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The Stimulatory Effects of the Antimicrobial Agents Bavistin, Cefotaxime and Kanamycin on *In Vitro* Plant Regeneration of *Centella asiatica* (L.)—An Important Antijaundice Medicinal Plant

Chandra Sekhar Panathula, Manikyam Doraiswamy Naidu Mahadev, Challagundla Varadarajulu Naidu*

Department of Biotechnology, Dravidian University, Kuppam, India.
Email: *challagundlav@yahoo.co.in

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ABSTRACT

Antimicrobial agents such as bavistin, cefotaxime and kanamycin were evaluated for their effects on the rapid shoot regeneration from nodal explants of *Centella asiatica* (L.). Filter sterilized bavistin (250 mg/L) was augmented alone and in combination with cytokinins such as BAP and TDZ into the media to trace the effect on regeneration. On this basis, the potential use of bavistin (150 mg/L) along with BAP (2.0 mg/L) was evaluated which showed the maximum shoot number (6.6) and shoot length (4.4 cm) respectively. Cefotaxime at the concentration of 100 µM/L was found to be effective to obtain the maximum shoot number formation (5.8) with the regeneration frequency (90%). Kanamycin at the concentration of 80 µM/L induced maximum shoot regeneration (5.12). Kanamycin at 100 µM/L or at higher concentrations reduced the shoot regeneration. The best rooting response was noticed when *in vitro* regenerated microshoots were transferred to the rooting medim which was supplemented with IBA (2.0 mg/L). This combination generates 90% of regeneration frequency and maximum number of roots per shoot (14.2) and high root length (4.2 cm). The rooted plants were acclimatized and transferred to field for survivalance. The addition of antibiotics was found to be more effective and safer for using since their effects on regeneration were found to be negligible.

KEYWORDS

***Centella asiatica* (L.); Bavistin; Cefotaxime; Kanamycin; 6-Benzyl Amino Purine; *In Vitro* Regeneration**

1. Introduction

Centella asiatica (L.) Urban is a small creeping perennial and profoundly branched prostrate herb belonging to family Apiaceae or Umbelliferae and commonly called as Indian pennywort [1]. This plant is known as a source for various chemical constituents like carotenoids, vitamins B and C, bitter compound vellarin, fatty oils as glycerides of oleic, linolic, centoic, linolenic, palmitic and steric acids [2]. In addition to that, it is also used in many ayurvedic preparations and is reported to possess anti leprotic, antifilarial, antibacterial, adaptogeneic, anti-feedant and antiviral properties [3]. It is also used for improving the memory, for curing bronchitis, leucoderma, fever, asthma, anemia, measales, dysentery, jaundice and small pox [4,5]. Due to its increasing demand by the Indian pharmaceutical industry coupled with limited cultivation, now it is listed as threatened species by the International union for conservation of native and natural resources IUCN [6] and an endangered species [7,8].

Micropropogation technique would play an important role in conservation, genetic improvement and pharmaco-therapeutical need of the endangered medicinal plants.

*Corresponding author.

By considering the above importance of this plant, *in vitro* cultivation was found to be effective to provide the plant with uniform phytochemical components. Antibiotics have been employed to eliminate or inhibit microbial growth in plant tissue culture studies. Generally antimicrobial agents are frequently used in micro propagation techniques to obtain contaminated free elite clones. The contaminants are present in explants or arise during incubation time. These antibiotics also have been reported to exhibit plant growth regulators like properties which may vary with the species.

The present protocol was aimed not only to eliminate microbial contamination but also to standardize the optimum concentration of bavistin, cefotaxime and kanamycin on the induction of multiple shoot production and to promote the frequency of the rapid regeneration of *C. asiatica*, an important medicinal plant.

2. Materials and Methods

2.1. Collection of Plant Material and Surface Sterilization

In the present investigation young *C. asiatica* plants were collected from herbal garden, Department of Biotechnology, Dravidian University, Kuppam, Andhra Pradesh, India. Axillary buds were collected from the young sprouts of the stock plants were used as explants. These explants were washed under running tap water for 10 min, followed by immersing in liquid detergent solution 5% (v/v) teepol (Qualigens India Ltd.) for 10 min and then washed under running tap water. The explants were surface sterilized with 0.4% (w/v) bavistin and then with 70% (v/v) ethanol for 60 Sec. The explants were surface sterilized with 0.1% (w/v) mercuric chloride (Merck India) for 1 - 3 minutes and thoroughly washed with sterile double distilled water for 3 - 4 times, to remove the traces of $HgCl_2$ before inoculation. After trimming the cut ends of the explants they were blotted on sterile filter paper discs.

2.2. Culture Medium and Culture Conditions

Murashige and Skoog medium [9], containing 3% (w/v) sucrose with bavistin at different concentrations ranging from 10 - 300 mg/L alone and in combination with BAP was used. In addition to that medium supplemented with various concentrations of cefotaxime and kanamycin added individually was used in all the experiments to trace their effects on rapid shoot induction and regeneration.

The PH of the medium was adjusted to 5.8 and gelled with addition of 0.8% (w/v) agar. Molten medium was dispensed approximately 15 ml into culture tubes (25 × 150 mm) and closed with non-absorbent cotton plugs. The medium was autoclaved at 15 lbs. pressure and 121˚C for 20 minutes. All the cultures were incubated in an *in vitro* culture room maintained at 26˚C ± 2˚C temperature and 55% - 60% relative humidity with a photo period of 16 hours day light and 8 hours dark with a light intensity of 3000 lux provided by cool white fluorescent tubes.

2.3. Shoot Proliferation and Data Recording

MS basal medium supplemented with various plant growth regulators at their different concentrations either alone or in combination were used for culture initiation and shoot proliferation. All the cultures were transferred to fresh culture medium after 21 days of culture duration. Experiments are set up in a completely randomized block design (RBD) and each treatment repeated as thrice and at least 20 replicates per each treatment. Visual observations were recorded on the frequency (number of cultures responding for axillary shoot proliferation, shoot development, number of shoots per explant, length of the regenerated shoots, number of roots per shoot and root length). Each value of data represented the mean ± SE of 20 independent determinations.

2.4. Statistical Analysis

All experiments were conducted with a minimum of 20 explants. All assays were repeated at least three times. The experimental data were statistically analyzed by one-way ANOVA using the DMRT (Duncan's Multiple Range Test) ($P < 0.05$) and were presented as the average ± standard error (SE).

3. Results and Discussion

All the antibiotics tested which had been not showed any negative effect on regeneration and even further more enhanced the induction of multiple shoot production. Interestingly when these antibiotics were added to the medium the results were showed highly significant effects of genotype and antibiotics on explants initiation and development.

3.1. Effect of Bavistin on the *in Vitro* Plant Regeneration of Nodal Explants of *C. asiatica*

The axillary bud explants that were cultured on MS medium containing (250 mg/L) bavistin is the most effective in terms of regeneration frequency (75%), and regeneration of number of shoots per explants (5.8 ± 0.31) (**Figure 1(B)**). The maximum shoot length was obtained in 200 mg/L of bavistin (4.4 ± 0.45 cm) (**Table 1**). Bavistin (150 mg/L) and in combination with BAP (2.0

Table 1. Effect of Bavistin alone and in combination with plant growth regulator BAP on *in vitro* shoot organogenesis from nodal explants of field grown plants of *C. asiatica*. Data represent treatment means ± SE followed by different letter(s) within column indicate significant differences according to ANOVA and DMRT test (P < 0.05).

Bavistin (mg/L)	BAP	TDZ	Regeneration frequency (%)	Mean No. of shoots/explants	Mean shoot length (cm)
-	-	-	-	-	-
25	-	-	40.00	1.5 ± 0.26^{a}	2.0 ± 0.18^{a}
50	-	-	50.00	2.5 ± 0.64^{b}	2.4 ± 0.96^{b}
100	-	-	58.00	3.8 ± 0.45^{d}	2.8 ± 0.87^{b}
150	-	-	70.00	4.2 ± 0.72^{d}	3.2 ± 0.42^{c}
200	-	-	90.00	5.4 ± 0.24^{e}	4.4 ± 0.21^{e}
250	-	-	75.00	5.8 ± 0.31^{f}	3.9 ± 0.45^{d}
300	-	-	40.00	3.5 ± 0.68^{c}	3.4 ± 0.28^{c}
150	1.0	-	80.00	5.4 ± 0.34^{ef}	3.1 ± 0.92^{c}
150	2.0	-	85.00	6.6 ± 0.48^{g}	4.0 ± 0.29^{de}
150	-	1.0	50.00	3.6 ± 0.72^{c}	2.0 ± 0.12^{a}
150	-	2.0	60.00	4.0 ± 0.12^{d}	2.4 ± 0.14^{ab}

BAP = 6-Benzyl amino purine; TDZ = Thidiazuron.

mg/L) showed maximum frequency of shoot regeneration (95%), increased shoot number (6.6 ± 0.48) and shoot length (4.0 ± 0.29 cm) (**Figure 1(A)**). The above results were compared with bavistin alone and devoid of plant growth regulator. MS basal medium devoid of both plant growth regulators and bavistin does not show much significance in the regeneration. In the present study bavistin was used not only to reveal the contamination and their elimination but also to promote the shoot proliferation. Similar results were reported in the same species [10], and also in other medicinal plants [11,12]. Bavistin appeared to have much stronger cytokinin like activity. It has been reported that the molecular structure of bavistin or carbendazim has some resemblance to that of kinetin, adenine, and many other adenine based cytokinins as adenine thiosulphate (ATS). Earlier studies have shown that the bavistin enhances differentiation of roots and shoots in calli derived from carrot segment [13].

The shoot regeneration promoting activity of bavistin is results in increased biosynthesis of endogenous cytokinins with in the cultured explants. Due to its broad spectrum antifungal activity it seems to be eliminates fungal contamination during initiation. In addition to that it is least toxic to the plant cells [14]. It was also been reported that bavistin can have beneficial effects on the physiology of the plant [15].

3.2. Effect of Cefotaxime on *in Vitro* Plant Regeneration of Nodal Explants of *C. asiatica*

In the present study cefotaxime alone was used with free of plant growth hormones to trace the effect on regeneration of *C. asiatica*. The obtained data reveals that the significant effect of cefotaxime on the nodal cultures of this plant. Shoot initiation and proliferation was observed after 10 days of culture inoculation (**Figure 1(B)**). Maximum regeneration frequency (90%) and maximum number of shoots per explant (5.8 ± 0.38) and the maximum shoot length (6.18 ± 0.62 cm) was obtained at the concentration of 100 µM/L (**Table 2**). Increased concentration of cefotaxime results in decreased frequency (65%), shoot number (2.2 ± 0.35) and shoot length (2.98 ± 0.32) respectively. The standardized concentration for shoot regeneration was between (40 - 100 µM/L).

Regeneration efficiency of *in vitro* cultured plantlets was depends on many significant factors such as regeneration response of an explant to the medium, genotype of the explant and the nature of the antimicrobial agent that was used in the medium. Many reports were indicated enhanced regeneration frequency when antibiotics were included in the media formulation. Cefotaxime is a broad spectrum antibiotic having activity against both gram-positive and gram-negative bacteria. These antibiotics were blocking the nucleopeptide synthesis by cross linking peptidoglycon layer and inactivating the transpeptidase enzymes. Cefotaxime belonging to the β-lactum group and have minimal toxicity on most plant tissues. The possibility of that cell metabolism converts cefotaxime to a compound with growth regulator activity has been discussed [16]. Low concentration of cefotaxime enhanced shoot regeneration in wheat [16,17] maize [18], apple [19] and strawberry [20]. Similarly regeneration efficiency was reduced by increasing the

Table 2. Influence of Cefotaxime added to MS medium on *in vitro* regeneration of plantlets from nodal explants of *C. asiatica*. Data represent treatment means ± SE fallowed by different letter(s) within column indicate significant differences according to ANOVA and DMRT test (P < 0.05).

Cefotaxime (µM/L)	Frequency of regeneration (%)	Mean No. of shoots/explant	Mean shoot length (cm)
-	-	-	-
5	35.00	1.8 ± 0.21^{a}	2.0 ± 0.26^{a}
10	40.00	3.0 ± 0.34^{b}	2.8 ± 0.46^{b}
20	55.00	3.2 ± 0.40^{b}	3.6 ± 0.42^{c}
40	60.00	4.0 ± 0.39^{c}	3.8 ± 0.15^{cd}
60	65.00	4.6 ± 0.45^{d}	4.34 ± 0.27^{d}
80	75.00	5.2 ± 0.55^{e}	5.21 ± 1.2^{e}
100	90.00	5.8 ± 0.38^{f}	6.18 ± 0.62^{f}
125	80.00	4.7 ± 0.29^{d}	4.27 ± 0.39^{d}
150	65.00	2.2 ± 0.35^{a}	2.98 ± 0.32^{b}

concentration of cefotaxime in three plants such as maize, apple and wheat and inhibits in common snapdragon [21] and tobacco [22]. Cefotaxime at higher concentration seriously inhibit callus growth of papaya [23]. Recently it has been reported that cefotaxime also improved elongation and multiplication in sugarcane [24]. Likewise cefotaxime have been observed to have a significant positive effect on *in vitro* regeneration of *Stevia rebeudina* [25], *Solanum nigrum* [26] and *Mentha peperita* [27]. An obvious explanation for the function of cefotaxime is that the molecule mimics like a plant growth regulator [16]. However the mechanism of cefotaxime function is not clearly known [28].

3.3. Effect of Kanamycin on the *in Vitro* Plant Regeneration of Nodal Explants of *C. asiatica*

The influence of kanamycin on shoot regeneration was examined and the obtained data clearly indicate the effect of kanamycin on nodal cultures of *C. asiatica*. The range of kanamycin added to media was (5 - 200 µM/L). MS medium supplemented with (80 µM/L) of kanamycin was induced maximum regeneration frequency (85%), regenerated mean number of shoots per explant (5.12 ± 0.23) the maximum shoot length (4.12 ± 0.53 cm) (**Figure 1(C)**, **Table 3**) respectively. Increased concentration of kanamycin reduces the elongation and multiplication rate of regenerated shoots.

Kanamycin is an aminoglycoside antibiotic (AA) isolated from *Streptomyces kanamyceticus*. It binds to 30 s ribosome subunit and consequently inhibiting the protein synthesis. Phytotoxicity and enhancing effects of antibiotics varies significantly within the plant species and even different explants of same plant [29]. Many reports revealed that kanamycin sensitivity seems to be species specific. A wide range of organogenesis inhibiting concentrations can be found in the cited literature. The possible mechanism of stimulatory effect of antibiotics on regeneration may involve generation of stress that makes

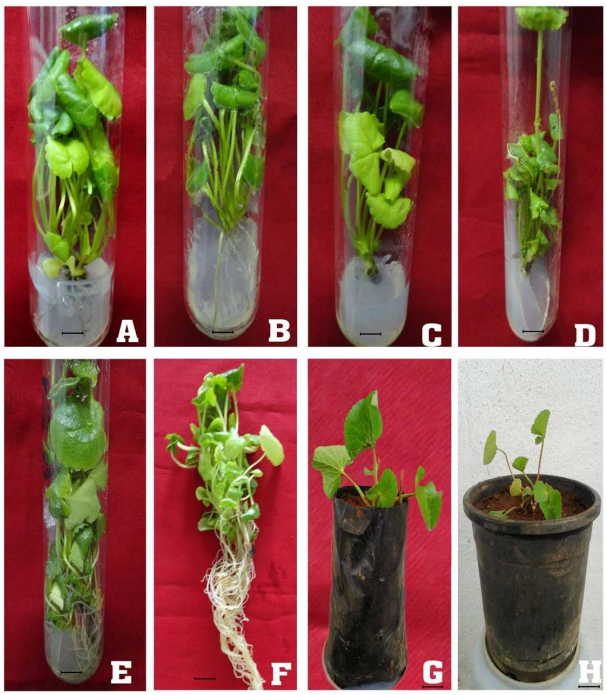

Figure 1. Nodal explants cultured on MS medium supplemented with various concentrations of antimicrobial agents such as bavistin, cefotaxime and kanamycin. Shoot initiation and multiplication from axillary bud explants cultured on; A) MS + Bavistin 150 mg/L+ BAP 2.0 mg/L (bar 1 cm = 1.5 cm); B) MS + Bavistin 250 mg/L alone (bar 1 cm = 1.5 cm); C) MS + Cefotaxime 100 µm/L (bar 1 cm = 1.3 cm); D) MS + Kanamycin 80 µm/L (bar 1 cm = 1.5 cm); E) and F) *In vitro* rooting of regenerated microshoots on MS + IBA (2.0 mg/L) (bar 1 cm = 2.0 cm); G) and H) Hardened Plantlet in earthen pot containing soil and vermiculite in 1:1 ratio (bar 1 cm = 2.0 cm).

cells competent for regeneration alternatively the antibiotics may mimic like plant growth regulators [30].

3.4. *In Vitro* Rooting and Hardening

In vitro derived microshoots of length (4.0 - 6.0 cm) were separated and sub cultured on MS medium supplemented with different concentrations of auxins as IBA and NAA (0.5 - 2.0 mg/L). Increased rooting frequency (90%) with highest number of roots (12.0 ± 0.68) was observed on IBA (2.0 mg/L), the results are tabulated (**Figures 1(D)** and **(E)**, **Table 4**). For hardening well established rooted plantlets were separated, washed and transferred to polybags containing soil + vermiculite in 1:1 ratio (**Figure 1(F)**). During the hardening process, there was very less mortality in the plants regenerated from bavistin, cefotaxime and kanamycin treated cultures. Upon transfer to field, nearly 90% plants were survived.

4. Conclusion

This investigation disclosed that the application of antimicrobial agents eliminated the contaminating microorganisms and does not show any negative effect on nodal explants of *C. asiatica* and furthermore they promoted the shoot regeneration efficiency. The addition of these antimicrobial agents in micropropogation technique has been found to be an efficient protocol for the regeneration and conservation of this valuable medicinal plant.

Table 3. Influence of Kanamycin added to MS medium on *in vitro* regeneration of plantlets from axillary bud explants of *C. asiatica*. Data represent treatment means ± SE fallowed by different letter(s) within column indicate significant differences according to ANOVA and DMRT test (P < 0.05).

Kanamycin (µM/L)	Frequency of regeneration (%)	Mean No. of shoots/explant	Mean shoot length (cm)
-	-	-	-
5	55.00	1.56 ± 0.32^{a}	1.86 ± 0.12^{a}
10	50.00	1.82 ± 0.39^{b}	2.0 ± 0.34^{a}
20	45.00	2.10 ± 0.47^{c}	2.64 ± 2.22^{b}
40	60.00	3.18 ± 0.40^{d}	3.12 ± 0.67^{d}
60	70.00	4.0 ± 0.27^{f}	3.89 ± 0.90^{e}
80	85.00	5.12 ± 0.23^{a}	4.2 ± 1.53^{e}
100	60.00	4.16 ± 0.25^{g}	3.01 ± 0.12^{c}
125	55.00	3.70 ± 0.55^{e}	2.42 ± 0.29^{b}
150	-	-	-
200	-	-	-

Table 4. Root organogenesis of *in vitro* derived shoot lets of *C. asiatica* on MS medium supplemented with various concentrations of auxins such as IBA, IAA & NAA. Data represent treatment means ± SE fallowed by different letter(s) within column indicate significant differences according to ANOVA and DMRT test (P < 0.05).

Plant growth regulators (mg/L)		Root regeneration frequency (%)	Mean No. of roots/shoot	Mean No. of root length (cm)
IBA	NAA			
-	-	-	-	-
0.5	-	55	4.5 ± 0.21^{a}	3.1 ± 0.42^{a}
1.0	-	60	7.0 ± 0.38^{c}	3.6 ± 0.64^{a}
1.5	-	68	8.6 ± 0.41^{d}	4.0 ± 0.82^{b}
2.0	-	90	14.2 ± 0.30^{g}	4.2 ± 0.31^{b}
-	0.5	56	4.8 ± 0.82^{a}	3.4 ± 0.35^{a}
-	1.0	65	6.5 ± 0.18^{b}	3.8 ± 0.90^{a}
-	1.5	78	9.0 ± 0.96^{e}	4.0 ± 0.45^{b}
-	2.0	80	12.0 ± 0.68^{f}	4.4 ± 0.98^{b}

IBA = 3-indole butyric acid; NAA = α-naphthalene acetic acid.

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REFERENCES

[1] K. Purushotam and D. Anilkumar, "Medicinal Plants and Raw Drugs of India," Shiva Offset Press, Dehradun, 2000.

[2] N. D. Prajapati, S. S. Purohit, A. K. Sharma and T. Kumar, "A Hand Book of Medicinal Plants," Hindustan Press, Agro bios, 2006.

[3] P. K. Warrier, V. P. K. Nambiar and C. Ramankutty, "Indian Medicinal Plants. A Compendium of 500 Species," Orient Longman Pvt. Ltd., Madras, 1994.

[4] V. V. Sivarajan and I. Balachandran, "Ayurvedic Drugs and Their Plant Sources," Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 1994.

[5] K. R. Kirtikar and B. D. Basu, "Indian Medicinal Plants," New Delhi. M/s Bishen Sing Mahendrapalsing, Vol. 3, Dehradun, 1975.

[6] N. K. Pandey, K. C. Tewari, R. N. Tewari, G. C. Joshi, V. N. Pande and G. Pandey, "Medicinal Plants of Kumaon Himalaya, Strategies for Conservation," In: U. Dhar, Ed., *Himalayan Biodiversity Conservation Strategies*, Himavikas Publication, Nainital, 1993, pp. 293-302.

[7] H. G. Singh, "Himalayan Herbs and Drugs, Importance and Extinction Threat," *Journal Medicinal Plants Research*, Vol. 10, 1989, pp. 47-52.

[8] B. L. Sharma and A. Kumar, "Biodiversity of Medicinal Plants of Triyugi Narain (Garhwali Himalaya) and Their Conservation," *National Conference on Recent Trends in Spices and Medicinal Plant Research*, Calcutta, 2-4 April 1998, p. 78.

[9] T. Murashige and F. Skoog, "A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures," *Plant Physiology*, Vol. 15, No. 3, 1962, pp. 473-479. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x

[10] S. George, A. B. Remashree, D. Sebastian and M. Hariharan, "Micropropagation of *Centella asiatica* (L). through Axillary Bud Multiplication," *Phytomorphology*, Vol. 54, 2004, pp. 31-34.

[11] J. Patnaik and B. K. Debata, "Micropropagation of *Hemidesmus indicus* R.Br. through Axillary Bud Culture," *Plant Cell Reports*, Vol. 15, No. 6, 1996, pp. 427-430. http://dx.doi.org/10.1007/BF00232069

[12] Y. Sahoo, and P. K. Chand, "Micropropagation of *Vitex negundo* (L). A Woody Aromatic Medicinal Shrub, through High Frequency Axillary Shoot Proliferation," *Plant Cell Reports*, Vol. 18, No. 3-4, 1998, pp. 301-307. http://dx.doi.org/10.1007/s002990050576

[13] R. K. Tripathi and S. Ram, "Induction of Growth and Differentiation of Carrot Callus Cultures by Carbendazim and Benzimidazole," *Indian Journal of Experimental Biology*, Vol. 20, No. 9, 1982, pp. 674-677.

[14] R. Shields, S. J. Robinson and P. A. Anslow, "Use of Fungicides in Plant Tissue Culture," *Plant Cell Reports*, Vol. 3, No. 1, 1984, pp. 33-36.

[15] P. C. Garcia, R. M. Rivero, J. M. Ruiz and L. Romero, "The Role of Fungicides in the Physiology of Higher Plants: Implications for Defense Responses," *Botanical Review*, Vol. 69, No. 2, 2003, pp. 162-172. http://dx.doi.org/10.1663/0006-8101(2003)069[0162:TROFIT]2.0.CO;2

[16] R. J. Mathias and L. A. Boyd, "Cefotaxime Stimulates Callus Growth, Embryogenesis and Regeneration in Hexaploid Bread Wheat (*Triticum aestivum* L. EM. THELL)," *Plant Science*, Vol. 46, No. 3, 1986, pp. 217-223. http://dx.doi.org/10.1016/0168-9452(86)90195-0

[17] G. M. Borrelli, N. Di Fonzo and E. Lupotto, "Effect of Cefotaxime on Callus Culture and Plant Regeneration in Durum Wheat," *Journal of Plant Physiology*, Vol. 140, No. 3, 1992, pp. 372-374. http://dx.doi.org/10.1016/S0176-1617(11)81095-3

[18] S. A. Danilova and Y. I. Dolgikh, "The Stimulatory Effect of the Antibiotic Cefotaxime on Plant Regeneration in Maize Tissue Culture," *Russian Journal of Plant Physiology*, Vol. 51, No. 4, 2004, pp. 559-562. http://dx.doi.org/10.1023/B:RUPP.0000035752.09295.55

[19] D. James, A. Passey, D. Barbara and M. Bevan, "Genetic Transformation of Apple (*Malus pumila* Mill) Using a Disarmed Ti-Binary Vector," *Plant Cell Reports*, Vol. 7, No. 8, 1989, pp. 658-661.

[20] D. James, A. Passey and D. Barbara, "*Agrobacterium* Mediated Transformation of the Cultivated Strawberry (*Fragria x Anannassa* Duch.) Using Disarmed Binary Vectors, *Plant Science*, Vol. 69, No. 1, 1990 pp. 79-94. http://dx.doi.org/10.1016/0168-9452(90)90106-X

[21] P. Holford and H. J. Newbury, "The Effects of Antibiotics and Their Breakdown Products on the *in Vitro* Growth of *Antirrhinum majus*," *Plant Cell Reports*, Vol. 11, No. 2, 1992, pp. 93-96. http://dx.doi.org/10.1007/BF00235261

[22] B. Nauerby, K. Billing and R. Wyndaele, "Influence of the Antibiotic Timentin on Plant Regeneration Compared to Carbenicillin and Cefotaxime in Concentrations Suitable for Elimination of *Agrobacterium tumefaciens*," *Plant Science*, Vol. 123, No. 1-2, 1997, pp. 169-177.

[23] T.-A. Yu, S.-D. Yeh and J.-S. Yang, "Effects of Carbenicillin and Cefotaxime on Callus Growth and Somatic Embryogenesis from Adventitious Roots of Papaya," *Botanical Bulletin of Academia Sinica*, Vol. 42, No. 4, 2001, pp. 281-286.

[24] P. Mittal, S. S. Gosal, A. Senger and P. Kumar, "Impact of Cefotaxime on Somatic Embryogenesis and Shoot Regeneration in Sugarcane," *Physiology and Molecular Biology of Plants*, Vol. 15, No. 3, 2009, pp. 257-265.

[25] D. Preethi, T. M. Sridhar and C. V. Naidu, "Effect of Bavistin and Silver Thiosulphate on *in Vitro* Plant Regeneration of *Stevia rebaudiana*," *Journal of Phytology*, Vol. 3, No. 5, 2011, pp. 74-77.

[26] T. M. Sridhar and C. V. Naidu, "Antimicrobial Agents Alters the *in Vitro* Plant Regeneration in *Solanum nigrum*

(L.)," *Journal of Phytology*, Vol. 3, No. 5, 2011, pp. 65-68.

[27] P. Sujana and C. V. Naidu, "Influence of Bavistin, Cefotaxime, Kanamycin and Silver Thiosulphate on Plant Regeneration of *Mentha piperita* (L.)—An Important Multipurpose Medicinal Plant," *Journal of Phytology*, Vol. 3, No. 5, 2011, pp. 36-40.

[28] X. Zhan, D. Jones and A. Kerr, "Regeneration of Flax Plants Transformed by *Agrobacterium rhizogenes*," *Plant Molecular Biology*, Vol. 11, No. 5, 1988, pp. 551-559.

[29] R. J. Mathias and C. Mukasa, "The Effect of Cefotaxime on the Growth and Regeneration of Callus from Varieties of Barley (*Hordeum vulgare* L.)," *Plant Cell Reports*, Vol. 6, No. 6, 1987, pp. 454-457.

[30] A. M. Rao, K. P. Sree and P. B. K. Kishor, "Enhanced Plant Regeneration in Grain and Sweet Sorghum by Aspargine, Proline and Cefotaxime," *Plant Cell Reports*, Vol. 15, No. 1-2, 1995, pp. 72-75.