The role of plant growth regulators on direct and indirect plant regeneration from various organs of *Leucaena leucocephala*

Sharmistha Maity, Samit Ray and Nirmalya Banerjee

Cytogenetics and Plant Biotechnology Laboratory, Department of Botany, Visva-Bharati University, Santiniketan-731235, West Bengal, India, e-mail: nirmalya_b @ rediffmail.com

Key words: Callus, *Leucaena leucocephala*, Organogenesis, Plant growth regulators, Plant regeneration

Abstract

Prolific differentiation of shoot buds of *Leucaena leucocephala* was induced from the different plant parts viz. cotyledon, hypocotyl and leaf. Adventitious shoot bud formation was recorded with prudent application of N\(^6\)-2-(isopentenyl) adenosine and 15 % (v/v) coconut water. Coconut water alone was unable to produce any beneficial effect with regard to the shoot bud proliferation but the response was augmented with the increase in concentration of N\(^6\)-2- (isopentenyl) adenosine. However supra-optimal level of N\(^6\)-2- (isopentenyl) adenosine was inhibitory. Best response was recorded from the cotyledon explant at 2 mg dm\(^{-3}\) N\(^6\)-2- (isopentenyl) adenosine compared to the other two explants. Comparative assessment was undertaken following the same experimental protocol in liquid shake culture. The regenerated shoot buds were subcultured in plant growth regulator-free medium where leafy shoot emergence was recorded. Optimum regeneration of roots was observed in these shoots in presence of 1 mg dm\(^{-3}\) α-naphthalene acetic acid. Plantlets were finally hardened following standard procedures before transplantation to the field.

In another experimental set up, the de-embryonated cotyledons regenerated shoot buds via callus formation. The regenerated shoots and plantlets obtained through callus mediated organogenesis could be used for rapid multiplication and also for the genetic improvement of individual clones of *Leucaena leucocephala*.

List of abbreviations: NAA: α-Naphthalene acetic acid, 2 iP: N\(^6\)-2-(isopentenyl) adenosine, CW: Coconut water, BA: 6- benzyl adenine, MS: Murashige and Skoog medium, ANOVA: Analysis of variance, DMRT: Duncan’s Multiple Range Test, PGR: Plant growth regulator, Kn: Kinetin

Introduction

Forest trees provide subsistence to millions of people with a wide range of significant economic value (Yasodha et al. 2004). To ensure sustainable and equitable use of resources for meeting the basic needs of present and future, the best option is to practice massive tree plantation of forest species. Seed propagation has a number of constraints such as prolonged dormancy and poor viability (Venkateswaran and Gandhi 1982). Vegetative propagation is unreliable in case of limited ‘elite’ material (Bonga et al. 1987). Therefore, propagation of tree species through *in vitro* technology is regarded as a reliable means for rapid multiplication of such plants (Sreedevi and Pullaiah 1999). Regeneration of forest trees in general and legumes in particular has been a difficult task as the seeds of such taxa are considered to be recalcitrant in nature (Ravishankar and Jagadishchandra 1989).
Leucaena leucocephala, belonging to the family Fabaceae, offers enormous economic potential as a source of pulpwod. It is indeed an under exploited promising forage legume of the tropics. Apart from being a wood crop, it has good heating value and is used as a fuel-source in the developing world thereby relieving the pressure on the consumption of fossil fuel (Negi et al. 1995). It can also be used for production of biomass in the social forestry programme (Nagmani and Venkateswaran 1979).

The species is self-incompatible and produces very little inbred seeds. This plant is presently under great threat due to inferior germplasm, high rate of out breeding and habitat specificity, which accounts for poor regeneration and seed viability (Singh et al. 1995). Unfortunately due to unrestricted deforestation without provision for aorestation, Leucaena has now become rare in most of its Indian range, making it a major candidate for conservation (Bonga 1987).

In view of that it was thought essential to develop a standard in vitro protocol for artificial propagation, which would enable us to generate homogenous clones within a short time. Although in vitro propagation of L. leucocephala has been reported by a number of workers (Ghosh and Bandyopadhyay 1984, Dutta and Dutta 1985, Hossain et al. 1992), a comprehensive study of the effects of various plant growth regulators as well as other media supplements on the direct and indirect plantlet regeneration in different cultural systems has not been made. The present investigation is therefore, undertaken to determine the optimal conditions for rapid regeneration of uniform plantlets of L. leucocephala through a systematic and critical study of role of plant growth regulators, type of explant and also the culture systems on direct and indirect regeneration of shoots and shoot buds. Hence, the plant regeneration through direct differentiation of shoot buds would be an effective measure for conservation purpose while the callus mediated organogenesis would possibly help in maintaining optimum genetic diversity causing minimum damage to the natural population.

Materials and Methods

Seeds of L. leucocephala were collected from the forests of Santiniketan, Birbhum, West Bengal, India. They were washed in 2 % (v/v) detergent solution ‘Teepol’ (Qualigens, India) and subsequently surface sterilized in 0.1 % (w/v) aqueous mercuric chloride solution for 12 min. After rinsing 4 times with sterile distilled water, seeds were transferred to moist cotton beds in 250 ml Erlenmeyer flask and incubated in the culture room for 7 days under 10 hours photoperiod of 37.5 μmol m⁻² s⁻¹ light intensity. Cotyledons, hypocotyls and the leaves were excised from the 7-day old axenic seedlings and were inoculated into MS basal medium (Murashige and Skoog 1962) supplemented with 2 iP (0.5 to 4 mg dm⁻³) combined with 15 % v/v CW.

Ten replicates were used per treatment. The pH of the media was adjusted to 5.8 prior to autoclaving. The media were solidified with 0.8 % w/v agar. Routinely, 25 ml molten medium was dispensed into each culture tube (25 × 150 mm), plugged with non-absorbent cotton and subsequently sterilized at 121 °C and 102 × 10⁶ kg m⁻² pressure for 15 min. Cultures were incubated at 25 ± 2 °C under a light intensity 37.5 μmol m⁻² s⁻¹ under a photoperiod of 10 hours.

For de novo formation of shoot buds, explants like cotyledon, hypocotyl and leaf were excised and cultured in MS media with various concentrations of 2 iP (0.5 to 4 mg dm⁻³) in combination with CW (15 % w/v). The in vitro multiple shoot buds produced at higher concentration of 2 iP were subcultured on MS media supplemented with lower levels of 2 iP (0.1 mg dm⁻³) or with complete omission of 2 iP for subsequent leafy shoot emergence. Similar experiments were carried out in liquid MS medium to compare the frequency of leafy shoot emergence with that of solid medium.

For the initiation of callus and subsequent shoot bud regeneration through organogenesis, the same explants were inoculated into MS basal media supplemented with various combinations and concentrations of NAA (0.2 - 4.0 mg dm⁻³) and BA (0.2 - 4.0 mg dm⁻³). The friable callus after 60 days were subcultured in MS media with various concentrations of BA (0.2 to 4 mg dm⁻³) in combination with