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Efficacy of *Azotobacter chroococcum* in Rooting and Growth of *Eucalyptus camaldulensis* Stem Cuttings

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ABSTRACT

Eucalyptus camaldulensis Dehn is a commercial tree crop mainly for paper and pulp industries. This tree crop is propagated by vegetative propagation method to obtain genetically superior clones. At the time of vegetative propagation a synthetic rooting hormone Indole Butyric Acid (IBA) is being used in nurseries for successful rooting in *E. camaldulensis* stem cuttings. To reduce the cost of IBA and improve the rooting and nutrient enrichment as an alternate method a nitrogen fixing bacteria *Azotobacter chroococcum* was applied in the stem cuttings of *E. camaldulensis* in present study. The influence of *A chroococcum* on rooting and subsequent growth of *E. camaldulensis* cuttings was observed under nursery conditions. An un-inoculated control and IBA treated cuttings were also maintained to compare the growth with *A. chroococcum* inoculated stem cuttings. Bacterial inoculum (5×10^7 cfu mL^{-1}) at the rate of 5 and 10 mL were applied to the rooting substrate (vermiculite) during cutting installation. Rooting and Biomass was evaluated after 30 days of cutting installation. Additionally, the isolates were screened for their ability to produce Indole Acetic Acid (IAA) under *in vitro* conditions either in the presence or absence of tryptophan at different concentrations. The results revealed that *A. chroococcum* produced significant quantities of IAA for root initiation and *A. chroococcum* inoculated cuttings had higher growth than IBA treated cuttings at $p<0.05$. From this study, it was concluded that the stem cuttings of *E. camaldulensis* responded positively to *A. chroococcum* inoculation through increased root proliferation and growth.

Key words: *Azotobacter chroococcum,* indole acetic acid, *Eucalyptus camaldulensis*, rooting hormone, Bio mass

INTRODUCTION

Eucalyptus camaldulensis Dehn. is a very popular choice for afforestation among Eucalyptus species due to its fast growth, high productivity and short rotation (Kebebew, 2010). This species produces quality pulpwood for paper and newsprint production. In the state of Tamilnadu (India) *E. camaldulensis* has been planted in an area of 35,000 ha by Tamilnadu Forest Plantation Corporation Ltd., India. The yield of *E. camaldulensis* on a rotation of seven years is about 20 ton ha^{-1} (Karthikeyan and Suryaprakash, 2008).

Generally synthetic rooting hormones are used for large scale of vegetative propagation of *E. camaldulensis* stem cuttings exhibit variation in rooting and fail to initiate rooting in certain clones (Mohammad and Prasad, 1998). Therefore, alternative strategies that optimize rooting percentage of *E. camaldulensis* stem cuttings needs to be developed for successful mass vegetative

propagation. Plant Growth Promoting Rhizobacterias (PGPRs) are known to induce root in many crops (Kloepper *et al*., 1980). The effect of PGPRs on rooting was also reported for seedlings and micro propagated plants belonging to the genera, *Pinus, Picea, Tsuga* and *Pseudotsuga* (Enebak *et al*., 1998). The PGPRs *Bacillus pumilis* and *B. licheniformis* were reported to produce high concentrations of active gibberellins and *B. polymyxa* produces cytokinin Bottini *et al*. (2004). Similarly *Brevibacterium* sp. reported as production of auxin in *Helianthus annus* (Faisal and Hasnain, 2010).

In this present study the PGPR, *Azotobacter chroococcum* was evaluated for its ability to effect on rooting in *E. camaldulensis* cuttings. *A. chroococcum* is free living Gram negative bacteria that fix aerobic nitrogen and ubiquitous in rhizosphere (Panaiyadiyan and Chellaia, 2011). There are many reports on the beneficial effects of *A. chroococum* in multiple ways on growth and yield of various agriculturally important crops. These include (i) ability to produce ammonia, vitamins and growth substances that enhance seed germination and (ii) production of Indole Acetic Acid (IAA) and other growth hormones such as gibberellins and cytokinins (Verma *et al*., 2001; Woyessa and Assefa, 2011) which enhance root growth and aid in nutrient absorption. IAA is the main auxin in plants controlling important physiological process including cell enlargement and division, tissue differentiation and responses to light and gravity (Taiz and Zeiger, 1998). A recent study by Fatima *et al*. (2009) showed that *Azotobacter* sp. produced 19.4-30.2 μg mL^{-1} of IAA and significantly increased root and shoot length in wheat. Further it was very recently reported that *Azotobacter* sp increased the auxin content in *Triticum aestivum* Shaukat *et al*. (2006). It was also reported that an increase of 44% in *E. camaldulensis* seedling bio mass was observed after co-inoculation of the potting medium with *A. chroococcum* (Mohammad and Prasad, 1998).

Hence in this present study *A. chroococcum* was used as an alternative to synthetic hormone for rooting in *E. camaldulensis* cuttings so as to reduce the production cost and improve the quality planting stock.

MATERIALS AND METHODS

Isolation of *A. chroococcum*: This study was conducted at Model Nursery of Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamilnadu (India) from September 2009 to February 2010. *A. chroococcum* was isolated from the rhizoplane of 7 years old *E. camaldulensis* growing in Coimbatore. Root segments with adhered substrate were placed in centrifuge tube and centrifuged for 5 min at 1000 rpm. Then the supernatant was collected and made up to 100 mL^{-1} with sterile distilled water. One mL of the diluted supernatant was placed in Nitrogen free Jensen's medium (sucrose 20 g, di-potassium hydrogen phosphate 1 g, magnesium sulfate 0.5 g, sodium chloride 0.5 g, ferrous sulfate 0.1 g, sodium molybdate 0.005, agar 20 g, for 1 liter, pH 6.9). After 5 days of inoculation the bacterial colonies appeared on the media. The morphological characters were carefully observed and recorded. Further confirmation of *A. chroococcum* was bio chemically characterized by Indole, Methyl Red-Voges Proskaur test, carbohydrate fermentation, oxidase and urease tests as per the standard protocols (Cappuccino and Sherman, 1992). The culture was deposited in the bacterial culture collection of IFGTB and numbered as AB I 08.

Experimental design: Stem cuttings of *E. camaldulensis* with uniform height (4 cm) and healthy leaf and axillary bud were collected from Karunya Nagar, Coimbatore, India and preserved in ice box for 1 h. The cuttings were dipped in 1% Bavistin (Carbendazim) fungicide to avoid fungal infection. Then *A. chroococcum* inoculum suspension at 5×10^7 cfu mL^{-1} (5 and 10 mL) was applied to the stem cuttings at the rate of 5 and 10 mL placed in root trainers (100 cc) containing

rooting substrate (vermiculite). Untreated cuttings and Indole Butyric Acid (IBA) treated (1000 ppm with talcum powder) were also maintained to compare the effect of *A. chroococcum* on rooting of *E. camaldulensis*. Each treatment was replicated at 15 times and arranged in a randomized block design. The cuttings were maintained for 30 days in mist chamber (33°C, 65%) and no nutrients were supplied.

Treatments: There were four treatments including an untreated control as follows:

- Control
- IBA
- *A. chroococcum* (5 mL broth)
- *A. chroococcum* (10 mL broth)

Harvest and assessment of stem cuttings: After 30 days of treatment the stem cuttings were harvested and the assessments of root number and length, leaf number and bio mass were made. The percent root initiation was used as a measure to asses the influence of *A. chroococcum* and IBA on the rooting of *E. camaldulensis* cuttings and was calculated using the formula:

$$\frac{\text{No. of root initiations } (\textit{A. chroococcum}) - \text{No. of root initiation } (+\text{IBA})}{\text{No. of root initiation}} \times 100$$

Estimation of *A. chroococcum* for Indole-Acetic Acid (IAA) production: *A. chroococcum* was estimated for IAA production by the method of Loper and Schroth (1986). The presence of IAA in *A. chroococcum* culture was determined by development of pink colour and the concentration was measured in UV spectrophotometer at 530 nm.

Statistical analysis: Each measured variable in the nursery experiment were subjected to analysis of variance and means were separated using Duncan's multiple range test using SPSS (ver. 10) software at the significant level of $p<0.05$.

RESULTS

Growth and rooting of *E. camaldulensis* stem cuttings: *E. camaldulensis* stem cuttings inoculated with *A. chroococcum* showed significantly increased higher growth and bio mass rather than IBA treated and control stem cuttings. Stem cuttings inoculated with 10 mL broth of *A. chroococcum* showed two to three fold increase of (significant at $p<0.05$) higher shoot length, root length, No. of lateral roots and bio mass than IBA treated cuttings. The R/S ratio was also found significantly ($p<0.05$) lower in *A. chroococum* (0.754) inoculated stem cuttings than IBA treated cuttings (0.929) (Table 1).

Percent root initiation: Significantly ($p<0.05$) 90% root initiation was obtained in 10 mL broth of *A. chroococcum* inoculated stem cuttings whereas 5 mL broth of *A. chroococcum* induced 60% of root initiation and IBA treated stem cuttings showed only 28% of root initiation (Fig. 1).

IAA production of *A. chroococum*: IAA production was found in *A. chroococcum* broth with tryptophan addition. Higher concentrations of IAA occurred with increasing concentrations of tryptophan (Fig. 2).

Table 1: Growth, rooting and biomass of *E. camaldulensis* stem cuttings inoculated with *A. chroococum* (mean of 15 replicates)

Treatments	Root length (cm)	Shoot length (cm)	Root collar diameter (cm)	Stem girth (cm)	No. of lateral roots/plant	No. of leaves/plant	Plant biomass (mg plant^{-1}) Root	Shoot	R/S ratio
Control	1.07^{a}	5.3^{a}	0.98^{a}	1.85^{a}	1.10^{a}	1.48^{a}	0.64^{a}	0.27^{a}	2.370^{a}
IBA	2.34^{b}	6.8^{a}	1.22^{b}	2.06^{ab}	4.45^{b}	2.54^{a}	1.58^{b}	1.70^{a}	0.929^{b}
A. chroococum (5 mL plant^{-1})	2.72^{b}	7.1^{b}	1.39^{b}	2.13^{b}	5.52^{b}	3.98^{ab}	1.68^{b}	2.12^{b}	0.792^{c}
A. chroococum (10 mL plant^{-1})	5.18^{c}	8.8^{b}	2.67^{c}	2.89^{c}	8.24^{c}	4.78^{b}	1.72^{b}	2.28^{b}	0.754^{c}

Means followed by same letters are significantly not different at p<0.05 according to Duncan's Multiple Range Test

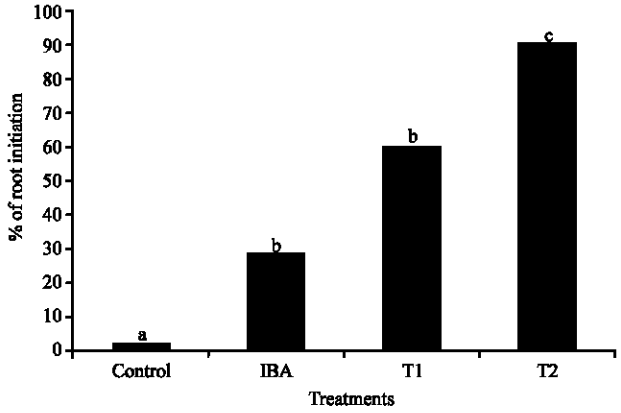

Fig. 1: Percent root initiation of *E. camaldulensis* stem cutting inoculated wr *A. chroococum* (mean of 10 replicates) T1 *A. Chroococum* (5 mL), T2 *A. Chroococum* (10 mL), means followed by same letters are not significantly not different at p<0.05 according to Duncans multiple range test

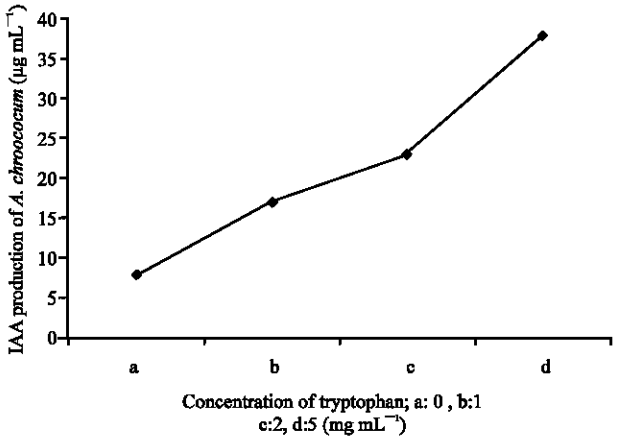

Fig. 2: IAA production of *A. chroococum* (mean of ten isolates)

DISCUSSION

Microbes always play in nutrient management of soil to improve soil fertility (Vikram and Hamzehzarghani, 2008). The application of the N_2 fixing bacteria *A. chroococcum* was first proposed in the mid 1970s as a new approach to provide fixed N to reduce fertilizer requirement and to increase yield (Dart, 1976). In present study *A. chroococcum* induced better rooting and

growth of *E. camaldulensis* stem cuttings. This is in accordance with earlier reports where increased plant growth and bio mass have reported in response of N_2 fixing bacterial inoculation (Akbari *et al*., 2007; Fallik *et al*., 1998). This study also confirms the observations of Karthikeyan and Suryaprakash (2008) where inoculation of N_2 fixing bacteria *Azospirillum brasilense* resulted in better growth of *E. camaldulensis* seedlings. The stimulation of rooting in *E. camaldulensis* stem cuttings by *A. chroococcum* suggests its ability to produce plant hormones that could stimulate root initiation. The root exudates also promote the growth of microbe in the inert media which helps to survive the bacteria as reported in previous study (Kumar *et al*., 2007). Further In an earlier study it was indicated that about 80% of the bacteria present in soil are able to produce IAA (Glick *et al*., 1999). The production of IAA and important hormone for rooting by *A. chroococcum* was confirmed in this study. It was reported that IAA is the important hormone which promote the rooting in *Vitellaria paradoxa* cuttings (Yeboah *et al*., 2009) and *Balanites aegyptiaca* cuttings (Mostafa and Alhamd, 2011). Earlier studies have shown that *Azotobacter* species are able to produce IAA, GA and Cytokinin like substances both under culture conditions and in the plant rhizosphere (Brown, 1976; Jain and Patriquin, 1985). Inoculation of *A. chroococum* stimulated rooting in *E. camaldulensis* stem cuttings better than IBA. This suggests that continuous release of small quantities of IAA might enhance root initiation better than a single application of root hormone. In present study sufficient amount (7.8 μg mL^{-1}) of IAA to promote root initiation and elongation was produced even without supplement of tryptophan. This is evidenced by the significant variations observed in percentage of root initiation in *E. camaldulensis* cuttings with inoculation of *A. chroococcum*. Similar responses to *A. chroococcum* inoculation have been reported for mulberry (Das *et al*., 1990) and Bamboo (Dhamangaonkar and Misra, 2009) and *Ocimum sanctum* (Vinutha, 2005). Therefore, this study emphasized the need of rooting hormone synthesizing inoculants such as *A. chroococcum* for the propagation of *E. camaldulensis* and to reduce the usage of costlier synthetic root hormones and chemical fertilizers.

CONCLUSION

Studies on *E. camaldulensis* cuttings with inoculation of *A. chroococcum* do indicate that it can reduce fertilizer requirement in plant production. For quality cuttings production of commercial tree crops like *E. camaldulensis* consideration should be given to N_2 fixing microbes like *A. chroococcum* rather than costlier synthetic root hormones and chemical fertilizers. Moreover, this microbial inoculation techniques is cheap and easy to handle, could be adopted in clonal nurseries to improve the rate of growth and quality of *E. camaldulensis* cuttings without chemical fertilizers thus avoiding the associated costs.

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