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PLANTLET FORMATION THROUGH SHOOT TIP CULTURE IN VASAKA (*ADHATODA VASICA* NEES)

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Shoot tips of *Adhatoda vasica* Nees were cultured on B5 medium supplemented with KN alone & in combination of NAA. Multiple shoot buds were obtained on supplementing the media with Phloroglucmol (396.48 µM). Reduced concentration of KN (4.65 µM) was favourable for elongation of shoots. Shoots rooted when transferred to B5 medium with half strength inorganic and organic constituents. Complete plantlets were formed in quarter strength medium.

Key Words : *Adhatoda vasica,* shoot tip, plantlet, *in vitro.*

About a quarter of the prescription drugs used in industrialized nations contain ingredients extracted from higher plants (Anderson *et al.*, 1982). Micropropagation of medicinal plants is of considerable importance for mass propagation of high yielding clones (Chomchalow and Sahavacharin, 1981). Tissue culture techniques have been reported for conservation and multiplication of medicinal plants and for the production of physiologically active important compounds (Cervelli, 1987; Pandey *et al.*, 1992). Shoot tip culture has been widely used for rapid propagation of many species due to its advantage over traditional methods (Hu and Wang, 1983).

Vasaka (*Adhatoda vasica* Nees syn. *Justicia adhatoda* L, family Acanthaceae) is a multipurpose shrub of tremendous medicinal significance and is used for the treatment of cough, bronchitis, asthma and tuberculosis (Pandita et *al.*, 1983). There is a considerable demand for this plant within the country. Its indiscriminate use has led to its large scale depletion in the wild and has necessitated its replenishment and cultivation.

Although some work has been done on regeneration of roots of *Adhatoda* its tissue culture has scarcely been attempted (Jaiswal *et al.*, 1983). The present communication describes an efficient procedure for regeneration of plantlets from shoot tip cultures of *A. vasica.*

MATERIALS AND METHODS

Esatablishment of cultures

Small twigs of *A. vasica* were collected from naturally growing healthy and active plants. *In vitro* cultures were initiated from shoot tips (3-8 mm) possessing 2-3 primordial leaves. Explantswere thoroughly washed under running tap water for 1 hr. Surface sterilization was carried out with 2% Teepol (10 min.) followed by 70% Ethanol (10 min) and finally with 0.1% $HgCl_2$ (10 min). Explants were washed with d H_2O in between and then rinsed several times with sterilized double distilled water before inoculation. For the present study B5 medium (Gamborg *et al.*, 1968) supplemented with different concentrations (0.46, 2.32, 4.65, 23.23 and 46.46 µM) of cytokinin viz Kinetin (6 furfurylaminopurine) alone and in combination with auxin viz NAA (alpha) naphthalene acetic acid), (0.54-5.37 µM). The medium was supplemented with 3% sucrose (Qualigens) and solidified with 0.8% agar (Qualigens). Additives like Phloroglucinol (396.48 µM) was also included in the medium. The pH of the media was adjusted to 5.6-5.8 before autoclaving. Cultures were maintained at 25 ± 2°C with 16 hr photoperiod (approx 1500 lux) and subcultured every 4 weeks. A total of 15 replicates were kept in each treatment and each treatment was repeated twice.

OBSERVATIONS AND DISCUSSION

Shoot differentiation

Explant sewelling was the first change in the shoot tips cultured on medium containing KN (0.46 - 46.46 µM) alone within 8-10 days of inoculation. However, neither low (0.46-4.65 µM) nor high (46.46 µM) concentrations proved suitable for initial regenerative response and only a moderate concentration of

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Table 1 Frequency of shoot formation from apical buds of *A vasica* on B5 medium supplemented with growth regulators (Mean ± SE).

PGRs (μM)	Shoot Formation (%)
1. KN	
0.46	46.67 ± 20.06
2.32	13.34 ± 3.35
4.65	16.67 ± 10.02
23.23	83.34 ± 16.72
46.46	13.34 ± 3.35
2. NAA + KN	
0.54 + 0.46	80.00 ± 13.37
0.54 + 2.32	86.67 ± 6.69
0.54 + 4.65	93.34 ± 6.69
0.54 + 23.23	100
0.54 + 46.46	90.00 ± 10.03
2.69 + 0.46	100
2.69 + 2.32	86.67 ± 6.69
2.69 + 4.65	93.34 ± 6.69
2.69 + 23.23	93.34 ± 6.69
2.69 + 46.46	83.34 ± 16.72
5.37 + 0.46	90.00 ± 10.03
5.37 + 2.32	83.34 ± 16.72
5.37 + 4.65	96.67 ± 15.78
5.37 + 23.23	60 ± 6.69
5.37 + 46.46	73.34 ± 6.69

(23.23 μM) KN exhibited excellent (100%) shoot growth. A combination of KN (0.46-46.46 μM) with NAA (0.54-5.37 μM) also produced favourable results (Table 1). The higher frequency of shoot growth in combined treatments emphasizes the requirement of both auxins and cytokinins for shoot formation as reported earlier (Das and Mitra, 1990).

The explants were subcultured in the same media with the additive like Phloroglucinol (396.48 μM). After one subculture a cluster of shoot buds was observed with KN (23.23 μM) alone as well as KN (0.46-23.23 μM) combined with NAA (0.54 μM) Rosette bud clumps formed were compact and small in size (Fig-1) hindering the separation of clumps into smaller units. A higher frequency of multiple (2-9) shoot formation occurred from rosette clumps when KN level was reduced from 23.23 μM to 4.65 μM. Similar observation was made in combined treatments employing KN (4.65 μM) and NAA (2.69 μM).

Elongation of microshoots

Elongation of shoot buds was difficult and a slow process due probably to multiple shoot formation. The induction of multiple shoot buds has been reported in past to inhibit whole plant regeneration (Reddy and Bahadur, 1989). To overcome this problem KN

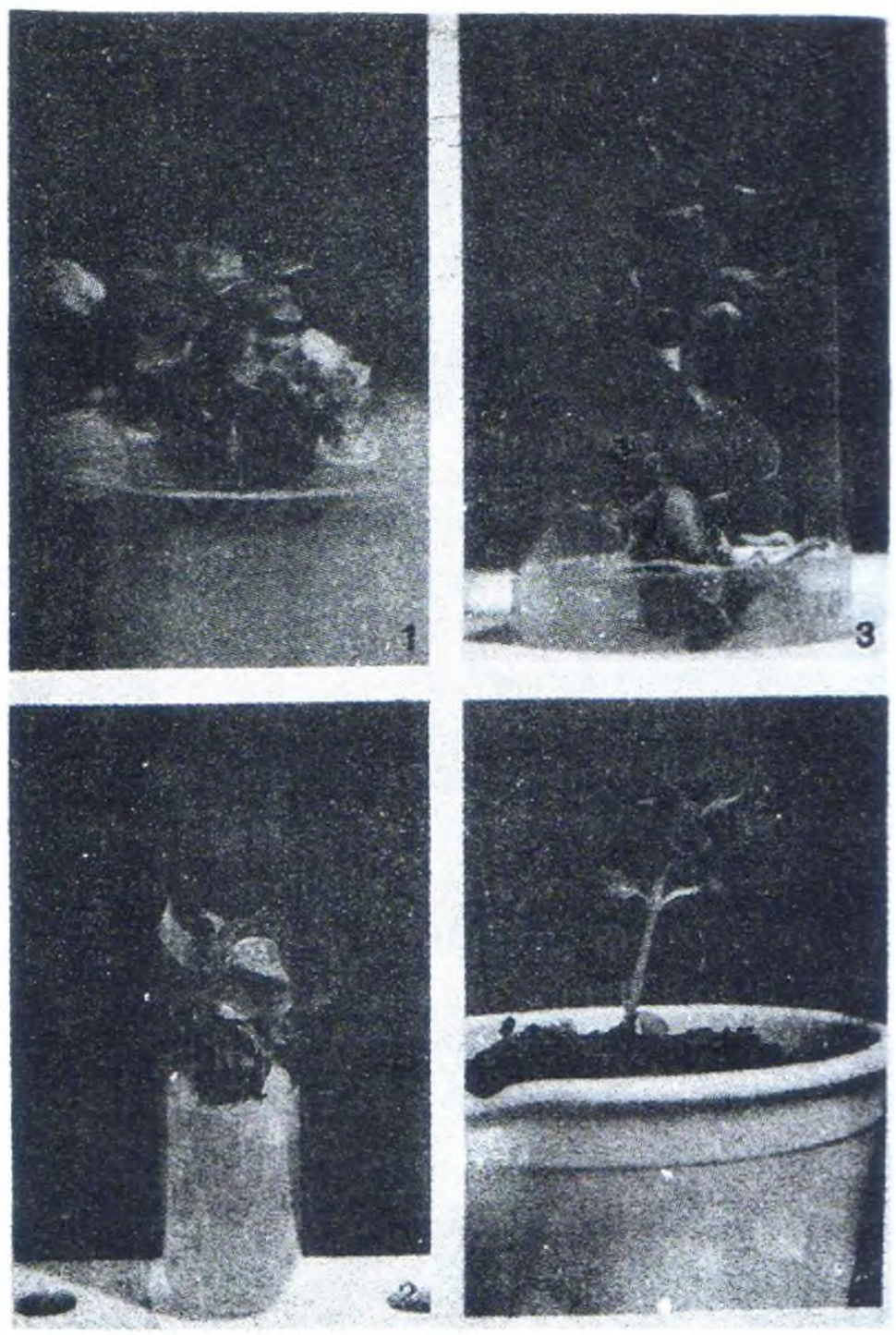

Fig 1. Multiple shoot formation on B5 + KN (4.65 μM) + PG (396.48 μM). Fig 2. Elongation of shoot buds on B5 (half) + KN (4.65 μM) + PG (396.48 μM). Fig 3. Root regeneration from elongated shoots on B5 (quarter) + KN (4.65 μM) + PG (396.48 μM). Fig 4. Four week old plantlet transferred to soil.

levels were altered during subsequent subcultures. Elongation of the shoots were favoured by KN at low (4.65 μM) concentrations (Fig 2) whereas its high (46.46 μM) concentrations was found to be inhibitory. similar observations were made in other species (Lee and Wetzstein, 1990).

The addition of Phloroglucinol (396.48 μM) in the elongation medium further supported growth and elongation of the microshoots as described earlier (Thomas and Mehta, 1983). Microshoots were subcultured on the the same medium thrice to obtain proper growth of the shoots. It was necessary to separate the elongating shoots (2 cm) from the rosette clumps and culture in reduced (1/2) strength medium. This step was essential for the growth of the remaining shoots of the clump.

Root induction

Induction of long and thin roots occurred in the

shoot elongation medium containing half strength of micro and macro elements within a period of 10-15 days. This was followed by their culture in quarter (1/4) strength media which led to further strengthening of the root system within the subsequent four weeks (Fig 3). This observation supports the earlier finding of the suitability of reduced media strength for root regeneration (Phillips *et al.*, 1996). However, this report differs with the earlier finding that exogenous KN inhibits the normal development of apical meristem into a complete plant (Smith and Murashige, 1970).

Soil transfer

Roots of the plantlets (4-5 cm) were freed from adhering agar and washed with sterilised water. They were then treated with bavistin (0.1%) and finally transferred to FYM : soil (presterilized) (1:1). The plantlets frequently irrigated with water have been maintained in Plant Growth Chamber where they have been showing vigorous growth for over two months now (Fig 4).

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REFERENCES

Anderson L A, A T Keene & J D Phillipson 1982 Alkaloid production by leaf organ root organ and cell suspension cultures of Cinchona ledgeriana. *Planta Medica* **46** 25-27.

Cervelli R 1987 *In vitro* propagation of Aconitum noveboracense and Aconitum napellus. *Hort Science* **22** 304-305.

Chomchalow N D & O Sahavacharin 1981 The role of tissue culture in the development of medicinal plants and spices. *In Proc COSTED Symp on Tissue Culture of Economically Important Plants* p 162-166.

Das T O & G C Mitra 1990 Micropropagation of *Eucalyptus tereticornis* Smith. *Plant Cell Tissue and Org Cult* **22** 95-103.

Gamborg O L, R A Miller & K Ojima 1968 Nutrient requirements of suspension cultures of soyabean root cells. *Exp Cell Res* **50** 151-158.

Hu C Y & P J Wang 1983 Meristem, shoot tip and bud cultures. In *Handbook of Plant Cell Culture* Vol I (Evans D A, R S Williams, P V Ammirato & Y Yamada eds) MacMillan Publishing Co New York p 177-227.

Jaiswal V S, P Narayan & M Lal 1989 Micropropagation of *Adhatoda vasica* Nees through nodal segment culture. In *Tissue Culture and Biotechnology of Medicinal and Aromatic Plants* (Kukreja A K, A K Mathur, P S Ahuja and R S Thakur eds) CIMAP Lucknow p 7-11.

Lee N & H Y Wetzstein 1990 *In vitro* propagation of muscadina grape by axillary shoot proliferation. *J Am Soc Hort Sci* **115** 324-329.

Pandey R K, P S Chandel & S R Rao 1992 *In vitro* propagation of *Allium tuberosum* Rottl ex Spreng by shoot proliferation. *Plant Cell Rep* **11** 375-378.

Pandita K, M S Bhatia R K Thappa S G Agarwal K L Dhar & C K Atal 1983 Seasonal variation of alkaloids of *Adhatoda vasica* and detection of glycosides and N-oxides of vasicine and vasicinone. *Planta Medica* **48** 81-82.

Phillips G C, J F Hubstenberger & E E Hansen 1996 Adventitious shoot proliferation In *Plant Cell Tissue and Organ Culture* (Gamborg O L and G C Phillips eds) Narosa Publishing House New Delhi p 55-65.

Reddy V R K & B Bahadur 1989 *In vitro* multiplication of castor. In *Recent Adv Genet & Cytogenet* (Farook S A and Khan I A) Premier Publ House Hyderabad p 479-482.

Smith R H & T Murashige 1970 *In vitro* development of the isolated shoot apical meristem of Angiosperms. *Amer J Bot* **57** 562-568.

Thomas V & A R Mehta 1983 Effect of Phloroglucinol on shoot growth and initiation of roots in carob tree cultures grown in vitro. In *Plant Cell Culture in Crop Improvement* (Sen S K and K L Giles eds). Plenum Press New York p 451-457.