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In vitro multiple shoot induction from excised shoot tips and nodal segment explants of - Lagerstroemia indica (L) - A medicinal cum Ornamental Shrub.

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Abstract: An efficient *in vitro* multiplication protocol was developed through excised shoot tip and nodal segment of *Lagerstroemia indica*. The effect of different growth regulators alone or in combination on multiple shoots production from different explants of *L.indica* was studied. Multiple shoots were obtained from excised shoot tips and nodal segment on MS medium supplemented with BAP (2.0 mgl^{-1}) and NAA (0.5 mgl^{-1}), Maximum number of shoots (16-24 per explants) was obtained from excised shoot tips.

Key words: *Lagerstroemia indica* L., Nodal segment, Excised shoot tip, Plant growth regulators, *In vitro* multiplication.

Introduction

The *Lagerstroemia indica*. L. (Crape myrtle) is an ornamental cum medicinal shrub or small tree[1,2] native of China belonging to the family Lythraceae commonly cultivated in gardens throughout India. The preparation from its bark is used in Indo-China as purgative and hydrogogue, the roots are astringent and used as gargle and seed contain narcotic principle[3].This plant feed as secondary food plants of non-mulberry Tasar silk worm[4], and also this plant is tolerant to O_3 and NO_2 [5].

In vitro organ culture attempt were made early workers [6-9]. The present study was aimed at enhancing both regeneration efficiency and the multiple shoot production for mass propagation of *L. indica*.

Materials and Methods

Plant material and Culture condition

Excised shoot tips and Nodal segments (1.0 cm long containing at least one node with the axillary buds) were collected from 20 years –old shrub of *L. indica* grown plant in the University Campus, Manasagangotri, Mysore.

These explants were washed in running tap water for 30 minutes followed by washing with 10% solutions of Tween-20 for 25 minutes. These explants were washed thoroughly there after until the detergent was completely washed out, to prevent fungal contamination. Excised shoot tip and nodal explants were treated with 10% Bavistin solution for 30 minutes. Finally the explants were treated with 70% ethanol for one minute followed by 0.4% sodium hydrochloride for 5 to 8 minutes in a laminar air flow chamber. After each treatment explants were washed thrice with autoclaved double distilled water. The explants were then cultured on MS medium supplemented with various concentrations of the growth regulators BAP, Kn, NAA and IBA separately or in combinations. The pH of the medium was adjusted to 5.8 before autoclaving. The medium was autoclaved at 1.1Kg cm^{-2} pressure and 121 ^oC for 15 minutes; cultures were incubated at 25 $\pm 2^oC$ under 8h light/ 16h dark photoperiod (50µ mole m^{-2} S^{-1})

Multiple shoots induction from shoot tip and nodal segment

Properly sterilized excised shoot tips and nodal segment (containing axillary buds) explants were inoculated in glass culture tubes containing MS semi solid basal medium supplemented with different concentrations of auxins (NAA and IBA) and cytokinins (BAP and Kn). Each treatment was repeated thrice. The regenerated plants were subculture every three weeks interval.

Results and Discussion

Multiple shoot induction through shoot tip and nodal segment

Excised shoot tips inoculated on basal MS medium failed to produce multiple shoots. Where in excised shoot tips inoculated on MS medium supplemented with either BAP (1.0 -2.5 mgl^{-1}) or Kinetin (1.0 – 2.5 mg l^{-1}) alone also produced multiple shoots, but in the presence of both BAP (2.0 mgl^{-1}) and NAA (0.5 mg l^{-1}) the multiple shoot regeneration efficiency was higher (91.40%) (Table 1 and Figure. IA).

Nodal segments inoculated on MS basal medium showed 4-6 shoots per culture were obtained. MS medium supplemented with either BAP (0.1 to 2.5mgl^{-1}) or kinetin (0.1 to 2.5mgl^{-1}), alone showed variation in the induction of multiple shoots which ranged between 25 to 66% in case of BAP and 17 to 56.35% in case of kinetin alone. The highest percent of shoot induction (86.68%) was obtained in MS medium supplemented with BAP (2.0 mgl^{-1}) and NAA (0.5 mgl^{-1}) and 5 shoots per culture were obtained (Table 2 and Figure. IB).

Multiple shoots were obtained from two explants though in different proportions. Higher numbers of multiple shoots were obtained from excised shoot tip explants (16-24 shoot per explants); Nodal segment explants produced the least number of multiple shoots (5 per explant).

From excised shoot tip and nodal segment explants, about 5-24 shoots per culture were obtained. Excised shoot tip and nodal segment explants produced 91.40% and 86.68% multiple shoots respectively inoculated in MS medium. BAP (2.0 mgl^{-1}) and NAA (0.5mgl^{-1}) were found to be the best combination for multiple shoot induction. The least response for multiple shooting in excised shoot tip and nodal explants was Kinetin (0.1 mgl^{-1}) and the multiple shoots obtained were 12% and 16% respectively. Nodal segment explants, were inoculated on basal MS medium multiple shoots were produced except excised shoot tip. MS medium supplemented with cytokinins alone BAP or Kinetin produced shoots in nodal and excised shoot tip explants. But by supplementing the medium with cytokinins (BAP or Kinetin) and auxins (NAA and IBA), both the percentage of shoot regeneration as well as number of shoots per culture was increased, which shows that auxins (NAA) also plays significance role in multiple shoot induction. The enhanced rate of multiple shoot induction in cultures supplemented with BAP and NAA may be largely ascribed due to increased rates of cell division induced by cytokinin (BAP) in the terminal and axillary meristematic zone of explant tissues. Cells in this zone divide with the faster pace and thus, produced large number of shoots. The increased multiple shooting in excises shoot tip explants may be due to rapid division of cells in the excised shoot tip and production of several primordial out growth, which eventually develop into shoots.

Conclusion

In the present investigation higher numbers of multiple shoots (16-24 shoots per explant) were obtained from excised shoot tip explants of *L. indica*. There are no earlier reports in which excised shoot tip and nodal segment explants were used for the multiple shoot induction in *L. indica*. Thus, this protocol can be suitably exploited for the mass multiplication on a large scale for commercial and may be worth full in conservation of natural reserves of *L. indica*.

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Table – 1: Multiple shoot induction in excised shoot tip explants of *L.indica.* in MS medium supplemented with different concentration of BAP, Kn, NAA and IBA in combination or alone.

Growth regulators ($mg1^{-1}$)	% Shoot formation of excised shoot tip*	Average shoot per culture of excised shoot tip*	Average length of shoot (cm) of excised shoot tip*
BAP			
0.1	17.00	2.6	2.5
0.5	39.00	3.3	3.6
1.0	44.00	3.6	3.8
1.5	48.00	3.8	4.0
2.0	65.00	5.6	4.3
2.5	53.00	3.7	3.8
3.0	27.00	2.4	2.3
Kn			
0.1	12.00	2.3	1.3
0.5	36.00	2.9	1.9
1.0	42.00	3.1	2.3
1.0	53.00	3.4	2.8
2.0	6.35	6.4	3.7
2.5	51.00	3.3	2.4
3.0	23.00	2.4	2.3
BAP + NAA			
0.1 + 0.1	35.33	2.8	2.8
0.5 + 0.2	39.00	4.6	3.4
1.0 + 0.3	48.22	6.9	3.6
1.5 + 0.4	68.00	16.25	4.8
2.0 + 0.5	91.40	24.00	5.7
BAP + IBA			
0.1 + 0.1	27.05	1.6	1.5
0.5 + 0.2	30.54	2.2	2.4
1.0 + 0.3	44.28	2.8	2.8
1.5 + 0.4	63.00	3.5	3.3
2.0 + 0.5	68.37	5.9	4.2
Kn + IBA			
0.1 + 0.1	28.31	1.8	1.9
0.5 + 0.2	34.38	2.1	2.4
1.0 + 0.3	48.14	2.6	2.7
1.5 + 0.4	64.13	3.1	3.3
2.0 + 0.5	72.00	8.3	3.7
Kn + IBA			
0.1 + 0.1	18.73	1.3	1.5
0.5 + 0.2	25.84	1.8	1.8
1.0 + 0.3	43.59	2.7	2.4
1.5 + 0.4	53.23	2.9	2.6
2.0 + 0.5	58.19	5.1	4.1

*Mean of three replicates of nodal segment explants.

Table – 2: Multiple shoot induction in nodal segment explants of *L. indica* on MS medium supplemented with different concentration of BAP, Kn, NAA & IBA in combinations or alone**.**

Growth regulators ($mg1^{-1}$)	% Shoot formation of excised shoot tip*	Average shoot per culture of excised shoot tip*	Average length of shoot (cm) of excised shoot tip*
BAP	-	-	-
0	15.0	2.0	2.4
0.1	25.00	2.4	4.4
0.5	38.25	2.8	4.6
1.0	48.00	3.1	4.7
1.5	58.00	3.4	4.7
2.0	66.00	3.9	4.8
Kn			
0.1	16.00	2.0	1.4
0.5	36.00	2.4	1.8
1.0	46.00	2.5	2.1
1.5	54.00	2.6	2.6
2.0	56.00	3.2	3.7
2.5	49.00	3.1	2.4
3.0	21.00	2.2	2.1
Kn +IBA			
2.0 + 0.1	46.00	3.1	4.3
2.0 + 0.2	55.00	4.2	6.1
2.0 + 0.3	62.00	3.9	5.2
2.0 + 0.4	72.00	4.2	5.6
2.0 + 0.5	86.68	5.0	5.8
Kn + NAA			
2.0 + 0.1	30.31	1.8	1.9
2.0 + 0.2	38.38	2.1	2.3
2.0 + 0.3	51.37	2.6	2.5
2.0 + 0.4	66.49	3.1	3.1
2.0 + 0.5	74.00	4.3	3.4
Kn + IBA			
2.0 + 0.1	17.79	1.3	1.4
2.0 + 0.2	26.84	1.8	1.8
2.0 + 0.3	47.13	2.7	2.1
2.0 + 0.4	55.23	2.9	2.6
2.0 + 0.5	61.19	3.5	4.5

*Mean of three replicates of nodal segment explants.

Figure. I. In vitro multiple shoots from shoot tip and nodal explants

A - Multiple shoots from shoot tip on MS basal medium supplemented with 2.0 mgl^{-1} + 0.5 mgl^{-1} NAA

B – Induction of multiple shoots from nodal segment on basal medium supplemented with 2.0 mgl^{-1} + 0.5 mgl^{-1} NAA

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