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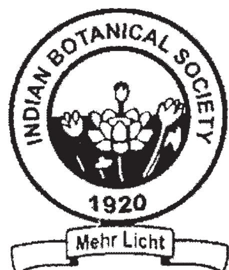

IN VITRO PROPAGATION FROM APICAL BUDS OF ASHWAGANDHA(*WITHANIA SOMNIFERA* (L.) DUNAL)

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In vitro propagation of *Withania somnifera* (L.) Dunal has been successfully established from apical bud explants on MS medium. Maximum number of multiple shoots was obtained on MS medium containing BAP (0.44 µM) + KN (2.32 µM). Half MS supplemented with IBA (0.49 µM) gave maximum frequency of root initiation & root number. These plantlets were successfully transferred to the field.

Key words: Apical buds, kinetin, micropropagation, *Withania somnifera.*

India has a rich heritage of knowledge on plant based drugs for use in preventive as well as curative therapies (Rai and Nath 2005). About 6000 plants in India have been in use in traditional, folk and herbal medicines (Dubey *et al.* 2004)

Plant tissue culture is a powerful tool for studying and solving basic applied problems in plant biotechnology (Das *et al.* 1996). Ashwagandha (*Withania somnifera* (L.) Dunal) belonging to the family Solanaceae is a plant of tremendous economic importance (Sivanesan and Murugesan 2008). The plant is reputed to have adaptogenic, tonic, analgesic, antipyretic, diuretic, anti inflammatory, abortifacient and sedative properties (Siddique *et al.* 2004). Leaves of the plant contain withanolides like withaferin A that exhibit anti bacterial and anti tumour properties (Supe *et al.* 2006).

Owing to its high medicinal value Ashwagandha has been overexploited resulting in depletion of its natural population (Sen and Sharma 1991). It can be propagated both by sexual and asexual methods. Seed propagation, however, is not always satisfactory, since the seeds show poor viability and produce a great deal of variation (Manickam *et al.* 2000). On the other hand multiplication through cuttings gives rise to less productive plants than the seed derived plants. Looking to its importance present study was undertaken to develop a protocol of the *in vitro* multiplication of *Withania somnifera.*

MATERIALS AND METHODS

The plants were collected from the nursery of the Jawaharlal Nehru Krishi Vishwavidyalaya (J.N.K.V.V.) Adhartal, Jabalpur and Tropical Forest Research Institute (T.F.R.I.), Jabalpur. Both the field grown plants and10-15 days old seedlings provided the explants in the form of apical buds (5-10 mm), axillary buds (10 mm) and leaves (5×5 mm). The explants were washed by a wetting agent labolene (1%) and then rinsed in running water (20-25 min.). They were then surface disinfected with 0.1% (w/v) mercuric chloride for 1-2 min. and rinsed with autoclaved distilled water thrice. These sterile explants were then dried on sterile filter paper and subsequently inoculated on MS medium supplemented with different concentrations of PGRs viz. cytokinin eg. 6-

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Table 1. Effect of cytokinin (BAP) on **r**egeneration parameters from **a**pical buds of *Withania somnifera* (Values are Mean ± SE)

S/No	BAP (µM)	FSI (%)	MSN	MSL (cm)	MNN	FRI (%)	RN	RL
1	0.44	36.11	0.52±0.092	2.0±0.235	4.0±0.408	-	-	-
2	2.22	27.7	0.38±0.016	1.0±0.235	1.0±0.235	-	-	-
3	4.44	75	1.62±0174	2.0±0.235	3.95±0.235	-	-	-
4	8.88	54.1	0.71±0.148	2.0±0.235	2.0±0.235	-	-	-
5	22.2	70.83	0.45±0.023	3.0±0.235	3.0±0.235	12.5	0.43±0.028	3.0±0.235

Incubation period: 20 weeks

Table 2. Effect of Kinetin (KN) regeneration parameters from apical buds explants of *Withania somnifera* (Values are Mean ± SE)

S/No	BAP (µM)	FSI (%)	MSN	MSL (cm)	MNN	FRI (%)	RN	RL
1	0.46	47.22	1.19±0.161	4.18±0.386	4.77±0.095	27.7	-	-
2	2.35	30.5	0.30±0.023	0.76±0.008	3.0±0.235	-	-	-
3	4.64	41.6	0.83±0.069	1.70±0.244	1.04±0.408	-	-	-
4	9.28	66.6	0.66±0.065	2.33±0.024	2.0±0.235	12.5	0.5±0.040	4.0±0.408
5	23.2	62.6	0.70±0.120	1.0±0.235	1.25±0.117	-	-	-

Incubation period: 20 weeks

Benzyl amino purine (0.44, 2.22, 4.44, 8.88, 22.2 µM), Kinetin (0.46, 2.32, 4.64, 9.28, 23.2µM) and auxins like 2,4-Dichloro phenoxy acetic acid (0.45µM, 2.25µM, 4.54µM, and 22.7µM) alone and in combinations. The pH of the medium was adjusted to 5.6-5.8 using 0.1N NaOH or 0.1N HCl and was autoclaved at 121^{0}C for 15 minutes. The cultures were incubated at 25±2^{0}C relative humidity (RH) of 60-70% and a light intensity of approx 1500 lux provided by cool, white fluorescent tubes under a photoperiod of 16/8 hrs (light/dark). For multiple shoot induction plants were routinely subcultured after 20-25 days for rooting. Individual shoots above 3-4 cm. long were transferred to MS medium containing IAA, IBA or 2,4-D for rooting. All the experiments were performed in duplicates.

RESULTS AND DISCUSSION

Shoot differentiation- Apical bud explants were cultured on MS medium (basal). It differentiated into single shoots within two weeks. No multiple shoots were observed. Similar results were obtained by Sivanesan and Murugesan (2008). To induce multiple shoots

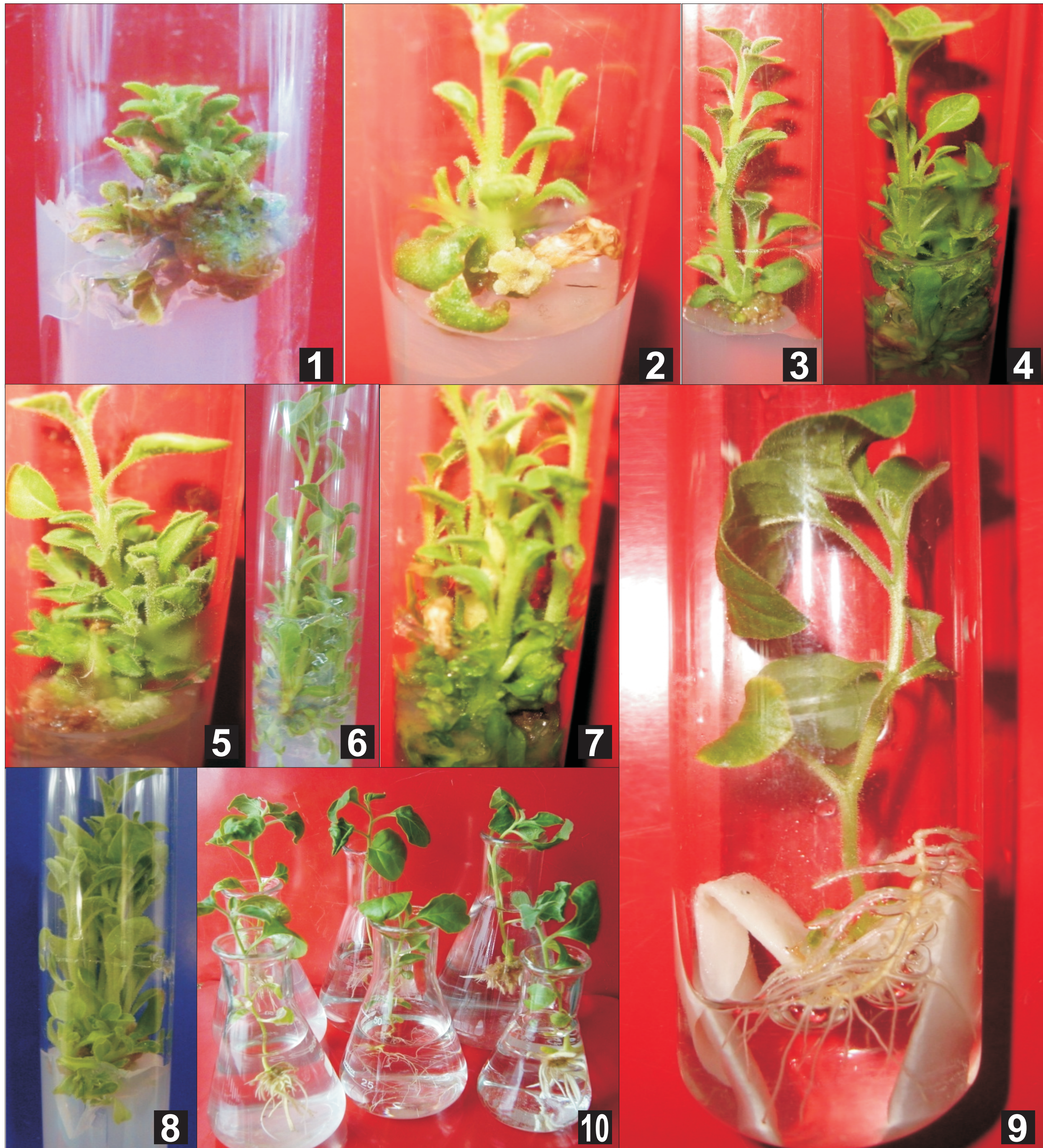

Figure 1-10 : Initiation with multiple shoot on MSM + BAP (2.22µM), 2. Elongation with multiple shoot on MSM + BAP (2.22µM), 3. Elongation with multiple shoot on MSM + BAP + KN (0.44µM+2.32µM), 4. Elongation with vigorous multiple shoot on MSM+BAP+KN (0.44µM+4.64µM), 5. Elongation with vigorous multiple shoot formation on MSM+BAP (8.88 µM), 6. Elongation with vigorous multiple shoot on MSM+BAP+KN (0.44µM+2.32µM), 7. Elongation with vigorous multiple shoot formation on MSM+BAP (8.88 µM), 8. Elongation with vigorous multiple shoot on MSM+BAP+KN (0.44µM+0.46µM), 9. Shoot elongation with rooting on MSM+½ MS+ IAA, 10. Hardening of *Withania somnifera.*

Table 3. Effect of cytokinin combination (KN+BAP) on rgeneration parameters from apical bud explants of *Withania somnifera*. (Values are Mean± SE)

S/No	BAP (0.44μM)			
	KN (μM)			
	0.46	2.32	4.64	23.2
FSI (%)	70.83	54.1	60	58.33
MSN	0.79±0.146	2.0±0.235	1.3±0.082	0.58±0.065
MSL (cm)	5.0±0.235	5.0±0.235	2.25±0.107	1.25±0.117
NN	3.12±0.075	10.31±0.149	3.25±0.117	0.87±0.061

Incubation period: 20 weeks

Table 4. Effect of Auxin (2, 4 D) on regeneration parameters from apical bud explants of *Withania somnifera.* (Values are Mean ± SE)

S/No	2,4D (μM)	FSI	MSN	MSL	MNN	FRI	RN	RL
1	0.45	54.16	0.54±0.016	2.0±0.235	1.35±0.47	20.83	0.83±0.096	4.0±0.408
2	2.25	70.83	0.87±0.061	1.75±0.118	3.06±0.08	-	-	-
3	4.54	37.05	0.37±0.034	0.25±0.010	0.37±0.034	-	-	-
4	22.7	-	-	-		-	-	-

Incubation period: 20 weeks

Table 5. Effect of Auxins IAA and IBA in combination with ½ MS on regeneration parameters from apical budexplants of *Withania somnifera* . (Values are Mean± SE)

S/No	PGRs (μM)	FSI	MSN	MSL	MNN	FRI	RN	RL
1	1/2 MS	66.6	0.66±0.065	6.5±0.235	3.0±0.235	66.6	2.0±0.235	3.5±0.235
2	1/2 ,MS +IBA	83.33	0.83±0.069	4.5±0.236	3.75±0.424	83.33	5.8±0.094	3.0±0.235
3	1/2 ,MS +IAA	66.6	0.66±0.006	6.5±0.235	3.0±0.235	66.6	5.6±0.282	4.0±0.235

Incubation period: 20 weeks

MS medium was supplemented with cytokinins (BAP and KN) alone and in combination. Multiple shoot formation observed was maximum at BAP (4.44μM). Similar results were obtained by Manickam *et al.* (2000). However, maximum shoot length was observed with its high concentration (22.2 μM) along with rooting contrary to the previous reports (Sivanesan and Murugesan 2008). (Table -1).

MS medium containing KN (0.46 μM) induced the highest number of shoots, shoot length, node number and also supported rooting whereas only shooting was reported at higher concentration (4.64 μM) (Sivanesan 2007). Besides good shoot initiation, regeneration was also observed by us at higher concentration of KN (9.28-23.2 μM) (Table -2).

Different cytokinins in combination induced multiple shoot formation with BAP (0.44 μM) + KN (0.46 μM) providing the maximum number of shoots, whereas maximum shoot length and node number were obtained on lower concentration of BAP (0.44 μM) + KN (2.32 μM). This is contrary to the reports of Sabir *et al.* (2008) who obtained these at higher concentration of BAP and KN. Thus, the present protocol appears to be more efficient than that reported previously (Table 3).

For shooting different concentrations of auxins were also used. 2,4- D at 2.25 μM gave maximum shoot initiation, shoot number and node number. Similar results were obtained by Sen and Sharma (1991). However, 2,4-D at 0.45 μM gave maximum shoot length as well as rooting.

Root induction-The roots were thick, short and fleshy with 2,4-D at 0.45 μM which was not reported previously (Table 4).

For rooting, the shoots were transferred to liquid media containing half strength of MS alone as well as in combination with different auxins viz. IAA and IBA. The maximum frequency of root initiation (83.33 %) was obtained on half strength of MS in combination with IBA (0.49 μM) with maximum root number (5.8). The maximum shoot length was, however, obtained on half MS with IAA (0.57 μM) (Table- 5).

HARDENING AND ACCLIMATIZATION

When the shoots of the *in vitro* regenerated plantlets attained a height of 6-7 cm bearing healthy leaves and good root system, the plants were subjected to hardening by keeping them in conical flasks filled with distilled water initially and covered with beaker for 4 days at 25±2°C and then transferred in tap water for 4 days (Gokhale and Bansal 2010). Plant lets were transferred to pots containing sterilized Soil: sand (1:1) covered with polytenens fo 15 days. Finally the plantlets were transferred to field. Approximately 60% plantlets survived Figures 1-10.

The present study revealed that a combination of BAP and KN at lower concentration gave maximum shoot number and shoot length and 2, 4-D gave thick and fleshy roots. Both these results were not previously reported. Thus our protocol appears to be more cost efficient in comparison to the previous protocols.

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