J. Phytol. Res. 21(2) : 239-243, 2008

IN VITRO MULTIPLICATION OF *ACALYPHA WILKESIANA* 'DWARF' THROUGH SHOOT TIP CULTURE

ANJU SHARMA and D.K. ARORA
Plant pathology, Tissue culture and Biotechnology Laboratory,
Department of Botany, University of Rajasthan, Jaipur-302004 India

A rapid and reproducible regeneration protocol has been established in *Acalypha wilkesiana* 'Dwarf' through shoot tip culture. It was observed that maximum number of shoot buds sporuted from a single shoot tip on Murashige & Skoog's medium supplemented with BAP (0.5 mg/l) and IAA (0.5 mg/l). An optimum number of 40.2±0.59 shoot buds were obtained per explant. Rooting of the *in vitro* multiplied shoots was achieved on MS-medium supplemented with NAA (1.0 mg/l). An efficient regeneration protocol for *in vitro* multiplication of *A. wilkesiana,* an ornamentally important plant, has been developed in the present study.

Keywords : *Acalypha wilkesiana* 'Dwarf'; *In vitro* multiplication; Ornamental plant; Shoot tip culture.

Introduction

Ornamental plants and flowers are among the loveliest objects on this earth[1]. Floriculture, which related to production and use of ornamentals is now emerging as an important venture in the world[2]. Economic aspect of ornamentals have been exploited both in the domestic and in the international market[3]. Two important components of the floriculture industry are the trade of cut flowers and foliage[4]. *Acalypha wilkesiana* 'Dwarf' is an important foliage plant and used indoors[5]. The common name of the plant is cooper leaf and Jacob's cat and it belongs to family Euphorbiaceae. The plant has beautiful foliage due to which it is very much in demand in the market and nursery trait. Taking into consideration its demand for mass propagation is very essential. Therefore, tissue culture technique has been employed for its mass propagation. Plant tissue culture technique has long been recognized as an efficient tool for rapid clonal propagation[6]. Over past several years tissue culture has rapidly evolved into one of the major research tool and is being used in various fields. It has an important role to play in solving problems related to economically important plants[7-10]. The present study describes a protocol for *in vitro* multiplication through shoot tip exaplant.

Materials and Methods

Explants were collected from healthy plants growing in the Botanical garden of University of Rajasthan. Isolated shoot apices were first soaked in Teepol solution (0.1%, v/v) for 5 minutes followed by rinsing in running water. Surface sterilization was done with 0.05% (w/v) mercuric chloride solution for 2 to 3 minutes followed by repeatedly washing with sterilized distilled water. The surface sterilized explants were cultured on MS-medium[11] containing 3% (w/v) sucrose and 0.8% (w/v) agar-agar. To elicit morphogenesis in cultured explants, this basal medium was fortified with various growth regulators viz. BAP, Kn, IAA and IBA in different concentrations and combinations. The pH of the medium was adjusted to 5.8 using 0.1 N HCl or 0.1 N NaOH and autoclaved at 15 psi for 20 minutes. The cultures were incubated at 26±2°C under 16 hr. photoperiod provided by fluorescent light of about 3000 lux intensity. All the experiments were repeated thrice and eight replicates per treatment were taken. Mean and standard error (SE) of the number of shoots produced per explant were calculated from the replicates of each treatment.

For initiation of roots, the elongated shoots were transferred on MS-medium supplemented with various auxins viz. NAA, IBA and IAA. The healthy rooted plantlets were then transferred to pot containing sterile garden soil and vermiculite (3:1) and covered with inverted glass beaker to retain humidity. The acclimatized plantlets were then transferred to greenhouse.

Histological studies were carried out by using standard histological techniques[12].

Results and Discussion

The shoot tip explants were inoculated on MS-medium containing different concentrations of BAP and Kn alone or in combination with auxins. The most effective cytokinin in promoting shoot proliferation from this explant was BAP.

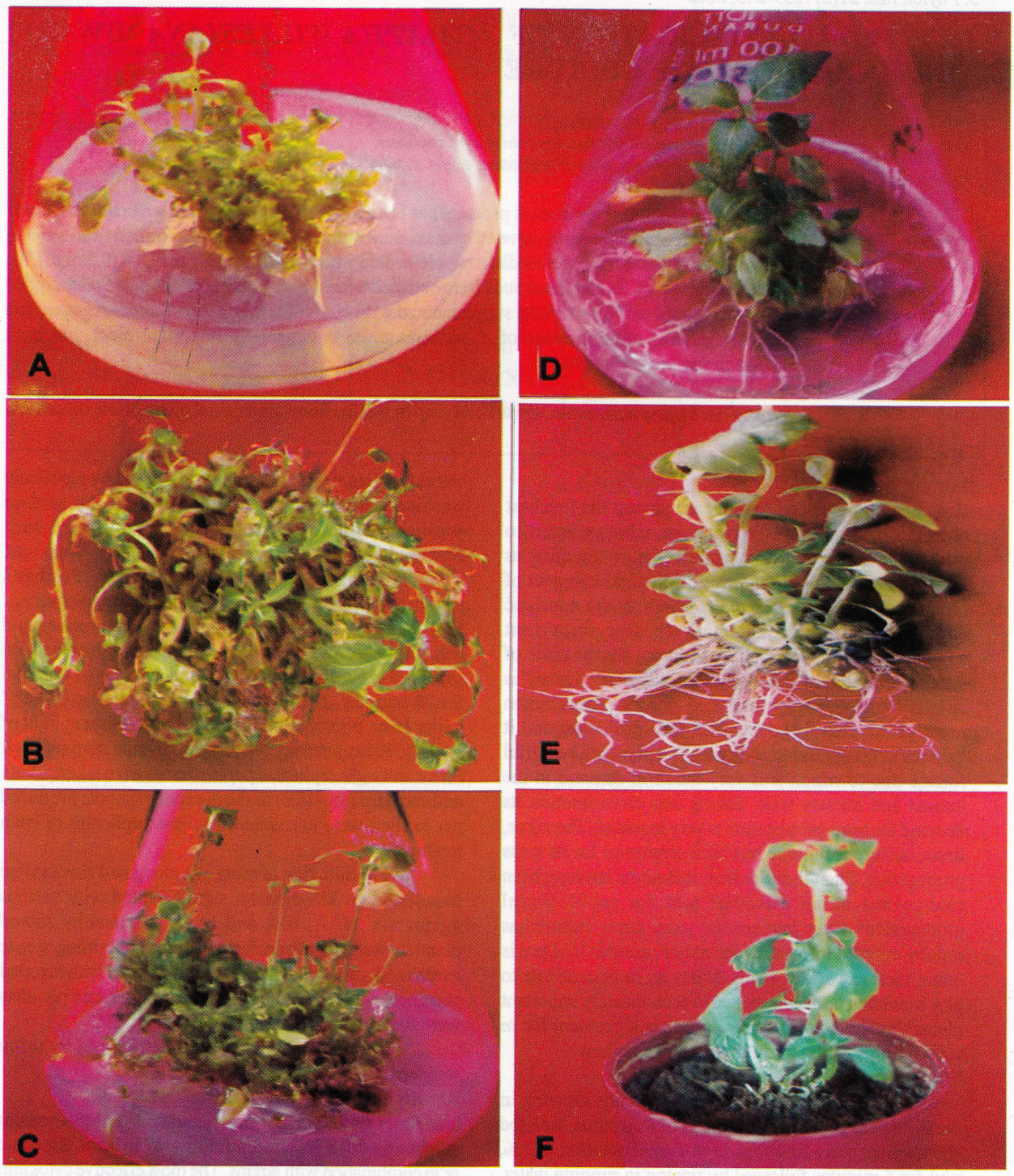

Fig.1. A-F. Multiple shoot formation from cultured shoot-tip explant in *Acalypha wilkesiana* 'Dwarf'
A. Multiple shoot formation from shoot-tip on MS+BAP (0.5 mg/l)+ IAA (0.5 mg/l) after 4 weeks; B. Closeup view of multiple shoots; C. Proliferation of multiple shoots after 6 weeks; D. Root formation in the elongated shoot on MS+NAA (1.0 mg/l) E. *In vitro* regenerated plantlets. F. *In vitro* regenerated acclimatized plant.

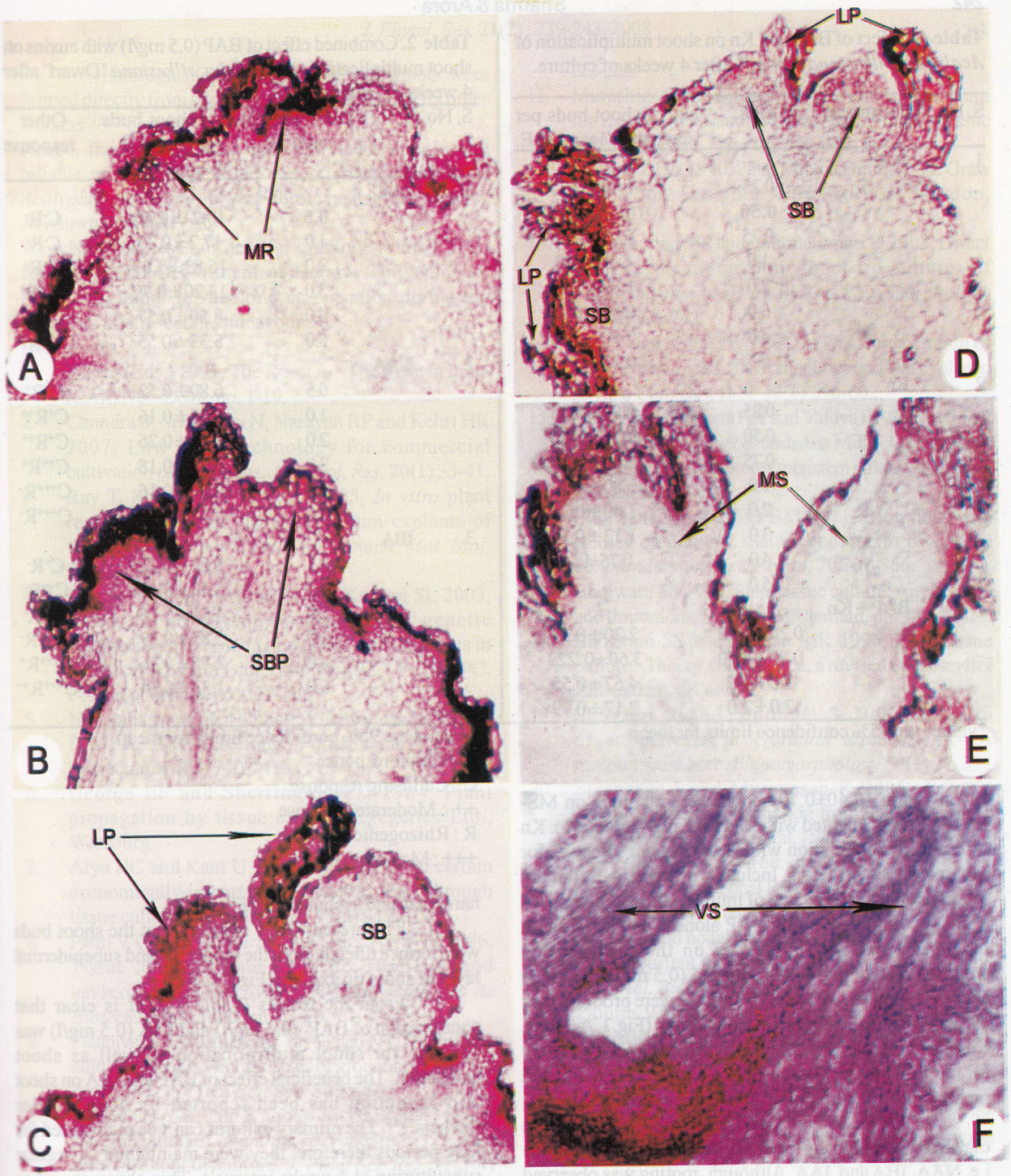

Fig.1. A-F. Multiple shoot formation from cultured shoot-tip explant in *Acalypha wilkesiana* 'Dwarf' A. Multiple shoot formation from shoot-tip on MS+BAP (0.5 mg/l)+ IAA (0.5 mg/l) after 4 weeks; B. Closeup view of multiple shoots; C. Proliferation of multiple shoots after 6 weeks; D. Root formation in the elongated shoot on MS+NAA (1.0 mg/l) E. *In vitro* regenerated plantlets. F. *In vitro* regenerated acclimatized plant.

Table 1. Effect of BAP and Kn on shoot multiplication of *Acalypha wilkesiana* 'Dwarf' after 4 weeks of culture.

S. No.	Treatment	mg/l	No. of shoot buds per explant *Mean ± S.E.
1.	**BAP**		
		0.25	1.40±0.75
		0.50	**10.40 ± 0.17**
		0.75	6.00±0.63
		1.0	5.2±0.33
		2.0	1.87±0.23
		3.0	0.80±0.37
		4.0	0.50±0.18
		5.0	0.12±0.11
2.	**Kn**		
		0.25	0.80±0.33
		0.50	2.20±0.52
		0.75	3.50±0.28
		1.0	5.80±0.52
		2.0	1.40±0.36
		3.0	1.12±0.39
		4.0	0.75±0.23
		5.0	
3.	**BAP + Kn**		
		0.25+0.25	2.20±0.33
		0.50+0.50	3.60±0.223
		1.0+1.0	4.67±0.55
		2.0+2.0	2.17±0.79

*values are 95% confidence limits for mean

Table 2. Combined effect of BAP (0.5 mg/l) with auxins on shoot multiplication of *Acalypha wilkesiana* 'Dwarf' after 4 weeks of culture.

S. No.	Treatment mg/l	No. of shoot buds per explant *Mean ± S.E.	Other response
1. **IAA**			
	0.5	**40.2 ± 0.59**	**$C^{+}R^{-}$**
	1.0	17.2±0.33	$C^{+}R^{-}$
	2.0	16.83±1.03	$C^{+}R^{-}$
	3.0	13.20±0.79	$C^{++}R^{+}$
	4.0	8.50±0.57	$C^{+++}R^{+}$
	5.0	5.33±0.55	$C^{+++}R^{++}$
2. **NAA**			
	0.5	6.80±0.52	$C^{+}R^{+}$
	1.0	2.25±0.16	$C^{+}R^{++}$
	2.0	1.12±0.26	$C^{+}R^{++}$
	3.0	0.75±0.18	$C^{++}R^{+}$
	4.0	0.62±0.16	$C^{+++}R^{-}$
	5.0	0.37±0.34	$C^{+++}R^{-}$
3. **IBA**			
	0.5	5.62±0.22	$C^{+}R^{-}$
	1.0	5.50±0.18	$C^{++}R^{-}$
	2.0	4.83±0.16	$C^{++}R^{-}$
	3.0	2.83±0.39	$C^{+++}R^{-}$
	4.0	2.75±0.36	$C^{+++}R^{+}$
	5.0	1.33±0.54	$C^{+++}R^{++}$

*values are 95% confidence limits for mean
+ : Slight response
C : Callusing response
++ : Moderate response
R : Rhizogenic response
+++ : Maximum response

A maximum 10.40±0.17 shoots were obtained on MS-medium supplemented with BAP (0.5 mg/l) (Table-1). Kn alone or in combination with BAP was not effective for multiple shoot formation. Inclusion of auxin in the culture medium enhanced the rate of muliplication as compared to the medium containing BAP alone. The rate of shoot multiplication was maximum on the MS-medium containing BAP (0.5 mg/l) and IAA (0.5 mg/l) (Table 2) and a maximum of 40.20±0.59 shoots were produced per explant within 4 weeks of inoculation (Fig.1 A & B). Combination of BAP with NAA and IBA was not effective for multiplication. The multiplied shoots were elongated on the same medium after further subcultured (Fig.1 C).

The *in vitro* raised elongated shoots were transferred individually to MS-medium with various auxins i.e. NAA, IBA and IAA. Although, rooting was observed on IBA and IAA supplemented medium but NAA was the best auxin for rooting. Thick, long and luxuriant rooting was observed on MS-medium supplemented with NAA (1.0 mg/l) (Fig.1 D & E). These plantlets were successfully hardened and acclimatized (Fig.1 F).

The present study revealed that the shoot buds were formed directly from the epidermal and subepidermal layer of shoot tip explant (Fig.2 A-F).

From the results (Table 1-2) it is clear that combination of BAP (0.5 mg/l) and IAA (0.5 mg/l) was suitable for shoot multiplication as well as shoot elongation. The beneficial effect of BAP and IAA on shoot bud induction has been reported by some earlier workers[13-15]. The primary cultures can't be maintained for long periods therefore, they were maintained by regular subculturing at 4 weeks intervals. This observation has been supported by several workers[16-18]. In the present study maximum percentage of rooting was reported on NAA (1.0 mg/l). The effectiveness of NAA on rooting in microshoots was reported by a number of workers[19-21].

Histological studies revealed that shoots were formed directly from shoot tip which is supported by other workers[13,22,23].

Hence, the present work demonstrated a simple and reliable procedure for rapid *in vitro* multiplication of *Acalypha wilkesiana* 'Dwarf' through shoot tip culture.

Acknowledgement

We wish to express our heartful gratitude to Prof. Uma Kant Ex-Head Department of Botany, University of Rajasthan, Jaipur for his kind co-operation during the course of this research endeavour.

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