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In vitro Regeneration of *Justicia gendarussa* Burm. f.

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Abstracts: An efficient plant regeneration protocol was developed for *Justicia gendarussa* Burm.f. (Acanthaceae), an important medicinal shrub. Nodal segments grown on Murashige and Skoog (MS) medium containing 1 mg LG^{1} 6-Benzyl adenine (BA) with 10 % coconut milk showed better growth response and produced 10.5 ± 0.6 shoots per explant with an average length of 4.4 ± 0.3 cm after 35 days. Rooting of shoots was achieved on growth regulator free half strength MS medium produced 5.3 ± 0.25 cm roots with an average height of 4.8 ± 0.2 cm after 30 days. The rooted plantlets were transferred for hardening, 80 % of plants were successfully established in the field.

Key words: *Justicia gendarussa* % Nodal segments % Regeneration % Coconut milk

INTRODUCTION

Justicia gendarussa Burm. F. (Acanthaceae) is an erect, branched, smooth undershrub, 0.8 - 1.5 meters in height with long leaves (7 to 14 cm) having acute tips; small flowers, terminal pinkish spikes with purple spots. It is found through out India and in Asian countries like Malaysia, Indonesia and Srilanka [1]. The extracts of roots and leaves are used as important ingredient of many ayurvedic preparations [2]. The plant is used in traditional medicinal practice for chronic rheumatism, inflammations, bronchitis, head ache, arthritis, facial paralysis, internal heamorrhages, vaginal discharges, dyspepsia, eye disease and common fever [3]. *J. gendarussa* has been reported to have higher mineral profiles in leaves (Ca, Mg & Zn) [4] and process antivenom properties [5]. The seeds of *J. gendarussa* show a very low germination percentage and not allow the production of homogenous populations [6]. In vitro culture of *J. gendarussa* has been attempted through organogenesis [7]. The present study aims to develop an alternative simple, rapid, economical and high frequency of plantlet regeneration through nodal explants for large scale propagation.

MATERIALS AND METHODS

Plant Material: Healthy plants of *J. gendarussa* collected from Surapet village near Chennai, Tamil Nadu, India and were raised in pots containing soil and farm yard manure (1:1) under green house conditions at Department of Biotechnology of our College.

Explant Preparation and Shoot Regeneration: Nodal segments were collected from potted plants, brought to the laboratory and processed. For surface sterilization, the explant were cleaned thoroughly under running tap water for 20 minutes; washed with a solution of Tween 20 (2 drops in 100 ml of water) for 1 min and again washed with sterile distilled water. The cleaned explants were finally treated with $HgCl_2$ (0.1% w/v) for 4-5 minutes under aseptic conditions and washed 5 times with sterile distilled water to remove traces of $HgCl_2$.

After surface sterilization, explants were trimmed to 0.8 - 1.0 cm and inoculated on MS basal medium [8] supplemented with different concentrations of BA (0.1, 0.25, 1.0, 1.5 and 2.0 mg LG^{1}) or Kinetin (Kin) (0.1, 0.25, 1.0, 1.5 and 2.0 mg LG^{1}) with Coconut milk (10.0 %) for shoot multiplication. The proliferated

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shootlets 4.5-5.0 cm length was transferred to half strength MS medium supplemented with no growth regulator for root development. Root number and length were recorded after 30 days in culture. Well developed plantlets were rinsed thoroughly with sterile water to remove residuals and potted with a mixture of red soil, vermiculite and farm yard manure (1:1:1), covered with moistened polythene bags for hardening. After 15 days, the fully acclimatized plantlets were transplanted to pots (6 cm dia).

Culture Medium and Conditions: MS basal medium supplemented with 3% (w/v) sucrose was used for all *in vitro* culture studies. The pH of the medium was adjusted to 5.6 ± 0.2 prior to adding 0.9 % (w/v) agar and autoclaved at 121°C at 1.06 kg cm^{-2} for 15 min. Cultures were maintained at 25 ± 1°C under 16h photoperiod with a photosynthetic hoton flux density (PPFD) of 50 µmol m^{-2} s^{-2} provided by cool white fluorescent lams (Phillips, India) and with 60 - 65 % relative humidity. The plant growth regulators were filter-sterilized using 0.2 µm filter (Minisart®, Sartorius) prior to addition to culture media.

RESULTS AND DISCUSSION

Multiple shoots developed from nodal explants cultured on MS medium supplemented with BA (0.25 - 2.0 mg L^{-1}), Kin (0.25 – 2.0 mg L^{-1}) and fortified with coconut milk (10.0 %).

Effect of Individual BA or Kin with 10 % Coconut Milk on Shoot Multiplication: Initiation of multiple shoots in most of the treatments was observed within 3 weeks of culture. High number of multiple shoots developed in MS medium containing BA 1.0 mg L^{-1} with 10% coconut milk (CoM), 80% response and produced 10.5 ± 0.6 shoots per explant with an average length of 4.4 ± 0.3 cm after 35 days (Table 1; Figure 1A, B & C). Whereas higher concentration of BA (2 mg L^{-1}) containing medium did not significantly induce the number of shoots per explants. A combination of other growth substances including Kinetin (KN) + CoM was not effective (Table 1). BA has been considered to be one of the most active cytokinins in organogenic differentiation in plant tissue culture [9-11]. The use of BA with 10 % coconut milk (CoM) in our study, the development of multiple shoot

Table 1: Effect of individual BA or Kin with 10 % coconut milk in MS medium on*in vitro* shoot multiplication from nodal explants of *J. gendarussa* after 35 days of culture

Plant growth regulators (mg L^{-1})				
BAP	% of Coconut water added with medium	Shoot Induction %	Number of shoots per explant	Shoot length (cm)*
0.25 mg L^{-1}	10%	46.8	3.4 ± 0.1	3.0 ± 0.6
0.5 mg L^{-1}	10%	55.9	4.0 ± 0.2	3.4 ± 0.2
1.0 mg L^{-1}	10%	80.0	10.5 ± 0.6	4.4 ± 0.3
1.5 mg L^{-1}	10%	51.8	3.7 ± 0.2	3.1 ± 0.8
2.0 mg L^{-1}	10%	39.8	2.9 ± 0.3	1.8 ± 0.2
KN				
0.25 mg L^{-1}	10%	-	-	-
0.5 mg L^{-1}	10 %	16.9	2.3 ± 0.1	0.8 ± 0.1
1.0 mg L^{-1}	10 %	29.6	3.4 ± 0.2	1.2 ± 0.1
1.5 mg L^{-1}	10 %	19.8	2.5 ± 0.1	1.1 ± 0.2
2.0 mg L^{-1}	10%	-	-	-

Results represent mean ± SD of three replicated experiments

Data were recorded after 35 days of culture

Table 2: Root formation from *in vitro* grown shoots of *J. gendarussa* after 30 days of culture

Medium	% response	Roots / shoot	Root length (cm)
½ MS	100.0 ± 0.0	5.3 ± 0.25	4.8 ± 0.2

Results represent mean ± SD of three replicated experiments

Data were recorded after 30 days of culture

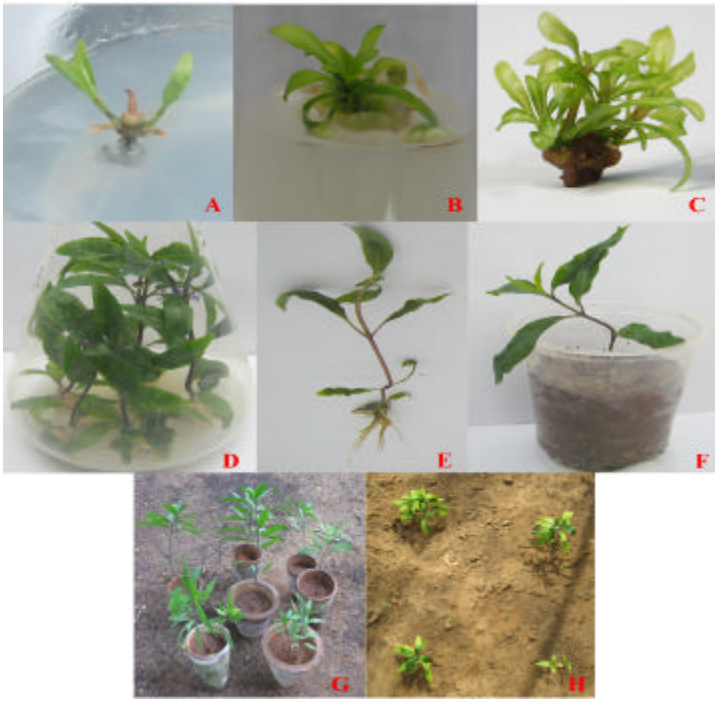

Fig. 1: Stages in the micropropagation of *J. gendarussa*
(A) Initiation of shoot from nodal explants after two weeks of culture.
(B) Multiple shoot initiation after three weeks of culture on MS medium containing 1.0 mg LG^{1} BA with 10% coconut milk
(C) Proliferation of multiple shoots from nodal explants after 35 days of culture.
(D) Rooted plantlets after 30 days of culture on ½ strength MS medium without growth regulator
(E) A well established plant
(F) Hardened *in vitro* plants successfully transplanted to the plastic cups
(G) Acclimatized plantlets successfully transplanted to the pots
(H) In field condition

production is superior to the earlier observation on using MS medium supplemented with higher concentration of BA (3 mg LG^{1}) were recorded 4.3 shoots per explant [12].

Root Formation: The regenerated shoots with 4-5 leaves were rooted on the growth regulator free half strength MS medium. The first roots appeared after 2 weeks of culture and after 25 days, the root system was well developed (Figure 1D & E). The percentage of rooting was 100% and 5.3 ± 0.25 roots per shoot induced after 30 days old culture on root induction medium (Table 2). The high number of roots per shoot produced on half strength growth regulator free medium in *J. gendarussa* with subsequent high survival rate.

Acclimatization: One hundred percent plantlet survival was seen after hardening of the regenerated *J. gendarussa* in red soil, vermiculite and farmyard manure (1:1:1) for 3 wk. However, the rate decreased as some plants died over the next 4-10 wk after transfer to soil. It was observed that very gradual acclimatization of *in vitro* grown plants to the external environment is most essential for *J. gendarussa*. Eighty percent of the plants transferred to pots survived and resumed growth (Figure 1F, G & H).

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