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RESEARCH ARTICLE

CONCENTRATION OF CYTOKININ SHOOT INDUCTION FROM STEM NODE EXPLANTS OF *LUFFA ACUTANGULA (L)*

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ABSTRACT

Currently, it is important to classify the mechanisms by which bacterial resistance occurs, since it represents a serious problem in terms of public health. The goal was to make a phylogenetic analysis of bacterial β-lactamases to prepare a classification based on their molecular sequence, unlike previous classifications. A phylogenetic tree of clinically important bacterial β-lactamases was constructed using their molecular sequences. A cladogram was made to analyze the characteristics of the families and subfamilies. The cladogram analysis shows the existence of three large families of β-lactamases**.** This work represents an advance to classify β-lactamases by their molecular sequence.

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INTRODUCTION

The conservation and use of the worlds remaining plant genetic resources conservation without use has little point and use will not come without evaluation of all forms of the cultured species. Plant regeneration from long term callus cultures of *Luffa acutangula (L)*. This variety resembles cucumber and is used as a vegetable. The primary aim of this study has been to gain some knowledge about the genotypic differences for callus initiation and high frequency. They are pale or dark green in colour, smooth or ridged, with soft downy hairs covering the skin when tender. Seeds are smaller than those of the musk melon tissue culture in vitro production of fruit crop plant M. Venkateshwarlu (2007). It grows on any kind of soil, but thrives best on well – manured rich loamy soils with abundant water supply. The seeds are small and edible, and are used in confectionery. Several workers in past have micropropagated some of the important Asclepiadaceae members such as *Ceropegia bulbosa* (Patil, 1998; Britto *et al.*, 2003), Venkateshwarlu (2020) & Thoyajalosa & Rai (2016). *Hemidesmus indicus* (Misra *et al.*, 2003; Patnaik and Kishore, 1996) Venkateshwarlu *et al* (2018) & Venkateshwarlu (2017). and *Holostemma ada-kodien* (Martin, 2002, 2003). Since very scarce information is available about micropropagation about this important medicinal plant, an attempt was made to develop a reproducible protocol for shoot and multiple shoot induction from nodal explants of one of the tissue culture recalcitrant medicinal plants of Cucurbitaceae family, *Luffa acutangula (L)*. Using various concentrations of Benzyl Amino Purine and Adenine Sulphate. In the recent years there has been a major crop plant development Application *In Vitro* culture an important field crop improvement.

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MATERIALS AND METHODS

Plant tissue culture technique involves the contribution of stem node explants both for the whole regeneration of plants and also the production of therapeutic pharmaceutical compounds. The shoot segments after removing the leaves were cut into 2cm pieces, each containing a single stem node region and washed under running tap water for 15 min, followed by brief washing with sterile distilled water. Node explants (1.25 cm) were surface sterilized in 80% (v/v) ethanol for 60 sec followed by 0.1% (w/v) mercuric chloride for 4-6 min. explants were thoroughly washed in sterile distilled water and blot dried on sterile Whatmann 1 mm filter paper. For shoot induction, Stem nodal explants were again trimmed into 1.0 cm and transferred to MS medium supplemented with 0.5 – 2.0 mg/l BAP. Cultures were incubated at 26±2° under a 16/8 h photoperiod for 26-28 days at a relative humidity of 70%. Node explants (1.0 cm long) were used as explants for multiple shoot induction on MS medium fortified with 2.0 mg/l Benzyl Amino Purine and 5-20 mg/l Adenine Sulphate. After two weeks of culturing at 26± 2° under a 16/8 photoperiod shoots were sub cultured onto fresh medium for proliferation. All the experiments were repeated thrice (each with 10 explants) and the response was scored after 26-28

days of culture initiation. Multiple shoot induction MS medium supplemented with 1.0 mg/l BAP and 5-20 mg/l Adenine Sulphate were used. The pH of all media was adjusted to 5.75 before adding 0.8% agar and autoclaved at 151b and 121°C for18 min. All the media were kept at 26±2°C for 3 days before use. The growth inhibition is recurrently amalgamated with cyto differentiation leads the involvement of certain enzymes for the production of secondary metabolites in case of cell and plant cultures.

RESULTS AND DISCUSSION

The MS media composition along with the influence of light, temperature these two aspects are inversely related both in the case of cell and plant cultures. The results scored on the above mentioned aspects (shoot and multiple shoot induction) are summarized in the following order. In order to assess the effect of different concentrations of Benzyl Amino Purine (0.5-2.0 mg/l) on shoot induction from *Luffa acutangula (L)* stem nodal explants were surface sterilized and inoculated onto MS media supplemented with various concentrations of Benzyl Amino Purine. Shoot induction was monitored after 15-20 days of inoculation by counting the number of shoots induced from each explants. Shoot induction was observed in all the concentrations of Benzyl Amino Purine tested with variation in per cent response of shoot induction. The number of shoots produced from stem nodal explants on medium with 1.0 mg/l BAP was 3.8 with an average height of 2.5 cm. We found an increase in the per cent response of shoot induction and number of shoots with an increase in the concentration of Benzyl Amino Purine from 0.1 mg/l to 1.2. The percentage of explants exhibiting shoot induction was found to be between 40-80 is most of the concentrations of Benzyl Amino Purine tested except MS medium supplemented with 2.0 mg/l Benzyl Amino Purine. After 26-28 days of culture, nodal explants derived shoot cultures were sub cultured to MS medium fortified with same concentration of hormone for shoot elongation.

Significant elongation has been achieved in medium with 1.0 and 2.0 mg/l Benzyl Amino Purine. There was no significant variation in shoot length between the different concentrations of Benzyl Amino Purine except in the case of medium with 2.0 mg/l producing average shoot length of 2.74 cm. The shoots sub cultured to fresh medium with same concentration of Benzyl Amino Purine proliferated additional 3-4 shoots after 26 days of culture. (Table-I, Plate-I). The nodal explants cultured on medium with Benzyl Amino Purine developed pale yellow intermediate callus at the basal portions due to the accumulation of auxins at the basal cut ends. The effect of Benzyl Amino Purine in inducing shoot induction was already reported in some of the important medicinal plants of Asclepiadaceae family members such as *Bulbosa* Komalavalli and Rao (2000) and in *Holostemma ada-kodien* (Martin, 2002). The promotive effect of Benzyl Amino Purine on shoot induction and multiplication was well understood in various plants like *Phytolocca decanta* (Demeke and Huges, 1990), *Saussuriea lappa*, *Clerodendran colebrookianum* (Mao *et al.*, 1995), *Trichopus zeylanicus* (Krishnan *et al.*, 1995) and in *Woodfordia fruticosa* (Krishnan and Seeni, 1994). To analyze the shoot induction ability of stem node explants from *in vitro* multiplied plants, stem nodal explants were used as an ideal source of explants for reculturing. Additional 2-3 shoots per node explants on MS medium fortified with 2.0 mg/l indicate the effectiveness of explants on multiple shoot induction without surface sterilization. A similar effect of the hormone in enhancing shoot induction has been reported in one of the Asclepiadaceae family members, *Ceropegia candelabrum* (Beena *et al.*, 2003). As expected, contamination rate has been drastically reduced in recultured stem node explants Venkateshwarlu M *et al* (2019). Harmful changes in water caused by too much fertilizers or nutrients getting into aquatic ecosystem P M Kahate (2019). The specific effects vary depending on what pollutants enter the environment. Sometimes water pollution causes on explosion of new plant growth by providing necessary nutrients and food.

Table 1. Shoot induction from nodal explants of *Luffa acutangula (L)*

S.No.	BAP+KN+L-Glutamic acid-(GL) concentration (mg/l)	Growth Response
1	BAP+Kn 1.0 mg/l	Callus
2	BAP+Kn 2.0 mg/l	Callus
3	BAP+Kn 3.0 mg/l	Callus +Shoot
4	BAP+Kn 4.0 mg/l	Shoots 2-4
5	BAP+GL 1.0 mg/l	Callus
6	BAP+GL 2.0 mg/l	Callus
7	BAP+GL 3.0 mg/l	Shoots 1-2
8	BAP+GL 4.0 mg/l	Shoots
9	BAP+GL 5.0 mg/l	Shoots 2-4

Plant tissue culture, Dipika Rathod et al (2019). In the present study, Adenine Sulphate when used in combination with Benzyl Amino Purine induced multiple shoots. Among the combinations tested, Benzyl Amino Purine NAA 2.0-5.0 mg/l with 5.0 mg/l Adenine Sulphate produced maximum number of shoots with intermittent callus at the basal cut end. Of the various concentrations of Adenine Sulphate tested, 15 mg resulted in maximum number of shoots followed by 20mg/l (9.8), 10mg/l (6.5) and 5mg/l (6.8).

Plate1. Shoot induction from stem node explants *Luffa acutangula* (L)

Average number of shoots generated per explant on medium with 2.0 mg/l Benzyl Amino Purine and 15 mg/l Adenine Sulphate is an improvement of almost 3 fold in the multiplication rate as compared with shoots induced on MS

medium with 2.0 mg/l Benzyl Amino Purine alone. Addition of 5 and 10mg/l Adenine Sulphate had no significant effect on number of shoots produced per explants. The presence of Adenine Sulphate initiated friable callus and suppress the formation of new shoots. A similar observation was reported in *Hemidesmus indicus* using 5-20 mg/l Adenine Sulphate with Benzyl Amino Purine and naphthalene acetic acid. The desired product yield but does not support the cellular growth Fujita *et al* (1981).

Conclusion

In Vitro Biotechnological approaches numerous plant cell suspension tissue culture methods have been pursued so far the concentration of Cytokins ultimately causes reduction in crop productivity *In vitro* methods for large scale plant production multiplication can be tapped by cutting down the cost of production per plant by applying tissue culture methods. Explants from stem node explants from callus shoots and then plant regeneration. Improvements in the tissue culture approaches and standardization of cultural conditions have been affected quite competently.

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