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In Vitro Multiplication of Two Economically Important and Endangered Medicinal Plants – *Justicia gendarussa* Brum and *Adenia hondala* (Gaertn) De Wilde

Johnson M[1*], Manickam V.S.[2], Sonali Das[1], Nikhat Yasmin[1], and Andal N[3]

[1] Muthayammal College of Arts and Science, 637 408 Rasipuram, India
[2] Centre for Biodiversity and Biotechnology, St.Xavier's College, Palayamkottai, India
[3] Sri paramakalyani Centre for Environmental Biotechnology, Manomanium Sundaranar University, Tirunelveli, India
* biojohnson2001@yahoo.com
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ABSTRACT A method for the micropropagation of *Justicia gendarussa* Brum and *Adenia hondala (Gaertn) de Wilde* from nodal segments was developed. High percentage of proliferating microshoot cultures were obtained by placing nodal segments on Murashige and Skoog's medium supplemented with 4.44μM BAP, 8.88μM, 2.22μM, 22.2μM and 2.69μM NAA respectively (85 %). Individual shoots were excised and transferred onto half strength MS medium for rooting. The half MS medium augmented with 4.92μM IBA produced maximum number (8 & 3 respectively) and maximum percentage (80 & 70 respectively) of rooting. The plantlets could be subsequently hardened to green house conditions.

(*In vitro*, multiple shoots, nodal explants, *Justicia gendaruss, Adenia hondala)*

INTRODUCTION

Justicia gendarussa Brum belonging to the family Acanthaceae is an evergreen shrub. Mature plants are an important source of drugs. Roots and leaves of the plant find wide use in both traditional and modern systems of medicine as an anti-emetic, anti-bechic and haemostatic and are particularly useful for bronchitis. The plant rarely set seeds; natural regeneration and conventional propagation through vegetative cutting are slow, season dependent and insufficient for large-scale production.

Adenia hondala (Gaertn) de Wilde belonging to Passifloraceae is a tendril climber with large tuberous roots, stem thickened at nodes. The drug source is the subterranean tuber of woody climbers, which have the shape of the tusk of a boar. It promotes strength and improves complexion, voice and cures burning sensation. It is vulnerable regionally. The conventional method of propagation is not satisfactory. The *in vitro* multiplication technique is used as an alternative tool for the large-scale multiplication. A number of reports are available for large-scale multiplication of medicinally important plants using tissue culture. Some examples Sivasubramaniam *et al.* [12] in *Plectranthus vetiveroides;* Singh and Sudarsana [11] in *Baliospermum axillare;* Beena *et al.* [2] in *Ceropegia candelabrum;* Martin [8] in *Rotula aquatica;* Gupta [6] in *Pelargonium graveolnes;* Johnson [6] in *Rhinacanthus nasutus;* Pawar *et al.* [10] in *Solanum surattense.* With this strong background the present study is intended to multiply such economically important and endangered medicinal plants of *Justicia gendurussa* Brum and *Adenia hondala* (Gaertn) de Wilde through biotechnological tools such as micropropagation and reintroduce them back into the natural habitats.

MATERIALS AND METHODS

Wild plants of *Justicia gendarussa* and *Adenia hondala* were collected from Tirunalvelli Hills and established in St. Xavier's college herbal garden. Young shoots of 5cm length were collected, defoliated and washed in running tap water for 10 minutes. Surface contamination of shoots consisted of passage through 0.1% (w/v) $HgCl_2$ for two minutes (*Justicia gendarussa* and *Adenia hondala*) and washed thrice in sterile

distilled water. The nodal segments of 0.8 - 1cm long were prepared and cultured onto [9] agar medium supplemented with 3% sucrose, 0.6% (w/v) agar (HIMEDIA, MUMBAI) and different concentration of and combination of BAP, NAA, IAA and 2,4-D. The pH of the medium was adjusted to 5.8 before adding agar. The medium was autoclaved at 121°C for 15 minutes in Astell scientific autoclave U.K. The cultures were incubated at 24 ± 2°C under the cool white fluorescent light (2000 Lux, 12 hours photoperiod). Multiple shoots of (4-5cm length) which formed 5-6 weeks after explants culture was excised and cultured on half strength MS supplemented with different concentration of IBA and IAA for rooting. The resulted rooted plants were washed thoroughly in water before transplanting into small polycups containing mixture of sterile sand and garden soil (1:1) and irrigation with 1/10 diluted liquid MS medium and covered with polybags for *in vitro* hardening. After hardening they were subsequently transferred to 15cm diameter pots containing garden soil, sand and compost (2:1:1) and maintained under mist irrigation until they were shifted for field.

RESULTS AND DISCUSSION

Justicia gendarussa

Nodal explants of *Justicia gendarussa* was inoculated onto MS medium with different concentration of plant growth regulators (BAP, 2, 4-D, NAA and IAA). After 5-10 days, the axillary bud proliferated. The MS medium supplemented with BAP (4.44μM) recorded maximum survival and shoot formation (85%) (Figure 1A). A combination of BAP (8.88μM) and NAA (2.69μM) induced the multiple shootlets (4-5) (Figure 1B). Multiple shootlets were also obtained in the MS medium supplemented with BAP (13.3μM) and NAA (2.69μM) (Table 1). MS medium supplemented with different concentration of BAP (4.44μM, 2.22μM and 8.88μM) produced aerial roots (Table 1).

Maximum number of aerial roots was formed at 4.44μM BAP concentration (Figure 1C). Maximum callus was obtained in MS media supplemented with 2, 4-D (0.45μM and 4.52μM). The 3-5cm long shoot cutting was transferred in MS media supplement with various concentration and combination of auxins (IAA, IBA and NAA) for rooting. Maximum numbers of roots were obtained in the half strength MS medium supplemented with IBA (4.92μM) and IAA (5.71μM) (Table 2). Maximum root length was also observed in the same hormonal concentration (up to 8cm). These results are in accordance with the [3 and 4] results. The number of shoots obtained in the micropropagation of *Justicia gendarussa* is somewhat similar to those found by [13] in *Adhatoda beddomi* who found that IAA and IBA are equally important in inducing root formation. After 30 days, the *in vitro* raised plants were transferred to polycups for hardening (Figure 1E). Nearly 78% of the micropropagated plants were established in pots.

Adenia hondala

Nodal explants of *Adenia hondala* were inoculated in MS medium containing different concentration of plant growth regulators (BAP, 2,4-D, NAA and IAA). After 5 days, the axillary buds produce shoots (Figure 2A). The MS medium supplemented with BAP (8.88μM) recorded maximum survival and shoot proliferation (85%) (Figure 2B). MS medium supplemented with BAP (2.22μM) and BAP (8.88μM) combined with NAA (2.69μM) yield 3-4 shoots per explants (Figure 2C). Maximum shoot length was obtained from the nodal explants cultured in MS medium supplemented with BAP (2.22μM) (8cm). Maximum callus was obtained in the MS medium supplemented with 2,4-D (2.26μM, 4.52μM and 3.16μM). Maximum shoot length (8cm) was obtained in the MS medium supplemented with BAP (2.22μM) (Table 3).

3-5cm length *in vitro* raised shoots cuttings were transferred to the MS medium supplemented with IBA, IAA and NAA. The cultures were kept in dark condition for rooting. After 20 days, the roots were formed on half MS medium supplemented with IBA (4.92μM) and (5.71μM) IAA under dark condition (Table 4). Maximum number of roots (8 roots) were obtained in the same concentration (Figure 2D). After 30 days, the *in vitro* raised plants were transferred to polycups for hardening (Figure 2E).

Our observation directly consonance with those results of Anand and Hariharan [1] in *Alpinia galanga*, Kukreja and Mathur [7] in *Durbrisia myoporoides*, Sivasubramaniam [12] in *Plectranthus vetiveroides*, Singh and Sudarshana [11] in *Baliospermum axillare*, Beena *et al.* [2] in

Ceropegia candelabrum, Martin [8] in *Rotula aquatica*, Gupta *et al.* [5] in *Pelargonium graveolnes* and Johnson *et al.* [6] in *Rhinacanthus nasutus*.

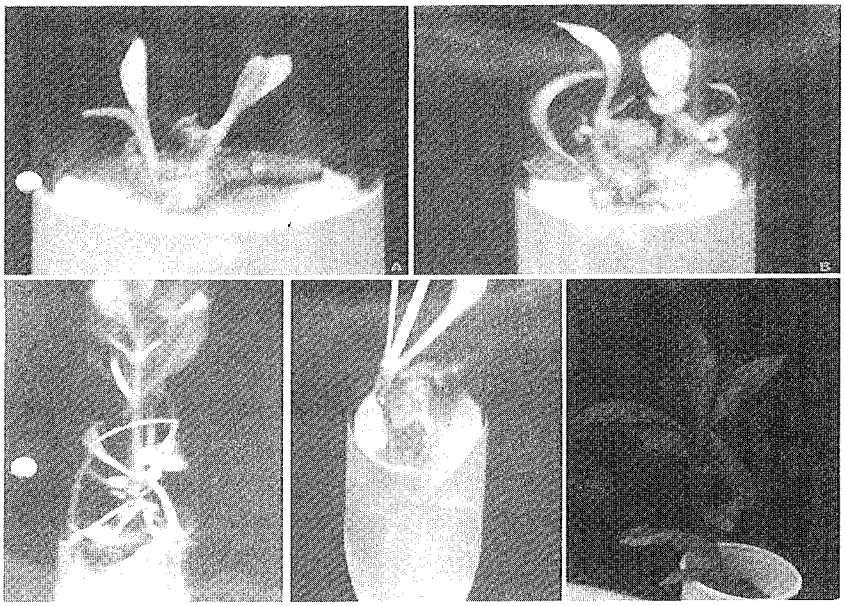

Figure 1. *In Vitro* Multiplication of *Justicia gendarussa* Brum
A: Initial stage
B: Multiple shoots on MS medium with BAP (4.44μM & 8.88μM)
C: Multiple shoots with aerial roots
D: Roots formation on half MS medium with IBA (4.92μM)
E: Polycup hardened plant (90 days old)

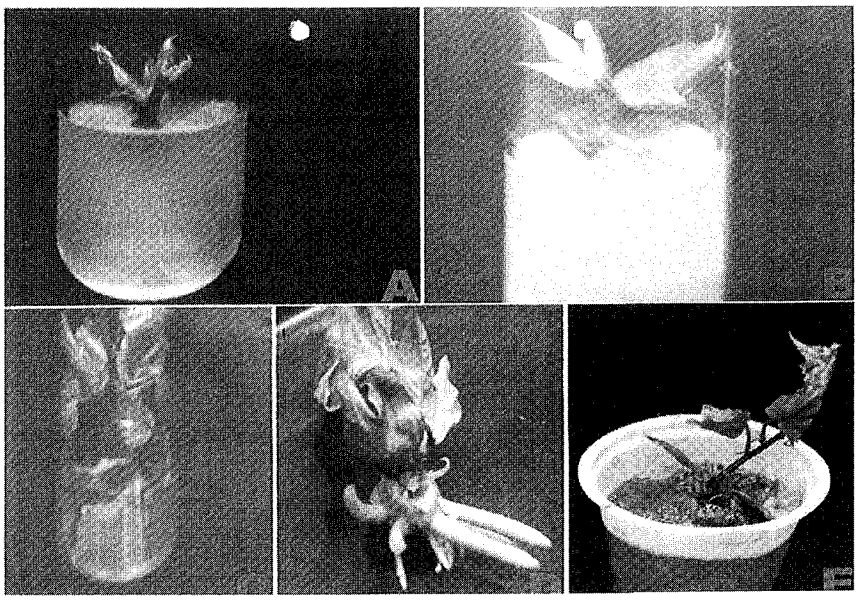

Figure 2. *In Vitro* Multiplication of *Adenia hondala* (Gaetrn) De Wilde
A: Initial stage
B: 10 days old culture
C: Multiple shoots on MS medium with BAP (22.2μM) and NAA (2.92μM)
D: Roots formation on half MS medium with IBA (4.92μM)
E: Polycup hardened plant (90 days old)

Table 1. Effect of Plant Growth Regulators on the Formation of Multiple Shoots from the Nodal Explants of *Justicia Gendarussa* in MS Medium

Plant Growth Regulators (μM)			% Of Shoot Formation	Mean Length Of Shoots ± S.E	No. Of Shoots/Explant ± S.E.
BAP	IAA	NAA			
0.44	0.0	0.0	75	7.5 ± 0.28	2.5 ± 0.50
0.88	0.0	0.0	70	6.0 ± 0.12	1.4 ± 0.17
2.22	0.0	0.0	55	6.5 ± 0.38	2.3 ± 0.25
4.44	**0.0**	**0.0**	**85**	**11 ± 0.2 5**	2.8 ± 0.09
8.88	0.0	0.0	50	7.5 ± 0.42	1.5 ± 0.75
33.3	0.0	0.0	55	2.0 ± 0.18	2.1 ± 0.26
8.88	2.85	0.0	40	3.0 ± 0.32	2.0 ± 0.20
8.88	**0.0**	**2.69**	**60**	**10 ± 0.50**	**4.8 ± 0.50**
13.3	0.0	2.69	40	5.0 ± 0.42	4. 8 ± 0.45

Table 2. Effect of Plant Growth Regulators on the Formation of Multiple Roots from the Shootlets of *Justicia Gendarussa* in Half MS Medium

Plant Growth Regulators (μM)			%Of Root Formation	Mean Length Of Roots After 15 Days(Cms)	No. Of Roots/Shoot±S.E.
IBA	IAA	2,4-D			
9.84	0.0	0.0	75	6.2 ± 0.53	6.5 ± 0.11
4.92	**5.71**	**0.0**	**70**	**8.3 ± 0.25**	**8.1 ± 0.23**
4.92	0.0	4.52	80	4.5 ± 0.41	4.2 ± 0.80

Table 3. Effect of Plant Growth Regulators on the Formation of Multiple Shoots from the Nodal Explants of *Adenia Hondala* in MS Medium

Plant Growth Regulators (μM)			% Of Shoot Formation	Mean Length After 15 Days(Cms) ± S.E	No. Of Shoots/Explants±S.E.
BAP	2,4-D	NAA			
0.44	0.0	0.0	65	1.8 ± 0.25	3.5 ± 0.45
2.22	0.0	0.0	60	4.0 ± 0.50	3.2 ± 0.32
4.44	0.0	0.0	50	3.8 ± 0.36	2.3 ± 0.17
8.88	0.0	0.0	85	4.5 ± 0.15	3.0 ± 0.26
2.22	**0.0**	**2.69**	**55**	**7.2 ± 0.17**	**4.0 ± 0.20**
4.44	0.45	0.0	40	3.5 ± 0.43	1.5 ± 0.80
4.44	4.52	0.0	40	3.3 ± 0.68	2.0 ± 0.54
8.88	0.0	2.69	60	5.5 ± 0.12	4.2 ± 0.1
13.3	0.0	2.69	60	6.2 ± 0.43	4.4 ± 0.09
22.2	**0.0**	**2.69**	**40**	**7.5 ± 0.51**	**2.0 ± 0.45**

Table 4. Effect of Plant Growth Regulators on the Formation of Multiple Roots from the Shootlets of *Adenia Hondala* in Half MS Medium

Plant Growth Regulators (μM)				%Of Root Formation	Mean Length Of The Root After 15 Days(Cms) ± S.E	No. Of Roots /Shoot±S.E.
IBA	IAA	NAA	2,4-D			
2.46	1.43	0.0	0.0	—	—	—
4.92	0.0	0.0	4.52	50	0.6 ± 0.45	1.2 ± 0.36
0.0	5.71	5.37	0.0	60	1.3 ± 0.28	2.6 ± 0.14
1.25	1.43	0.0	0.0	—	—	—
4.92	**5.71**	**0.0**	**0.0**	**70**	**2.0 ± 0.79**	**3.5 ± 0.20**

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