Micropropagation of *Lagerstroemia parviflora* Through Axillary Bud Culture

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Abstract

Axillary bud cultures were initiated from 3 types of nodal explants of *Lagerstroemia parviflora*. The cultures derived from explants of seedlings, terminal twigs of a 50-year-old tree and basal-sprouts of another 50-year-old tree showed significant variation in responses at establishment, shoot proliferation and rooting stages. All the 3 types of explants exuded phenolic substances from their cut ends. The exudation was checked by suspending them in a solution of 25 µM PVP 40 and 522.5 µM citric acid; and by the addition of 100 µM PVP 40 and 522.5 µM citric acid in establishment medium. Leaching continued upto rooting stage, therefore, PVP 40 and citric acid were added in MS medium used for successive transfer and rooting of microshoots. Seedling and basal-sprout explants placed on MS medium with 0.44 µM BA showed maximum shoot lengths 1.45 cm ± 0.13 and 1.16 cm ± 0.22, respectively. Tree explants exhibited best axillary shoot elongation (0.8 cm ± 0.07 cm) on MS medium without plant growth regulators. The cultures derived from seedling and basal-sprout explants could be successfully maintained upto 6th successive transfers, whereas, those derived from tree explants died after 3rd transfer. Microshoots obtained from seedling and basal-sprout explants showed 10% rooting on MS medium supplemented with 4.9 µM IBA.

Key words: Basal-sprouts, microshoots, rooting, shoot induction, shoot proliferation, successive transfer.

FDC: 165.44; 232.328.9; 176.1 *Lagerstroemia parviflora*; (540).

Introduction

Lendia (*Lagerstroemia parviflora* ROXB., Family-Lythraceae) occurs frequently in deciduous forests (PARKASH et al., 1991). Its wood is used for making charcoal, furniture and agricultural implements. Because Lendia undergoes vigorous coppicing, it is fit for short rotation plantations. Coppicing saves cost of replantation considerably (MATHUR et al., 1984). In nature, Lendia propagates through seeds with only 2% germination success (PARKASH et al., 1991). Rooting has been reported in only 13% stem cuttings (BHATT and TODARIA, 1990) making conventional methods of vegetative propagation difficult and unproductive. Micropropagation has been reportedly achieved in 3 species of *Lagerstroemia*, such as, *L. flos-reginae* (PAILY and D'SOUZA, 1986), *L. indica* (ZHANG and DAVIES, 1986) and *L. speciosa* (LIM-HO and LEE, 1985). This paper reports the results of *in vitro* propagation studies in *L. parviflora*.

Materials and Methods

Nodal segments of *L. parviflora* were collected from 3 different types of sources, viz., terminal branches of a 50-year-old tree, basal-sprouts of another 50-year-old tree and 1-year-old (juvenile) plants. The former 2 sources were located in Botanical Garden of lt. Ravishankar Shukla University, Raipur, and the latter were raised from the seeds. Apparently healthy seeds were procured from Raigarh district of Madhya Pradesh and sown in nursery beds. The 3 types of explants were collected from the new growth of the same year, in the month of June 1994. They were washed in running water for 30 min, disinfested with 0.1% aqueous mercuric chloride for 10 min, rinsed thoroughly with sterile mixture of citric acid (522.5 µM) and PVP 40 (25 µM), and suspended in the same mixture for 15 min. Finally, explants cut to a size of approximately 1 cm long were explanted on MS (MURASHIGE and SKOOG, 1962) medium containing citric acid (522.5 µM) and PVP 40 (100 µM), and supplemented with 0.44 µM, 2.22 µM or 11.1 µM 6-benzyladenine (BA). Three % sucrose was used as

Table 1. – Effect of different concentration of BA in MS medium on shoot bud establishment from nodal explants of *L. parviflora,* after 4 weeks.

BA	Seedling Explant			Tree Explant			Basal-sprout Explant		
(µM)	Bud Break (%)	Shoot Number $\bar{X} \pm SE$	Shoot Length $\bar{X} \pm SE$ (cm)	Bud Break (%)	Shoot Number $\bar{X} \pm SE$	Shoot Length $\bar{X} \pm SE$ (cm)	Bud Break (%)	Shoot Number $\bar{X} \pm SE$	Shoot Length $\bar{X} \pm SE$ (cm)
0	100	1 ± 0	0.95 ± 0.12	100	2.5 ± 0.2	0.80 ± 0.07	90	1.0 ± 0	0.93 ± 0.17
0.44	100	1 ± 0	1.45 ± 0.13	100	1.1 ± 0.1	0.75 ± 0.05	100	1.0 ± 0	1.16 ± 0.22
2.22	100	1 ± 0	0.51 ± 0.03	100	1.2 ± 0.1	0.71 ± 0.04	100	2.5 ± 1.9	0.72 ± 0.04
11.1	100	1 ± 0	0.42 ± 0.02	90	1.9 ± 0.2	0.45 ± 0.03	80	2.3 ± 1.8	0.30 ± 0.02

Each treatment consisted of 10 replicates and each experiment was repeated 3 times.

ANOVA: Shoot No.: BA: df = 3; F = 36.66; P < 0.0001*
Explant: df = 2; F = 89.12; P < 0.0001
BA X Explant: df = 6; F = 58.97; P < 0.0001
Shoot length: BA: df = 3; F = 84.76; P < 0.0001
Explant: df = 2; F = 5.78; P = 0.0034
BA X Explant: df = 6; F = 10.86; P < 0.0001

carbon source. The media were solidified with 0.7% agar powder, adjusted to pH 5.7 before autoclaving for 15 min at 121 °C temperature under 1.05 kg/cm^2 pressure. After inoculation, the cultures were incubated at 25 °C ± 2 °C under 16-h photoperiod with a light intensity of 2000 lux under white fluorescent tubes. After 4 weeks, the bud break, shoot number and shoot length were recorded.

The microshoots elongated from all the 3 original explants were excised, and each micronode was cut and used for shoot proliferation. These micronodes were placed on MS medium supplemented with 0.44 µM BA, 522.5 µM citric acid and 100 µM PVP 40 for 4 weeks and then all the new shoots longer than 0.5 cm were harvested, and the remaining part of the shoot culture was transferred to fresh medium. This procedure was repeated 6 times at 4 weeks interval to determine the multiplication potential of nodes.

For rooting, approximately 1.5 cm long shoots were excised and placed vertically on MS medium containing citric acid (522.5 µM) and PVP 40 (100 µM), and supplemented with indolee-3-acetic acid (IAA), indole-3-butyric acid (IBA) or 1-naphthalene acetic acid (NAA).

In all the experiments described above, 10 replicates were taken and each experiment was repeated 3 times. The data were analyzed by ANOVA.

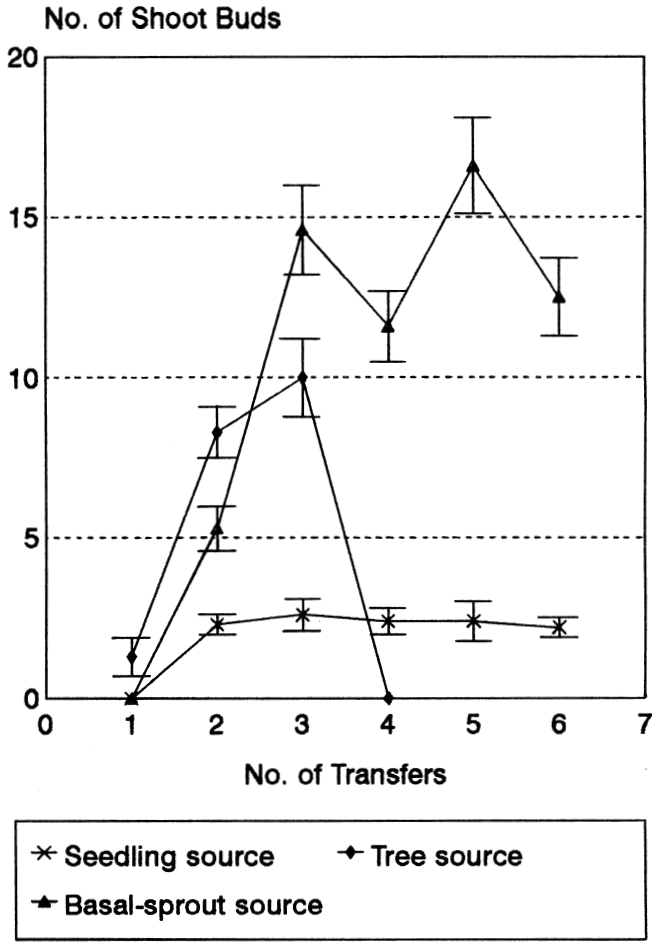
Results and Discussion

Within few hours of inoculation, the nutrient medium turned brown due to excessive leaching of phenolics from cut ends of all the 3 types of explants. Consequently, all the 3 types of explants died soon. Exudation of phenolic substances have been reported to be season (Das and Mitra, 1990) and age (Mascarenhas *et al*., 1987) dependent. But in this case, it occurred throughout the year, irrespective of the age of explant source. This could be effectively checked by dipping the explants into the mixture of citric acid and PVP 40; and by adding these chemicals to nutrient medium. Purohit and Tak (1992) also reported prevention of phenolic exudation from nodal segments of *Feronia limonia* explanted on nutrient medium containing an absorbent and antioxidant.

Axillary bud break from all the 3 types of nodal segments occurred irrespective of PGR level in the culture medium and age of the source of explants (*Table 1)*. The maximum axillary shoot elongation from juvenile and basal-sprout explants was seen on MS medium supplemented with 0.44 µM BA, and from tree nodes on PGR-free MS medium. Both types of tree explants produced >1 shoot per node, which were occasionally albinic in case of 50-year-old tree explants. Juvenile nodes produced a single green and healthy axillary shoot. In general, the juvenile nodes exhibited the best axillary shoot development response, followed by basal-sprout and tree explants. Occurrence of the better response of basal-sprout over tree explants may be attributed to the difference in their maturity levels, basal-sprout shoots being more juvenile than tree (Bonga and von Aderkas, 1993). Present results corroborate those reported by Amin and Jaiswal (1993) in *Artocarpus heterophyllus.*

Micronodes obtained from nodal cultures of the 3 types of explants and inoculated on MS + 0.44 µM BA, showed leaching of phenolics. Therefore, the same mixture of antioxidant and absorbent were added in the shoot multiplication medium. During multiplication, formation of shoot clusters was observed in all the 3 cases (*Fig. 1a*). Occasionally, from these clusters, leader shoots developed exhibiting normal leaf morphology (*Fig. 1b*). Similar phenomenon was reported by Das and Mitra (1990) in *Eucalyptus tereticornis*. Some of these clusters died and some new clusters were induced during each transfer (*Fig. 2*). The highest production of shoot cluster was recorded in sprout-originated cultures, followed by tree and seedling-originated cultures. When the cultures derived from juvenile explants were transferred every 4 weeks to fresh medium, for 6 times, they showed gradual increase in shoot number upto third transfer, whereas, the sprout-derived cultures exhibited sudden increase in shoot number, after the 2nd transfer (*Fig. 3*).

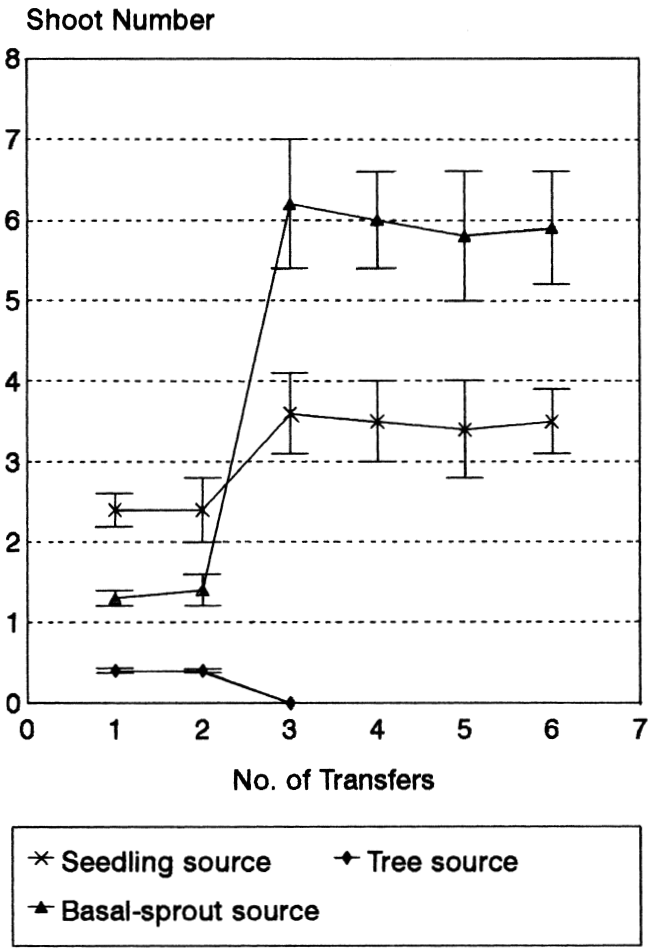
Figure 1. – Shoot multiplication from of basal-sprout source of *L. parviflora*: (a) shoot bud cluster, (b) shoot elongation.



Each treatment consisted of 10 replicates and each experiment was repeated 3 times.

ANOVA: No. of shoot buds: Transfer: df = 5; F = 245.05; P < 0.0001
Source: df = 2; F = 795.78; P < 0.0001
Transfer X Source: df = 10; F = 74.26; P < 0.0001

Figure 2. – Effect of successive transfers on number of shoot buds produced from axillary bud cultures derived from different explant sources of *L. parviflora*. The new shoots were harvested before each transfer and the cultures were maintained on MS + 0.44 µM BA, observations were taken after every 4 weeks.



Each treatment consisted of 10 replicates and each experiment was repeated 3 times.

ANOVA: Shoot No.: Transfer: df = 5; F = 283.68; P < 0.0001
Source: df = 2; F = 724.45; P < 0.0001
Transfer X Source: df = 10; F = –11.54; P = 1.

Figure 3. – Effect of successive transfers on number of shoots produced from axillary bud cultures derived from different explant sources of *L. parviflora*. The new shoots were harvested before each transfer and the cultures were maintained on MS + 0.44 µM BA, observation were taken after every 4 weeks.

This is perhaps due to the formation of large number of shoot buds from basal-sprout-derived micronodes during the first and the second transfers, and subsequent development of these buds into shoots. The basal-sprouts of hardwood trees have been found to mature at slower rate than others (BONGA and VON ADERKAS, 1993). In case of tree explants, very few shoots developed during the 1st and the 2nd transfers but these cultures could not be maintained after the 3rd transfer. In *Fagus sylvatica,* MEIER and REUTHER (1994) also reported better shoot multiplication from rejuvenated material than from mature material, which could not be subcultured *in vitro*. Thus, rejuvenation of plant material is necessary for cloning of mature trees.

After the third transfer, the microshoots placed vertically on rooting media exhibited exudation of black leachates. Therefore, antioxidant and absorbent were added in the rooting medium. Microshoots originating from both juvenile and basal-sprout explants showed 10% rooting on MS medium supplemented with 4.9 µM IBA. The low percentage of rooting suggests that in this species maturation starts much earlier, and the rootability is affected due to maturity. The microshoots derived from mature tree explants could not survive after the third transfer not withstanding the report that 13% stem cuttings possess rootability (BHATT and TODARIA, 1990). Further work would help to understand the role of other factors involved in rooting. Micropropagation of *L. parviflora* appeared to be difficult in comparison to *L. indica* (ZHANG and DAVIES, 1986) and *L. speciosa* (LIM-HO and LEE, 1985), characterized by easy explant establishment and 100% rooting.

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Genetic Control of Wood Basic Density and Bark Thickness and Their Relationships with Growth Traits of *Eucalyptus urophylla* in South East China

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Abstract

Wood basic density based on increment cores, Pilodyn penetration, bark thickness and relative bark thickness (measured as a ratio between bark thickness and diameter) were assessed at 4 *Eucalyptus urophylla* progeny trials in south east China. The results showed that both basic density and Pilodyn penetration are under strong genetic control, with heritabilities of 0.71 and 0.64, respectively. Heritabilities for bark thickness and relative bark thickness were also very high (h^2 = 0.45 and 0.40 respectively). Wood density had weak unfavourable genetic correlations with diameter, height and volume growth. Genetic correlations between relative bark thickness and growth were generally negative. Genotype by site interaction was unimportant for wood basic density, bark thickness, and relative bark thickness. The implications of these genetic parameters on the breeding strategies for this species are discussed.

Key words: heritability, genetic correlation, genotype by environment interaction, REML, *Eucalyptus urophylla*.

FDC: 165.5; 561; 176.1.

Introduction

In recent years, breeding objectives in tree improvement have moved from volume per hectare alone, to include also wood properties and their impact on industrial end products (BORRALHO *et al*., 1993; ZOBEL and JETT, 1995; DIETERS *et al*., 1996; GREAVES *et al*., 1996). Of the range of wood properties, basic density is one of the most important, affecting every aspect of the quality and quantity of pulp (HIGGINS, 1984; HARDING and LI, 1989; ZOBEL and JETT, 1995). Relative ease of measurement and generally high heritability also makes it the most studied wood characteristic in eucalypts (RUDMAN, 1970; HARDING and LI, 1989; BORRALHO, 1992; CHAFE, 1994; LI *et al*., 1995; RAYMOND, 1995; GREAVES *et al*., 1996). In general, density in eucalypts has been reported to be under strong genetic control (ZOBEL and JETT, 1995), with individual heritabilities ranging between 0.4 and 0.84 (RUDMAN *et al*., 1969; OTEBEYE and KELLISON, 1980; WANG *et al*., 1984; MALAN, 1988; DEAN *et al*., 1990; BORRALHO *et al*., 1992; RAYMOND, 1995; ZOBEL and JETT, 1995; GREAVES *et al*., 1996). Genetic correlations between basic density and growth rate have been weak but often unfavourable (MALAN, 1988, 1991; BORRALHO *et al*., 1992; GREAVES *et al*., 1996).

Measurement of wood density is expensive and time consuming and often needs to destroy sample trees, and that has restricted the number and accuracy of the studies published (RAYMOND, 1995). Recently, Pilodyn penetration, an indirect method for determining wood basic density, has proven effective in assessing large number of trees in eucalypts (MOURA *et al*., 1987; DEAN *et al*., 1990; GREAVES *et al*., 1996). Despite being less accurate than direct measurement for disks, GREAVES *et al*., (1996) demonstrated that Pilodyn assessment can yield the same amount of gain as direct selection due to its cheaper cost and higher selection intensity.

As *Eucalyptus urophylla* plantations in southern China and elsewhere are being established mainly for pulping (IKEMORI *et al*., 1986; LI *et al*., 1995), growth and wood properties

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