

In vitro Propagation of *Adenia hondala* (Gaertn.) de Wilde

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Abstract— *Adenia hondala* (Gaertn.) de Wilde belonging to the family Passifloraceae is a perennial climbing herb with potential medicinal value. The possibility of *in vitro* clonal propagation of *Adenia hondala* was investigated by the use of nodal explants cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of BAP and KN. Optimum treatment was the combination of 1 mg.L⁻¹ BAP and 0.5 mg.L⁻¹ KN that enhanced percent response of explants and the number of multiple shoots per explants. An average of 10.23 shoots per explants was resulted after 32 days of culture. *In vitro* shoots were elongated in MS medium supplemented with 1 mg.L⁻¹ KN. Half strength MS medium supplemented with 1 mg.L⁻¹ IBA was found to be the best medium for rooting. The rooted plantlets were gradually acclimated *ex vitro* in mist chamber and successfully established under field conditions with high survival rate.

Keywords— *Adenia hondala*, *in vitro*, multiple shoots, nodal segments.

I. INTRODUCTION

Adenia hondala belonging to the family Passifloraceae is found in the forests of Western Ghats. It has been red listed as vulnerable in South India. *A. hondala* is a perennial climber with tuberous roots, simple tendrils, simple and lobed leaves and circular glands between lobes of leaves. The monoecious flowers have oblong petals, 5 stamens, globular ovary and a trifid stigma. The tuber powder is used to treat cough and it increase lactation in nursing mother. The extract of tuber is used to cure intermittent fever, Anonymous, (1999) and the roots are used for the treatment of skin troubles, Anonymous, (2003).

Ayurveda is a system of Medicine with historical roots in Indian Subcontinent. 'Vidari', an Ayurvedic drug is an ingredient of more than 50 Ayurvedic formulations like Chyavanaprash and its annual requirement is about 500–1000 Metric Tonnes, Sulaiman *et al.*, (2014). Ayurveda correlates 'vidari' to tubers of *Pueraria tuberosa* (Roxb. Ex Willd.) DC (Fabaceae) and *Kshiravidari* to *Ipomoea mauritiana* Jacq. (Convolvulaceae). However, in Ayurvedic

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Pharmacopoeia both these species are attributed similar properties and are substituted by each other, Venkatasubramanian *et al.*, (2009). Apart from these, tubers of *Adenia hondala* (Passifloraceae) and the pith of *Cycas circinalis* L. (Cycadaceae) are also traded as 'vidari', Ved and Goraya, (2008). 'Vidari' is used as aphrodisiac, cardiogenic, diuretic and refrigerant, Chopra *et al.*, (1992). In traditional and folklore systems, 'vidari' has been used as a tonic, rejuvenator and galactagogue, Mithila *et al.*, (2014). It is reported that use of 'vidari' is beneficial in persons suffering from or prone to diabetes and coronary disease problems, Sulaiman *et al.*, (2014).

Indiscriminate collection, poor seed set and seed germination resulted in the disappearance of this plant from wild habitats. The reason for the diminishing number of *A. hondala* could be the dwindling forests due to excessive urbanization. Having established its potential as a medicinal plant there is a great necessity for large scale multiplication of this plant which is rapid, simple and genetically stable. *In vitro* protocols serve as a viable tool for conservation and propagation of germplasm, especially of endangered and threatened plants. There is a single report on *in vitro* propagation of this plant species (*A. hondala*) which focuses on *in vitro* organogenesis and somatic embryogenesis. The present experiment was conducted to develop a successful protocol for rapid clonal propagation of *A. hondala* through the culture of nodal explants.

II. MATERIALS AND METHODS

2.1 Plant sample and experiment design

Stem cuttings with four to six nodes were collected from three months old *A. hondala* plant. The stem was cut into single node pieces (2 to 3 cm length) and was washed in running tap water for 10 minutes. The nodal explants were then immersed in Bavistin solution (15 g.L⁻¹) for 3 minutes with Cefotaxime (200 mg.L⁻¹) and Tetracycline (200 mg.L⁻¹). After distilled water wash, the explants were sterilized in 1% mercuric chloride solution for three minutes followed by several rinses with double distilled water. The pH of the medium was adjusted to 5.8 before autoclaving at 15 psi

pressure and 121°C temperature for 15 minutes. All these culture vials were incubated in plant growth room at 25±2°C under 16/8 hour photoperiod with 50 μ mol m⁻² s⁻¹ light intensity supplied by cool white fluorescent lamps and 60±65% relative humidity. The nodal explants were then inoculated into MS media (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. The shoot bud initiation and multiple shoot induction were studied by inoculating the explants on MS media supplemented with 6- Benzylaminopurine -BAP (0.25, 0.5, 1.0, 2.0 mg.L⁻¹) either alone or in combination with Kinetin-KN (0.25, 0.5, 1.0, 2.0 mg.L⁻¹). Subcultures were carried out at an interval of 14 days. The proliferation rate for each of the treatment was observed. Same media compositions were used to study shoot elongation. During elongation, the length of shoot and the number of nodes were observed and recorded. Half strength and full strength MS media with (0.25, 0.5, 1.0 mg.L⁻¹) or without Indole Butyric Acid- IBA were experimented for *in vitro* rooting. Percentage of root induction, root length and root number were the parameters recorded for each treatment. The rooted shoots were taken out from the culture bottles and washed thoroughly with running tap water to remove traces of medium. These plants were transferred to plastic pots containing a mixture of soil and sand. Almost all rooted plants were acclimated and transferred to field conditions.

2.2 Statistical analysis

All experiments were performed with three replications, having 30 samples each. The effect of various treatments on selected growth parameters was measured quantitatively and statistically tested using analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences) version 11.0. The significance of the mean values of various treatments was assessed by Duncan's New Multiple Range Test (DMRT) at $p < 0.05$.

III. RESULTS

3.1 Effect of BAP and KN on multiple shoot induction and shoot proliferation from nodal explants

Cultures without any growth regulators were taken as control. No shoot induction was observed in control media. The effect of cytokinis on multiple shoot induction was experimented by culturing the nodal explants on MS medium supplemented with BAP and KN either alone or in different combinations. It was observed that, for the same concentration of BAP and KN tested (0.25, 0.5, 1.0, 2.0 mg.L⁻¹), percentage of explants showing shoot induction decreased (Table 1) on KN supplemented (75- 78%) media than the media with BAP (81- 83%). Among various

concentrations and combinations, best results were recorded on medium containing 1 mg.L⁻¹BAP and 0.5 mg.L⁻¹ KN noticed 87% of explants proliferated within 7 days (Table 1). This particular combination of growth regulators regenerated 10.23±0.40 shoots, (Fig 1) which was found to be the single optimum treatment that promoted highest number of multiple shoots within 32days. MS medium with different combinations of BAP and KN favoured comparatively high multiple shoot regeneration than the treatments with BAP or KN alone. Combinations of BAP (1.0 mg.L⁻¹) with KN (0.25, 0.5 mg.L⁻¹) significantly increased the shoot number as compared to other treatments with BAP and KN alone (Table 2). In the present study, increased concentrations of BAP (2 mg.L⁻¹) adversely affected the shoot multiplication rate.

3.2 Effect of KN on shoot elongation

Same media composition as that of the shoot induction media were experimented for shoot elongation. Shoots obtained from KN supplemented media were significantly longer among all treatments including their combinations (Table 2). Among all the concentrations and combinations of growth regulators used, MS with 1.0 mg.L⁻¹ KN showed better result for shoot elongation after 12 days of sub culturing with an average length of 4.87± 0.15cm and an optimum number of 4.10 ± 0.40 nodes per explants. In the present study, length of the shoots were shorter in BAP as compared to KN either alone or in their combination (Table 3). Shoots of KN supplemented media were significantly longer among all cytokinin treatment. The presence of KN in the medium allowed the *in vitro* shoots to elongate where the morphogenetic response was lower in lower concentrations (0.25, 0.5mg.L⁻¹) as compared to higher concentration (1.0 mg.L⁻¹). Moreover higher concentrations of BAP (2 mg.L⁻¹) inhibited the shoot length compared to its lower concentrations (0.25, 0.5, 1.0 mg.L⁻¹) with or without KN.

3.3 Effect of Media and IBA on rooting

To induce rooting, *in vitro* shoots were transferred to full strength and half strength MS media with (0.25, 0.5, 0.1 mg.L⁻¹) or without IBA. The effects of media and IBA treatment on root formation from *in vitro* shoots were summarized on Table 4. The inclusion of IBA in the rooting media increased the root number. It was observed that half strength MS supplemented with 1.0 mg.L⁻¹ IBA showed highest percentage of root induction (83.00±0.50). Half strength MS media supplemented with IBA (1.0 mg.L⁻¹) was optimum in inducing an average number of roots (3.83 ± 0.35) reaching up to length 4.43 ± 0.15 cm within two

weeks of culture. As compared to half strength MS, full strength MS supplemented with IBA resulted in a

significant decrease in the percentage of root induction, number of roots and length of roots.

Table.1: Effect of BAP and KN on multiple shoot induction using nodal explants

Treatments	MS + Growth regulators		% of explants showing shoot induction	Number of days for shoot induction
	BAP (mg.L ⁻¹)	KN (mg.L ⁻¹)		
T ₀	0	0	00.000 ⁱ	00.000 ^g
T ₁	0.25	0	83.36±0.41 ^c	8.20±0.26 ^d
T ₂	0.5	0	82.33±0.47 ^d	8.43±0.20 ^d
T ₃	1	0	81.26±0.20 ^e	8.90±0.10 ^c
T ₄	1	0.25	85.73±0.61 ^b	7.67±0.20 ^e
T ₅	1	0.5	87.05±0.10 ^a	6.90±0.26 ^f
T ₆	2	0	80.57±0.14 ^f	8.86±0.05 ^c
T ₇	0	0.25	78.37±0.40 ^g	9.26±0.24 ^b
T ₈	0	0.5	77.73±0.25 ^g	9.46±0.05 ^{ab}
T ₉	0	1.0	75.90±0.62 ^h	9.77±0.01 ^a
T ₁₀	0	2.0	75.50±0.34 ^h	9.76±0.14 ^a

Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly ($P > 0.05$)

Table.2: Effect of BAP and KN on shoot proliferation after 32 days of culture.

Treatments	MS + Growth regulators		Number of multiple shoots per explant
	BAP (mg.L ⁻¹)	KN (mg.L ⁻¹)	
T ₀	0	0	00.000 ^g
T ₁	0.25	0	3.50±0.30 ^e
T ₂	0.5	0	3.97±0.21 ^{de}
T ₃	1	0	4.67±0.25 ^c
T ₄	1	0.25	5.93±0.30 ^b
T ₅	1	0.5	10.23±0.40 ^a
T ₆	2	0	3.40±0.36 ^e
T ₇	0	0.25	2.40±0.40 ^f
T ₈	0	0.5	3.87±0.11 ^{de}
T ₉	0	1.0	4.23±0.49 ^{cd}
T ₁₀	0	2.0	2.17±0.50 ^f

Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly ($P > 0.05$)

Table.3: Effect of KN on shoot elongation after 12 days of culture

Treatments	MS + Growth regulators		Shoot length(cm)	Number of nodes
	BAP (mg.L ⁻¹)	KN(mg.L ⁻¹)		
T ₀	0	0	00.000 ^g	00.000 ^d
T ₁	0.25	0	2.50±0.10 ^e	2.20±0.30 ^b
T ₂	0.5	0	2.53±0.37 ^e	2.30±0.46 ^b
T ₃	1	0	2.80±0.20 ^{de}	2.23±0.51 ^b
T ₄	1	0.25	3.03±0.35 ^d	2.80±0.40 ^b
T ₅	1	0.5	3.53±0.40 ^{bc}	2.50±0.40 ^b
T ₆	2	0	2.06±0.15 ^f	1.20±0.10 ^c
T ₇	0	0.25	3.23±0.25 ^{cd}	2.77±0.25 ^b
T ₈	0	0.5	3.87±0.06 ^b	3.50±0.36 ^a

Treatments	MS + Growth regulators		Shoot length(cm)	Number of nodes
	BAP (mg.L ⁻¹)	KN(mg.L ⁻¹)		
T ₉	0	1.0	4.87±0.15 ^a	4.10±0.40 ^a
T ₁₀	0	2.0	3.06±0.20 ^d	3.63±0.31 ^a

Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly ($P > 0.05$)

Table.4: Effect of media and IBA on *in vitro* rooting.

Treatments	Media strength	IBA (mg.L ⁻¹)	% of root induction	Root number	Root length (cm)
T ₀	Half strength MS	0	51.03±0.47 ^d	1.27 ± 0.35 ^d	0.90 ± 0.26 ^d
T ₁		0.25	66.00±1.00 ^c	1.80 ± 0.36 ^c	1.83 ± 0.42 ^c
T ₂		0.5	71.80±0.80 ^b	2.80 ± 0.10 ^b	2.53 ± 0.29 ^b
T ₃		1	83.00±0.50 ^a	3.83 ± 0.35 ^a	4.43 ± 0.15 ^a
T ₀	Full strength MS	0	40.5±0.50 ^d	0.83 ± 0.35 ^c	0.90 ± 0.40 ^b
T ₁		0.25	51.63±0.20 ^c	0.93 ± 0.41 ^c	0.83 ± 0.20 ^b
T ₂		0.5	61.00±1.00 ^b	1.63 ± 0.05 ^b	1.53 ± 0.25 ^a
T ₃		1	65.9±0.65 ^a	2.40 ± 0.10 ^a	1.93 ± 0.37 ^a

Level of significance was measured at $p < 0.05$. Column values with same superscript are not differing significantly ($P > 0.05$)

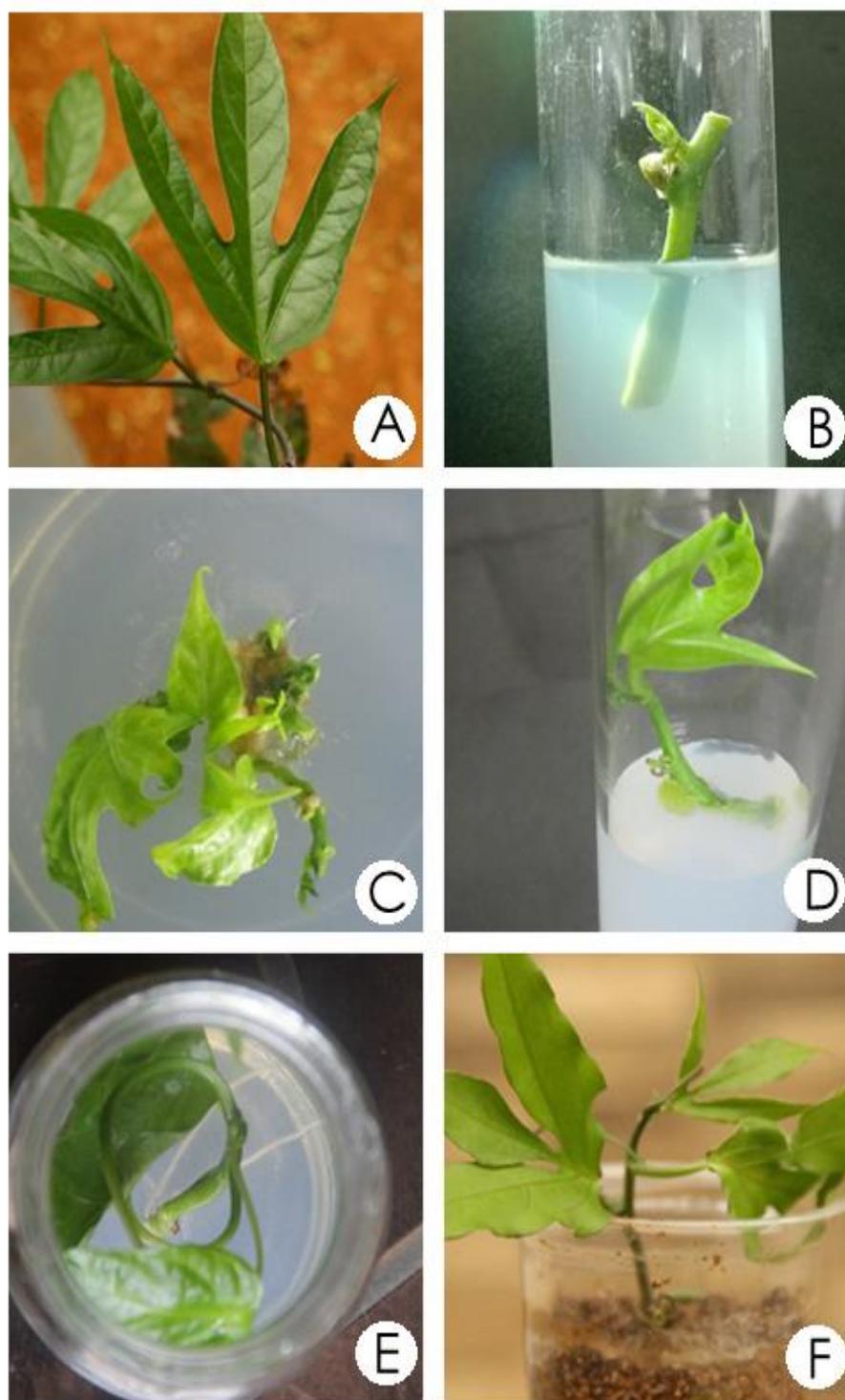


Figure.1. Micropropagation of *Adenia hondala*. A, Habit of *Adenia hondala*; B, Shoot bud initiation from nodal segments in MS medium supplemented with BAP 1 mg.L⁻¹ + KN 0.5 mg.L⁻¹; C, Multiple shoot induction from nodal segments in MS supplemented with BAP 1 mg.L⁻¹ + KN 0.5 mg.L⁻¹; D, Shoot elongation in MS supplemented with KN 1 mg.L⁻¹; E, Rooting in half MS basal media with 1 mg.L⁻¹ IBA; F, Hardened *in vitro* regenerated plant of *Adenia hondala* after acclimatisation

IV. DISCUSSION

Cytokinins have major role on plant development, such as the regulation of shoot induction, shoot multiplication and promotion of cell division, Mok and Mok, (2001). In the present study various concentrations of BAP and KN induced significant differences on shoot induction percentage, number of shoots per explants and shoot length. It was observed that the percentage of shoot emergence per explants increased on media with decreased concentrations of cytokinin. These findings are in line with the findings of Reddy and Saritha, (2013). The synergistic effect of BAP and KN produced high rate of shoot multiplication. The results are in accordance with Mehdi *et al.*, (2014); Shirin and Rana, (2007) and Saha *et al.*, (2007). BAP enhanced *in vitro* shoot induction and proliferation of many medicinal plant species (Lakshimi and Mythili, 2003) which is in conformity with the results of the present study. BAP is more effective on shoot induction and multiplication and less effective on shoot elongation as compared to KN. These results are in conformity with those of Mehdi *et al.*, (2014) and Sharma *et al.*, (1993). KN treatment significantly affected on increasing the shoot length and number of nodes as compared to any other concentration or combination of BAP either alone or with KN. The stimulatory effect of KN on shoot elongation was reported by Saha *et al.*, (2007). In the present experiment, optimum response for rooting resulted on half strength MS media with 1 mg.L⁻¹ IBA than full strength MS media with same hormone concentrations. It has been reported, Zayona *et al.*, (2014) half MS supplemented with auxin enhanced the number of rooted plants in micropropagation of *Paulownia elongata*. The number and length of roots was found to be increased on half strength MS with or without IBA than full strength MS. These results are in accordance with Shekhawat *et al.*, (2015) and Lattoo *et al.*, (2006).

V. CONCLUSION

In vitro propagation through nodal explants of *A.hondala* is an easy and economic way for obtaining large number of consistently uniform plants. Present protocol holds tremendous potential to select, multiply and conserve this genotypes, which are a potential resource of medicinally important constituents and it reduce the dependence on the natural habitat for the supply of raw drugs.

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