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REGENERATION OF A MEDICINAL HERB KALMEGH [*ANDROGRAPHIS PANICULATA* (BURM. F.) WALLICH EX NEES] THROUGH TISSUE CULTURE

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In vitro propagation of Kalmegh [*Andrographis paniculata* (Burm. F.) Wall. ex Nees] was achieved through tissue culture. Impact of different plant growth regulators on apical bud explants of *A. paniculata* Nees was investigated to develop a rapid propagation protocol for regeneration. The explants were obtained from 20-25 day old seedlings. The cytokinins (BAP, KN) were used alone and in combination with auxins (IAA, IBA, NAA) and additives like CH and $AgNO_3$. Although, optimum shoot induction occurred with BAP (0.44, 2.22µM), maximum direct multiple shoot formation was obtained with BAP (2.22µM). Maximum shoot length and rooting was achieved on MS medium with BAP (0.44, 2.22µM). Rooted plants were successfully transferred to soil.

Keywords: *Andrographis paniculata;* Kalmegh; Medicinal herb; Tissue culture.

Introduction

Kalmegh *(Andrographis paniculata* Nees) is an annual herb belonging to the family Acanthaceae. The plant is also known as rice bitters in West Indies and King of bitters or Chiretta in England. It is distributed throughout tropical India and Sri Lanka. The whole plant is the source of several diterpenoids, diterpene glycoside, lactone; andrographolide is important and distributed all over the plant body in different proportions. It is reported to have abortifacient, antimalarial, antiinflammatory, antibacterial, antithrombotic, cancerolytic, digestive, immune enhancer, laxative, sedative and vermicidal properties[1].

The important compounds isolated from different parts of the plant are apigenin-7,4'-di-o-methyl ether, carvacrol, eugenol, myristic acid, hentriacontane, tritriacontane, oroxylon A, wogonin and diterpenoids like andrographanin, andropanoside, andrographolide and neo-andrographolide[2]. Conventional vegetative propagation of this important plant is very difficult and too slow to meet the commercial quantities required. Except for the preliminary study of micropropagation[3] and somatic embryogenesis[4], no comprehensive *in vitro* studies have been reported on *Andrographis paniculata.* The present study attempts to establish a rapid *in vitro* propagation protocol for this valuable medicinal plant under different regimes of nutrients and PGRs.

Material and Methods

Seeds were washed under running tap water followed by a detergent labolene (1% v/v) for 4-5 mins. After thorough washing with sterile distilled water, seeds were surface-sterilized with 0.1% (w/v) mercuric chloride solution for 40-50 sec. and dried on a sterile filter paper.

Surface sterilized seeds were inoculated on sterile moist cotton in a conical flask. After 20 days 100% seeds germinated resulting in seedlings measuring 2.2-2.4 cm in length. Shoot tips (apical bud) obtained from the 20-25 day old seedlings were cultured on MS[5] medium supplemented with the different growth regulators viz. BAP (0.44-44.43µM), KN (0.46-46.43µM) either alone on in combination with IBA (0.49, 2.46µM), NAA (0.53,2.64µM) and IAA (0.57,2.46µM) and additives like casein hydrolysate (10-40mgl^{-1}) and $AgNO_3$ (1,2mg l^{-1}).

The medium was supplemented with 3% (w/v) sucrose and 0.8% agar. The pH of the medium was adjusted to 5.8 before the addition of agar. The medium was autoclaved at a pressure of 1.06kg cm^{-2} and temperature of 121°C for 20min. Sub cultures were carried out at an interval of 30-40 days.

Result and Discussion

Shoot induction- The shoot initiation was obtained within 10-15 days (1.5cm) on low concentrations of BAP (0.44,2.22µM) (Fig.1) with a frequency of 100% but it declined on its higher concentrations (4.43-44.46µM). The other cytokinin (KN) proved unsuitable when used alone or in combination with auxins like IAA, IBA, NAA and other additives like casein hydrolysate and silver nitrate in various concentrations.

Shoot elongation- Shoots elongated to 3.8cm within 20-

35 days of initiation. Shoots were healthier on low concentrations of BAP (0.44, 2.22 µM) (Fig.3) compared to the ones cultured on higher concentrations of BAP (22.2 µM) (Table 1).

Shoot Multiplication- Multiple shoots (20-25) were initiated from explant inoculated on higher concentrations of BAP (22.2 µM). Calli observed at shoot base in several cases led to multiple shooting (20-25) on this concentration (Fig.4). Kinetin alone and in combination with BAP did not support shoot multiplication from apical bud explants, whereas BAP (2.22 µM) or KN (2.23 µM) in combination with IAA (0.57µM), IBA (0.49, 2.46 µM) or NAA (0.53 µM) supported multiple shoot (2-5) formation (Table 1).

The maximum shoot number (3-4) was obtained from shoots 3-4 cm long on low concentration of BAP (0.5 mgl^{-1}) with CH (40mgl^{-1}) (Table 2). Addition of CH with (2.22µM) BAP caused an early shoot induction response. Addition of silver nitrate ($AgNO_3$) (2mgl^{-1}) with BAP (2.22µM) caused a remarkable enhancement in multiple shoot number but it reduced the shoot elongation (Table 3), number of roots (1-2) and length of roots (2-3cm). It is clear from above analysis that low concentrations of BAP (0.44, 2.22µM) were favorable for responses like direct multiple shoot formation (6-7), shoot elongation (4-6cm) and rooting also. Roots were initiated from elongated shoots cultured on BAP (0.44µM, 2.22µM) within 30 days of culture. Roots were 5-6cm long and were white, thin and branched (Figs. 8-10) (Table 5,6).

Subculture- Apical and axillary buds, obtained from *in vitro* regenerated shoots, were cultured on media containing BAP (0.44 µM, 2.22 µM) and KN (2.32 µM) alone and in combination with auxin (IBA) (2.46 µM) (Fig.5) (Table 4).

Growth of regenerated shoots increased after 7-8 days of inoculation. The explants responded best in 2nd & 3rd subculture on low concentration of BAP (2.22µM) (Figs.8-10) (Table- 4) in terms of shoot elongation, multiple shoot number (9-11), number of node (8-10) and healthy and good root system, compared to initial inoculation of explants on BAP alone.

The suitability of low concentration of BAP for regeneration through tissue culture has been reported on *Curcuma caesia* [6].

Regeneration through tissue culture for rapid multiplication has been reported to be under the control of plant growth regulator in several medicinal plants viz. *Catharanathus roseus*[7], *Centella asiatica*[8], *Chlorophytum borivilianum*[9], *Mentha arvensis*[10], and *Spilanthes acmella* (L) Murray[11].

Table 1. Effect of PGRs on regeneration responses from ApB of *Andrographis paniculata* (after 30days).

S. No.	PGRs (µM)	FSI (%)	MSN	MSL (cm)	MNN
1. BAP	0.44	90	2	2.3	2
	2.22	100	5	6	4
	4.43	50	1	2.1	1
	22.2	40	12	1	-
2. KN	0.46	80	2	3	2
	2.32	70	1	1.2	1
	4.46	-	1	1	-
	23.2	-	1	1	-
3. BAP+KN	0.44+0.46	10	1	1.5	1
	0.44+2.32	10	1	1.3	1
	2.22+0.46	89	2	1.1	1
	2.22+2.32	70	2	2.2	1
4. BAP+KN+IBA	0.44+0.46+0.49	90	3	1.3	2
	2.22+2.32+2.46	99	5	2.7	3
5. BAP+KN+IAA	0.44+0.46+0.57	31	1	1.1	1
	2.22+2.32+2.46	28	1	1	1
6. BAP+NAA+IBA	0.44+0.53+0.49	60	1	1.5	1
	2.22+2.64+2.46	40	2	1.1	1

Table 2. Effect of BAP and Casein hydrolysate on regeneration responses from ApB of *Andrographis paniculata* (after 30days).

S. No.	PGRs + Additives (BAP + CH) (mgl^{-1})	FSI (%)	MSN	MSL (cm)	MNN
1.	0.5 + 10	93	3	4.0	2
2.	0.5 + 15	90	4	3.9	1
3.	0.5 + 20	96	2	3.5	2
4.	0.5 + 30	87	2	3.8	2
5.	0.5 + 40	98	2	4.0	3

ApB - Apical bud
AxB - Axillary bud
FSI – Frequency of shoot initiation
MSN- Mean shoot number
MSL- Mean shoots length
MNN- Mean node number

Table 3. Effect of BAP and $AgNO_3$ on regeneration responses from ApB of *Andrographis paniculata* (after 30days).

S. No.	PGRs + Additives (BAP + $AgNO_3$) (mgl^{-1})	FSI (%)	MSN	MSL (cm)	MNN
1.	0.5 + 0.1	78	2	1.0	1
2.	0.5 + 0.2	80	2	1.3	1
3.	0.5 + 1.0	90	4	2.3	2
4.	0.5 + 2.0	97	6	1.5	2

Table 4. Effect of BAP, KN and IBA on regeneration responses from ApB and AxB of *Andrographis paniculata* (after 30days).

S. No.	PGRs (µM)	ApB				AxB			
		FSI (%)	MSN	MSL (cm)	MNN	FSI (%)	MSN	MSL (cm)	MNN
	BAP								
1.	0.44	90	2	4.2	3	70	3	1.5	1
2.	2.22	95	1	3.8	3	90	6	3.0	2
	BAP+KN+IBA								
3.	2.22+2.32+2.46	80	3	1.5	1	81	8	2.8	4

Abbreviations: $AgNO_3$: Silver nitrate; BAP: 6- benzyl-aminopurine; CH: Casein hydrolysate; IAA; 3-indole acetic acid; IBA; indole butyric acid; KN: Kinetin; MS medium: Murashige and Skoog medium; NAA: 1-naphthalene acetic acid; PGR: Plant growth regulator.

Table 5. Effect of PGRs on rooting responses from *in vitro* regenerated shoots of *Andrographis paniculata* (after 30days).

S. No.	PGRs (µM)		FRI (%)	MRN (cm)	MRL
1.	BAP	0.44	98	7	2
		2.22	99	14	6
		4.43	30	2	1
		22.2	-	-	-
2.	KN	0.46	-	-	-
		2.32	-	-	-
		4.46	-	-	-
		23.2	-	-	-
3.	BAP+KN	0.44+0.46	-	-	-
		0.44+2.32	-	-	-
		2.22+0.46	-	-	-
		2.22+2.32	-	-	-
4.	BAP+KN+IBA	0.44+0.46+0.49	-	-	-
		2.22+2.32+2.46	-	-	-
5.	BAP+KN+IAA	0.44+0.46+0.57	-	-	-
		2.22+2.32+2.46	-	-	-
6.	BAP+NAA+IBA	0.44+0.53+0.49	-	-	-
		2.22+2.64+2.46	-	-	-

Table 6. Effect of BAP and additives on rooting responses from *in vitro* regenerated shoots of *Andrographis paniculata* (after 30days).

S. No.	PGRs+Additives (mgl^{-1})		FRI (%)	MRN (cm)	MRL
1.	BAP+$AgNO_3$	0.5+0.1	-	-	-
		0.5+0.2	-	-	-
		0.5+1.0	60	1	0.8
		0.5+2.0	80	2	1.1
2.	BAP+CH	0.5+10	-	-	-
		0.5+15	-	-	-
		0.5+20	-	-	-
		0.5+30	-	-	-

FRI (%) - Frequency of root initiation
MRN - Mean root number
MRL - Mean root length

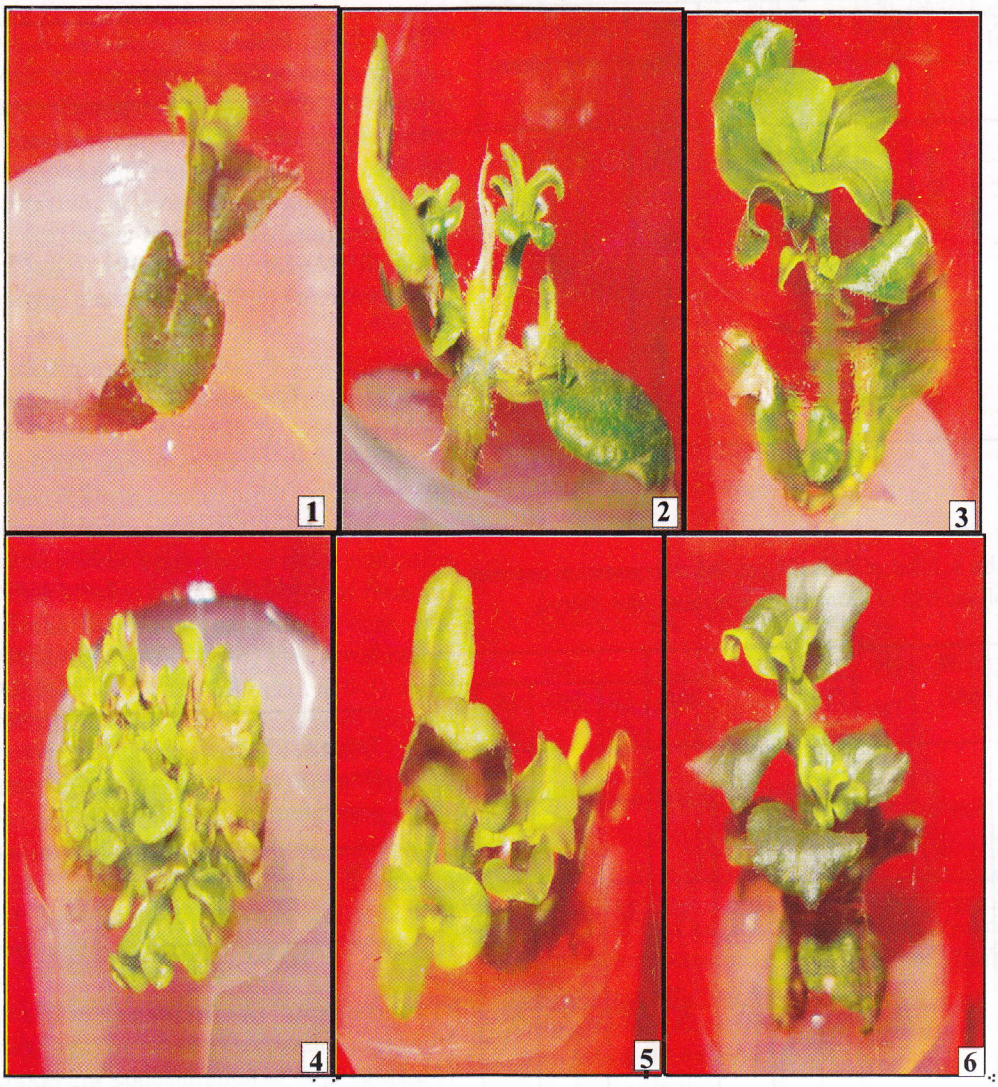

Fig.1-6. Plant regeneration of *Andrographis paniculat.*

1. Shoot initiation from Apical bud on MS medium with BAP (2.22 µM).
2. Multiple shoot on MS+BAP (2.22 µM)+$AgNO_3$ (2.0 mgl)[1].
3. Multiple shoot on MS+BAP (2.22 µM).
4. Multiple shoot on MS+BAP (22.2 µM).
5. Multiple shoot on MS+BAP (2.22µM) +KN (2.32µM) +IBA (2.46µM).
6. Shoot elongation onMS+BAP (2.22 µM).

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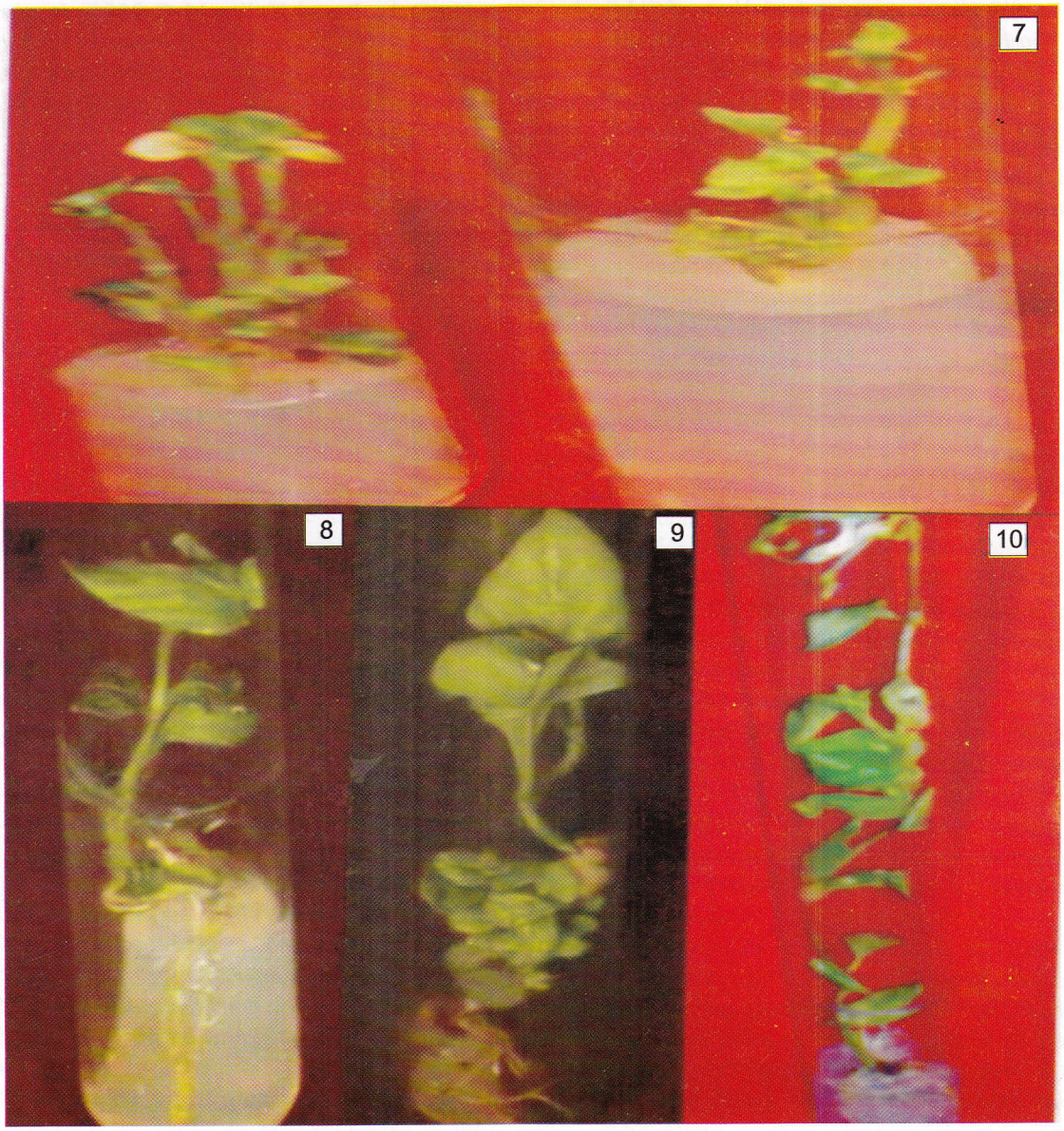

Fig.7-10. Plant regeneration of *Andrographis paniculata.*

7. Shoot multiplication and elongation on MS+BAP (0.44,2.22µM).
8. Root initiation on MS+BAP (2.22 µM).
9. Shoot multiplication and elongation and root multiplication on MS+BAP (2.22 µM).
10. Shoot multiplication, elongation, flowering and root multiplication on MS+BAP (2.22 µM).

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