on rotary shaker followed by washing them in running tap water for 4-5 minutes, 0.1% mercuric chloride (HgCl<sub>2</sub>) for 8-10 min and rinsed thrice with sterile distilled water, and reduced to 2-3 cm portion containing intact apex and one or more pairs of leaf primordia together with 3.5-4 cm of rhizomatous base under aseptic conditions. Shoot-tip explants were cultured on agar-solidified MS (Murashige and Skoog, 1962) + 6-benzylaminopurine, BA (5 mgL<sup>-1</sup>) medium for shoot regeneration for 7 cycles of 3 weeks each. Thereafter, fifty shoot cultures from culture jar were inoculated on each of the following media: (i) agar-solidified banana multiplication medium (Vuylsteke, 1998) supplemented with 0.5 mgL<sup>-1</sup> Indole-3-butyric acid (IBA) as control and (ii) agar-solidified banana multiplication medium + 0.5 mgL<sup>-1</sup> IBA + 100, 200, 300, 400, 500, 600 and 700 mgL<sup>-1</sup> cefotaxime medium (treatment). The experiment was replicated thrice. Shoot cultures were kept in an incubation room with 16/8 h light/dark regime having a light intensity of 68 μ mol m<sup>-2</sup>s<sup>-1</sup> and temperature of 25 ± 1 °C. Cefotaxime salt (1g, Omnatax™) was dissolved in 4 mL sterile water and sterilized by passing through membrane filter assembly having a pore size of 0.2 μm. Cefotaxime solution was added into the molten medium before solidification under aseptic conditions. Then, 15 mL of this medium was dispensed in pre-autoclaved culture jars ('HNG' glass jars, capacity 400 mL) and were closed with plastic caps. Also, medium was dispensed in pre-autoclaved culture tubes (150 x 25 mm, Borosil) and closed with cotton plugs wrapped in muslin cloth. The medium without cefotaxime served as control. Data were recorded on different parameters of shoot multiplication and elongation *viz.*, number of shoots per shoot culture, shoot length and fresh weight of plantlets, after 3 weeks of culturing. Data were analyzed using factorial experiment in Completely Randomized Block (CRD) Design to test the level of significance and critical difference for variety, media and variety x media interactions.

## Results and discussion

The creamish white tissue blocks (Fig. 1A) turned reddish (Fig. B) when placed in MS medium supplemented with 5 mgL<sup>-1</sup> BAP in 7-10 days and the explants exhibited unwhorling of leaf sheaths (Fig. 1C) after 3 weeks. The swollen explants growing on establishment medium were taken out from culture vessels and dead leaves and blackish basal tissues were removed. For subculturing, explants were cut longitudinally from the apex. Each half was subcultured in an upright position in a culture jar containing BMM for shoot-proliferation. The cultures showed enhanced tendency towards axillary shoot proliferation (Fig. 1D). After 28 days, multiple micro-shoots were formed, which were separated and transferred on control and treatment medium. Data were recorded on three traits *viz.*, number of shoot buds formed per shoot culture, shoot length and FW of plantlets after 3 weeks of culturing.

**Number of shoots:** Inclusion of antibiotic cefotaxime in the culture medium fostered the development of shoots in tissue culture regenerated shoots of 'Grand Naine' variety of banana at most of the concentrations. Number of shoots per culture in control and treatment media, after 3 weeks of culturing is presented in Table 1.

As is evident from Table 1, maximum number of shoots per culture were recorded in *cv.* Robusta (7.2, 41.6% increase over control), followed by 6.67 (51.7% increase) in Grand Naine on agar-solidified banana multiplication medium + 0.5 mgL<sup>-1</sup> IBA supplemented with 500 mgL<sup>-1</sup> cefotaxime medium. Previously, in apple higher number of shoots per culture were observed with 200 mgL<sup>-1</sup> cefotaxime as compared to control, and at higher cefotaxime doses, the rate of shoot multiplication and elongation was reduced (Yepes and Aldwinckle, 1994). In sugarcane, increase in number of microtillers may be associated with inhibition of ethylene production in cultures by cefotaxime, which is positively correlated with plantlet differentiation (Kaur *et al.*, 2008). Number of shoots followed a declining trend with the highest dose of cefotaxime (600 and 700 mgL<sup>-1</sup>). Thus, cefotaxime application in the medium beyond an optimum concentration may be toxic.

**Shoot length:** Different banana varieties respond almost equally well to establishment of explants, shoot regeneration and subsequent shoot proliferation. Under several instances, excessive shoot proliferation results in crowding, that leads to the formation of abnormally weak shoot clumps. Such clumps normally do not exhibit shoot elongation and rooting upon their subsequent transfer to respective shoot elongation and rooting media. In the present study, it was found that cefotaxime (up to a level of 500 mgL<sup>-1</sup>) promoted shoot length in both the genotypes tested. The maximum shoot length of 8.5 cm was achieved with the use of cefotaxime at the dose of 500 mgL<sup>-1</sup> in variety 'Robusta' (Fig. 2, Table 1). It was found to be higher over control (5.90 cm) by 30.58 %, and all other combinations of genotype x cefotaxime concentrations. Also, maximum increase in shoot length in variety 'Grand Naine' over control, *i.e.* 19.6 % increase over control, was recorded when culture medium was supplemented with 500 mgL<sup>-1</sup> cefotaxime. Increase in cefotaxime concentration beyond the optimum limits reduced the shoot length progressively. Minimum shoot length in all the varieties was noted with 700 mgL<sup>-1</sup> cefotaxime (Table 1).

Analysis of shoot length data in a factorial experiment in CRD design showed that there was a significant difference for shoot Table 1. Effect of antibiotic cefotaxime on number of shoots per shoot culture, shoot length and fresh weight of plantlets in banana varieties after 3 weeks of culturing

Cefotaxime (mgL <sup>-1</sup> )	Number of shoots		Shoot length (cm)		Fresh weight of plantlets (mg)	
	Grand Naine	Robusta	Grand Naine	Robusta	Grand Naine	Robusta
Control	3.22	4.20	6.03	5.90	302.30	305.30
100	4.11	4.53	6.20	6.26	335.30	319.90
200	4.40	5.23	6.60	6.73	360.50	349.40
300	5.20	5.78	6.63	7.20	382.70	372.20
400	5.40	6.50	6.90	7.60	415.90	402.90
500	6.67	7.20	7.50	8.50	494.80	456.30
600	2.85	3.36	6.57	7.20	292.90	291.30
700	2.35	2.54	6.13	6.30	345.60	200.10
LSD	0.71	0.20	0.15	0.30	4.54	9.08

(*P*=0.05)

\* Each value is a mean of 50 shoot cultures

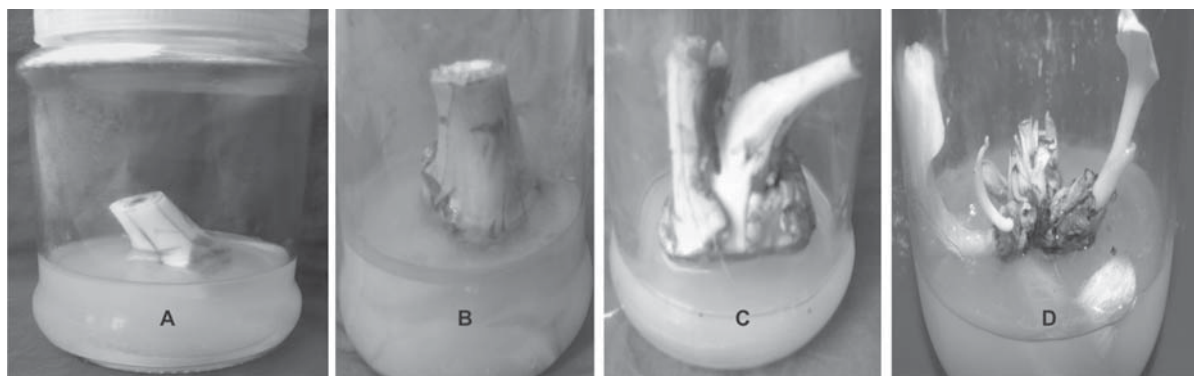


Fig. 1. Establishment of aseptic cultures of banana. A. Showing cultured explant on establishment medium [MS + BAP (5 mgL<sup>-1</sup>) + cefotaxime (500 mgL<sup>-1</sup>)] just after culturing. B. Swelling and reddening of the cultured explants after 5-7 days of culturing. C. Unwhorling of red leaf sheaths and exposure of green tissue from explant. D. Showing axillary shoot-proliferation in cultured explants of banana

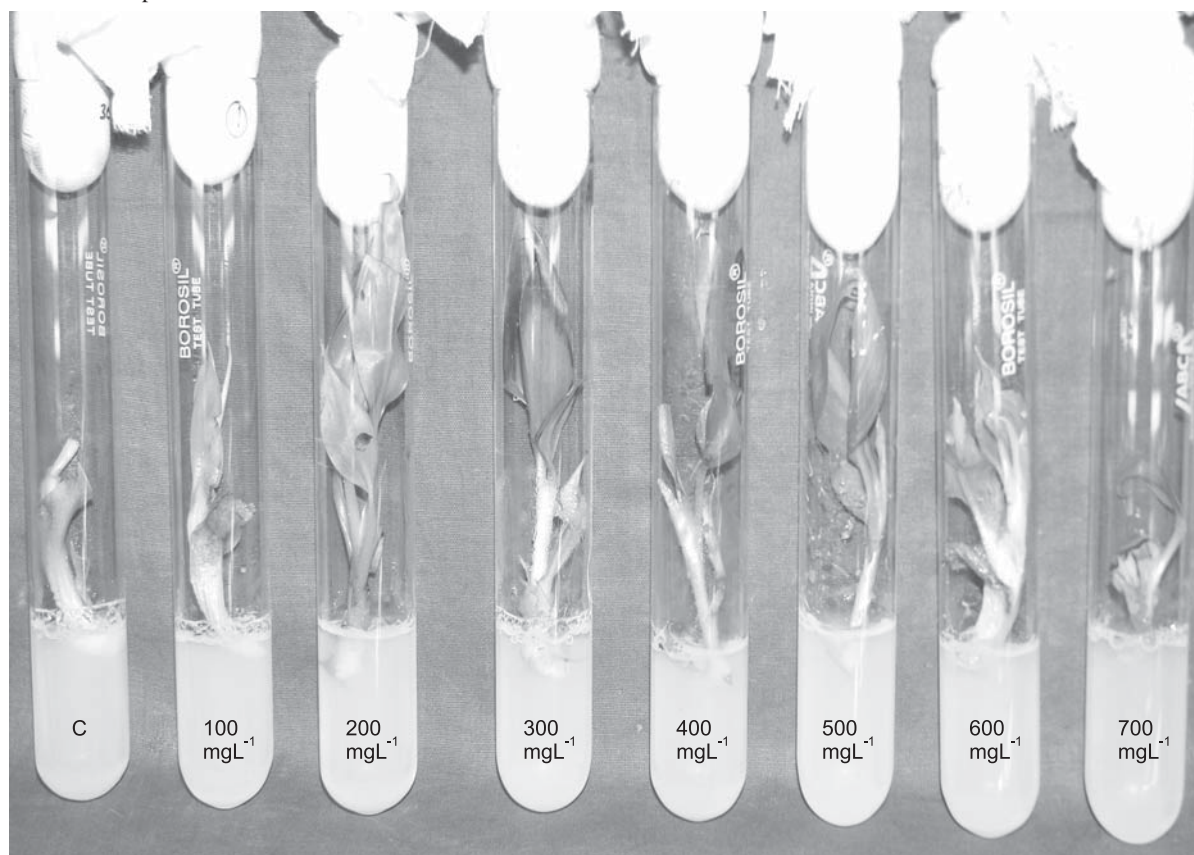


Fig. 2. Effect of different cefotaxime concentrations (0, 100, 200, 300, 400, 500, 600 and 700 mgL<sup>-1</sup>) on shoot length in banana *cv.* Grand Naine, after 3 weeks of culturing

length among varieties and among media containing different cefotaxime doses. Significance of variety x antibiotic interactions envisages that the varieties responded differently with respect to shoot length in 700 mgL<sup>-1</sup> cefotaxime.

**Fresh weight (FW) of plantlets:** Perusal of data in Table 1 reveals stimulatory effect of cefotaxime on FW of plantlets in banana  *cvs.* Grand Naine and Robusta. Maximum FW in both the genotypes *i.e.* 494.8 mg in Grand Naine and 456.3 mg in Robusta was achieved when the medium was supplemented with 500 mgL<sup>-1</sup> cefotaxime. This was significantly higher over the control *i.e.* 38.9% increase over control in Grand Naine and 33% increase over control in Robusta. In an earlier study, cefotaxime (250 and 500 mgL<sup>-1</sup>) has been reported to markedly enhance FW of callus in eggplant (Picoli *et al.*, 2008). Recently, the effect of cefotaxime in another monocotyledon *viz.* sugarcane regarding its

FW has been reported (Kaur *et al.*, 2008). The stimulatory impact of cefotaxime on FW may be associated with the inhibition of endogenous microbial growth (Yepes and Aldwinckle, 1994). There was a decrease in FW of plantlets in both the genotypes with 600 and 700 mgL<sup>-1</sup> cefotaxime. Thus, cefotaxime addition beyond an optimum concentration is undesirable.

Comparing the efficiency of different doses of the antibiotic, 500 mgL<sup>-1</sup> cefotaxime was found to be the best for improving fresh weight of the plantlets. The addition of cefotaxime in the culture medium also boosted up the photosynthetic machinery of banana plants, as they were greener than the plants of the control group after 3 weeks of transfer. More number of green plants were obtained when ryegrass calli were treated with 60 mgL<sup>-1</sup> cefotaxime for 6 weeks prior to placement on regeneration medium (Zaghmout and Torello, 1992). Without cefotaxime



pretreatment, only albino plants were produced. Therefore, for regeneration of plants from relatively older calli for induction of somaclonal variation in banana, use of cefotaxime in the shoot regeneration medium can give a high frequency of green and healthy shoots or somaclones. So, cefotaxime in somatic tissue culture may be acting at the level of chlorophyll synthesis. Further studies are required to know its actual mechanism of action. Cefotaxime did not inhibit *in vitro* root induction, multiplication and elongation in regenerated banana shoots as equally good rooting was observed in shoots on both the media. However, in tobacco, cefotaxime has been reported to inhibit rooting of shoots regenerated from leaf discs, but had no effect on rooting of shoots regenerated from cotyledons (Naurbey *et al.*, 1997).

Thus, it is concluded that addition of cefotaxime in the culture medium (100-500 mgL<sup>-1</sup>) enhanced the process of shoot elongation in both varieties of banana. In this regard, use of cefotaxime in the culture medium can promote shoot multiplication and elongation even from vitrified shoot clumps, and thus improve the rate of shoot multiplication through micropropagation in banana.

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