J. Phytol. Res. 13 (2) : 135-138, 2000

IN VITRO CLONAL PROPAGATION AND TUBER FORMATION OF *RAUVOLFIA SERPENTINA* L. BENTH. EX KURZ

A. C. DEKA, ABHIJIT TALUKDAR, MAHUA CHAKRABORTY and M.C. KALITA
Department of Biotechnology, Gauhati University, Guwahati 781014, Assam, India.

Axillary buds cultured in MS medium containing BAP (2.0mg/1), Kn (1.5mg/1) and sucrose (3%) formed multiple shoots after a period of 45 days. Number of shoots per explant was found to be highest in the medium supplemented with NAA (0.3 mg/1) along with BAP (2.0mg/1). Leaf explant also induced root when the medium was supplemented with IAA (1.0mg/1) and BAP (3.0mg/1). The root originated from leaf explant, showed tuber formation in BAP (3.0mg/1) along with NAA (1.0mg/1).

Keywords: Clonal propagation; *In vitro* tuber formation; *Rauvolfia serpentina*.

Introduction

Rauvolfia serpentina known as Sarpagandha in Assam is a rich source of indole alkaloids of medicinal value such as reserpine, ajmalicine, ajmaline and serpentine which are used in the treatment of circulatory disorders. The isolation of reserpine from dry roots of *Rauvolfia serpentina* L. marked a revolution in the antihypertensive and sedative drug therapy[1]. *In vitro* tissue culture works of *Rauvolfia serpentina* has been demonstrated by several workers [2-6]. They reported the establishment of complete autotetraploids from colchiploid shoots following the tissue culture technique. *Rauvolfia serpentina* is vegetatively Propagated by root cuttings, and has a poor seed viability and very low germination percentage that may be ascribed largely to the presence of cinnamic acid derivatives in the seeds[7-8].

In view of its medicinal and commercial importance, there has been felt an urgent need to apply non-conventional propagation methods for conservation and commercial availability. This communication has been designed to deal with micropropagation and tuber formation from leaf explant which is an interesting finding. and would hope to play an important role in *in vitro* compound production.

Materials and Methods

Axillary buds and leaves of the field grown young plants were collected and washed with detergent solution (5% Tween -20) for two minutes and surface sterilized with 0.1% $HgCl_2$ (W/V) for 5 minutes and washed several times with sterile double distilled water under aseptic conditions. The cut surfaces exposed to mercuric chloride damage were aseptically trimmed with a sharp, sterile surgical blade. Leaves about 0.75 cm^2 in area and internodal portion of the stems about 1.0 cm in length were used as explants. MS medium[9] supplemented with 3% sucrose and 0.9% agar (Bacteriological, Himedia, India) were used for all experiments. The medium was supplemented with different concentrations of plant growth regulators BAP (0.5-5.0 mg/1) NAA (0.1-4.0mg/1), IAA (0.1-4.0mg/1), Kn (0.1-4.0 mg/1) individually and in combination and also 2,4-D individually, to study the percentage regeneration of shoots and other morphogenetic response such as tuber formation from leaf explants. The shoot regeneration response from axillary buds were recorded at regular intervals of 15, 30, 45 and 60 days, which has been expressed as percentage regeneration. Similarly other parameters like number of shoots, length of shoots, rooting response, tuber formation and callus induction have been recorded. The pH of the medium was adjusted to 5.8 prior to autoclaving and gelled with 0.8% agar. The cultures were grown under white fluorescent light with 16/8 h light and dark cycle at 26 $\pm$ 1 °C and were maintained by regular subculture after 30 days of incubation.

Results and Discussion

Axillary buds cultured in MS medium showed high degree of explant response in the form of shoot initiation 80% shoot

initiation was observed in the treatment BAP (2.0 mg/1) along with NAA (0.3 mg/1) after 15 days of inoculation However the percentage response incresased to 85% after a continuous culture for 60 days. Highest 88% shoot initiation was observed in BAP (2.0 mg/1) with Kn (1.5mg/1) after 60 days of culture (Table 1, Fig.1). Number of shoots per explant was found to be highest in NAA (o.3 mg/1) with BAP (2.0mg/1). The multiple shoots were found to arise from the basal portion of the explant. However, multiple shoot induction was also found to occur in the other treatment containing NAA with BAP and NAA with IAA which ranged between 2-3 shoots per explant (Table 2). The length of shoots were recorded against each treatment. It was found that highest shoot length of 4.8 cm was recorded in the treatment NAA (1.0 mg/1) with BAP (3.0mg/1) (Fig 3).

Rooting was found to initiate after 45-50 days, when shoots were transferred to fresh treatments. Only few treatments containing NAA with IAA in MS showed root induction (Table2) Highest percentage of root initiation (40%) from the sub-cultured shoots were found in the treatment containing NAA (3.0 mg/1) and IAA (0.3mg/1) Lowest rooting percentage (10%) was observed in NAA (1.0mg/1) with BAP (3.0mg/1) The use of lower (0.1-1. 0mg/1) and higher (1.0-4.0mg/1) concentrations of NAA and IAA respectively resulted in diminished rooting response (Table 2).

Callus induction was observerd in the treatment containing 2,4 -D. Highest degree of callus induction was observed in 2,4 D (2.0mg/1) which was 64% However low frequency callus initiation was also found to occur in the treatment containing NAA+ BAP and also NAA + IAA which ranged between 5-15% of the total cultured explant (Table 2).

An interesting observation which was made during the study, was the formation of tuber like structure from roots, initiated from the leaf explant in the treatment NAA (1.0mg/1) + BAP (3.0mg/1) (Fig 2). Tuber formation was also observed at lower concentration of NAA and BAP, but as the concentration of NAA was substantially increased leaf explant showed callus induction instead of tuber formation.

In marked contrast to our report, induction of only 1-2 shoot buds in BA (13.2μM) as against maximum 9 numbers of shoots was obtained from a single explant in NAA (0.3 mg/1) + BAP (2.0mg/1)[6]. It also showed an 80% explant response. BA alone was insufficient to sustain the growth

Table 1. Combined effect of Auxin and Cytokinins on percent regencration of shoot.

MS MEDIA	Days of interval			
	15	30	45	60
	Percent regenaration			
MS 0	0.30	10.00	12.00	12.00
BAP (0.5mg/1) +NAA(0.1mg/1)	20;2	29.37	48.00	51.87
BAP (1.0mg /1) + NAA(0.2mg/1)	49.11	50.55	51.00	54.66
BAP (2.0mg/1) +NAA(0.3 mg /1)	80.00	80.00	82.55	85.00
BAP (3.0mg/1) +NAA(1.0 mg /1)	30.66	41.00	41.00	46.66
BAP (4.0mg/1) +NAA(2.0mg/1)	43.00	48.67	55.00	61.00
BAP (5.0mg/1) +NAA(0.2mg/1)	10.00	15.55	21.22	27.00
BAP (0.1mg/1) +KN(0.1mg/1)	8.00	10.55	11.00	14.55
BAP (0.5mg/1) +KN(0.2mg/1)	12.00	15.00	20.22	20.00
BAP (1.0mg/1) +KN(0.5mg/1)	28.00	35.00	40.00	40.00
BAP (1.5mg/1) +KN(1.0mg/1)	40.00	43.55	45.00	60.00
BAP (2.0mg/1) +KN(1.5mg/1)	58.00	80.00	80.00	88.00

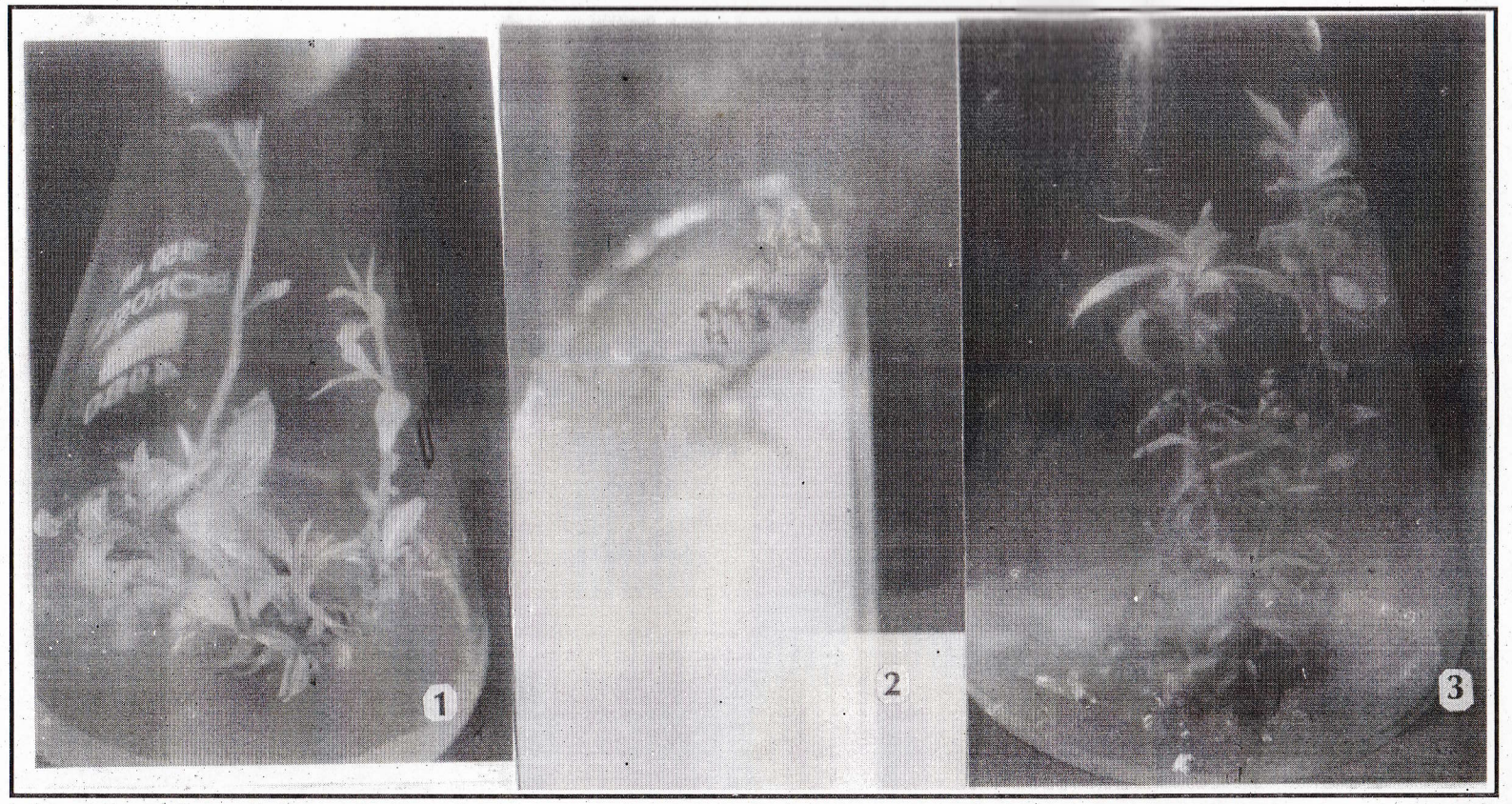

Fig.1. Multiple shoot initiated after 60 days of culture in BAP (2.0 mg/1)+Kn (1.5 mg/1);
2. Formation of Tubers from the roots induced from leaf explant in NAA (1 mg/1)+BAP (3mg/1);
3. Mass propagation of the shoots from nodal explant.

Table 2. Morphogenetic responce of nodal explnt to various combination of auxin and Cytokinins in MS media. (after 9weeks of culture).

MS MEDIA	SHOOT NO.	SHOOT LENGTH (cm)	ROOTING (%)	CALLUSING (%)
MS 0	1	1.3	-	-
NAA (0.1mg/1)+BAP(0.5mg/1)	2	1.8	-	-
NAA (0.3mg/1) +BAP(2.0mg/1)	9	2.3	-	10
NAA (1.0mg/1) +BAP(3.0mg/1)	3	4.8	10	-
NAA(2.0mg/1) +BAP(4.0mg/1)	3	4.0	16	5
NAA(3.0mg/1) +BAP(4.0mg/1)	2	2.0	12	15
NAA(4.0mg/1) +BAP(5.0mg/1)	3	3.2	12	14
NAA (1.0mg/1)+IAA(0.1mg/1)	1	16	-	-
NAA(2.0mg/1) +IAA(0.2mg/1)	1	3.0	10	-
NAA(3.0mg/1) +IAA(0.3 mg/1)	3	2.0	40	10
NAA(4.0mg/1) +IAA(1.0mg/1)	3	1.9	28	12
NAA(4.0mg/1) +IAA(4.0mg/1)	2	2.1	21	10
2,4-D(0.5mg/1)	1	0.5	-	-
2,4-D(1.0mg/1)	1	1.0	-	10
2,4-D(2.0mg/1)	-	-	-	64
2,4-D(5.0mg/1)	-	-	-	39
2,4-D(4.0mg/1)	-	-	-	45
2,4-D(5.0mg/1)	-	-	-	29

of buds into shoots in related species[10]. Each shoot so formed showed a marked tendency for enhanced axillary branching, especially from the basal nodal part. Combination of higher concentration was less effective with substantial reduction in the frequency of shoot formation as reported in the related species, *R.micrantha* [10]. It has also been reported that the shoots formed in high cytokinin medium were stunted in growth with short internodes and crowded leaves[11-13]. Multiple shoots formed a hard callus mass at the cut end which subsequently developed roots[5].

References

1. Muller JM, Schltittler E and Bein HJ 1952, *Experientia* **8** 338
2. Mitra G C and Chaturvedi HC 1970, *Curr. Sci.* **39** 128
3. Akram M and Ilahi I 1985, *Pak. Sci. Ind. Res.* **28** 412
4. Heble Benjamin, Roja PC and Chadha MS 1983, In: *Proceedings of National Seminar* PCRI, Kasaragod, Publisher of ICAR New Delhi.
5. Roja G and Heble MR 1996, *plant Cell, Tissue and Organ Culture* **44** 111
6. Mathur A, Mathur A K, Kukreja A K, Ahuja P S and Tyagi B R 1987, *Plant Cell, Tissue and Organ Culture* **10** 129
7. Mitra G C 1976, *Ind. J. Exp. Biol.* **14** 54
8. Sahu B N 1979, In: *Rauvolfias Botani and Agronomy* **Vol 1** New Delhi: Todays and Tomorrows Printers and Publishers.
9. Murashigee T and Skoog F 1962, *Physiol. Plant.* **15** 473
10. Sudha C G and Seene S 1996, *Plant Cell, Tissue and Organ Culture* **44** 243
11. Amin N M and Jaiswal V S 1987, *Plant Cell, Tissue and Organ Culture* **9** 235
12. Maari K A, Arnal Y and Miginiae E. 1986, *Sci. Horti.* **28** 315
13. Maria C F dos S, Maria A E and Antonio V P dos S 1990, *Plant Cell, Tissue and Organ Culture* **21** 75