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EFFECT OF DIFFERENT PLANT GROWTH REGULATORS ON MULTIPLE SHOOT INDUCTION IN *PLUCHEA LANCEOLATA* OLIVER & HIERN.

DEEPIKA ARYA and VIDYA PATNI
Plant Pathology ,Tissue Culture & Biotechnology Laboratory, Department of Botany, University of Rajasthan, Jaipur – 302004, India.

As for the effect of various plant growth regulators on shooting of *Pluchea lanceolata* is concerned, it was observed that maximum number of shoot buds sprouted from nodal segment explant on Murashige & Skoog's medium supplemented with BAP (0.25mg/l) and Kn (0.5mg/l). An optimum number of 30.2±0.59 shoots was obtained per nodal segment explant. NAA and IBA alone were not effective for multiple shoot induction. Rooting of the micropropagated shoots was observed on IBA(1.0mg/l). An efficient regeneration protocol for micropropagation of this threatened species has been developed during the present study.

Keywords : Multiple Shoot Induction; Plant Growth Regulators (PGR's); *Pluchea lanceolata.*

Introduction

The medicinal plants played a very important role from time immemorial among the illiterates and highly civilized population. *Pluchea lanceolata* is one of the important medicinal plants of India belonging to family Asteraceae. It is a xerophytic plant commonly grows in sandy soils. It has anti-inflammatory [1-3] and analgesic activity and is also used in rheumatism, neuralgic disorders, asthma, sciatica, edema inhibition etc. It contains high amount of medicinally important secondary metabolites like Flavonoids, Triterpenoids, Steroids etc.

Due to continuous exploitation of these plants in forestry sector, the wild population of the species have become vulnerable to extinction. The plant is listed in the priority species identified by Ministry of Health & family Welfare, Govt.of India[4]. Hence propagation through *in vitro* culture was attempted.

Over past several years, tissue culture has rapidly evolved into one of the major research tools and is being used in varied fields. It has an important role to play in solving problems related to economically important plant improvement[5-8]. *In vitro* multiplication technology is a powerful tool for achieving fast multiplication of medicinal plants[9-12]. This paper reports the response of the plant to Murashige and Skoog's (MS) medium supplemented with different concentrations and combinations of plant growth regulators for multiplication of shoot buds.

Material and Method

Explants (nodal segments) of *Pluchea lanceolata* were taken from plants growing in wild, in semi-arid regions of Jaipur, Rajasthan. The explants 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 thorough washing with sterilized distilled water. The surface sterilized explants were cultured on MS[13] medium containing 3%(w/v) sucrose and 0.8%(w/v) agar-agar. The pH of the medium was adjusted to 5.8 and autoclaved at 15 psi for 20 minutes. The explants were aseptically transferred singly to culture vials. Cultures were incubated at 25±2°C under 16 hr photoperiod provided by fluorescent light of about 3000 lux intensity. For each treatment 5 cultures were raised and all experiments were repeated thrice. MS medium was supplemented with various growth regulators like 6-Benzyl Amino Purine(BAP), Kinetin(Kn), Napthalene Acetic Acid(NAA) and Indole-3-Butyric Acid (IBA). The concentrations of these growth regulators in different combinations are detailed in the observation.

Results and Discussion

The nodal segment explants were inoculated on MS medium containing different concentrations of BAP (0.25-2.0 mg/l), Kn (0.5-2.0mg/l), NAA(0.5-2.0mg/l), IBA(0.5-2.0mg/l). As for the influence of growth regulators on shooting of *Pluchea lanceolata* is

Table1. Effect of Plant Growth Regulators (PGR's) on shoot formation from nodal segment explant of *Pluchea lanceolata* on MS medium.

PGR's (mg/l)				Mean Number of multiple shoot ± S.E.	Highest number of microshoot	Shoot with basal callus intensity
BAP	Kn	NAA	IBA			
0.25	-	-	-	9.6±0.49	12	+
0.5	-	-	-	13.8±0.80	15	+
1.0	-	-	-	20.0±0.50	25	++
2.0	-	-	-	8.4±0.80	10	+++
-	0.5	-	-	1.8±0.70	2	++
-	1.0	-	-	6.8±0.08	8	+++
-	1.5	-	-	14.6±0.49	20	+++
-	2.0	-	-	13.8±0.80	15	+++
-	-	0.5	-	0.8±0.23	1	+++
-	-	1.0	-	0.8±0.33	1	++++
-	-	1.5	-	0.6±0.22	1	++++
-	-	2.0	-	0.4±0.29	1	++++
-	-	-	0.5	0.4±0.33	1	+++
-	-	-	1.0	0.4±0.28	1	+++
-	-	-	1.5	0.8±0.29	1	++++
-	-	-	2.0	-	-	++++
0.25	0.5	-	-	30.2±0.59	35	+
0.25	1.0	-	-	18.6±0.70	20	+
0.5	0.5	-	-	20.2±0.59	25	++
0.5	1.0	-	-	16.0±0.50	20	+++
1.0	0.5	-	-	10.2±0.02	15	++
1.0	1.0	-	-	16.0±0.50	18	+++
2.0	0.5	-	-	8.4±0.80	10	++
2.0	1.0	-	-	6.8±0.08	8	+++

Callus intensity- + = very low callus, ++ = low callus
+++ = moderate callus , ++++ = high callus

concerned, it is evident that the use of BAP and Kn alone or in combination is largely effective in inducing multiple shoot other than NAA and IBA. Sprouting of shoot buds from the swollen basal part of the explant was noticed after 2 weeks of incubation in the presence of either BAP/Kn alone or in a combination of BAP and Kn. The results indicated that BAP in combination with Kn was effective in shoot bud production. Maximum shoot multiplication was obtained on MS medium fortified with 0.25mg/l BAP and 0.5mg/l Kn(Table 1,Fig.3). This medium was also suitable for elongation of shoots (Fig.4). When BAP alone was used for shoot multiplication, lesser number of shoots were produced and the shoots were very small in size (Fig.1). Same results were obtained when Kn alone was used for shoot multiplication (Fig.2). NAA and IBA were found ineffective for shoot mutltiplication. On the various auxins like NAA (0.5-2.0mg/l) and IBA(0.5-2.0mg/l) rooting was observed. Best rooting from regenerated shoots was observed on MS medium supplemented with IBA(1.0mg/l) within 4 weeks (Fig.5). On this medium root was thick, long and with root hairs.

From the results (Table 1) it is clear that a combination of BAP(0.25mg/l) and Kn (0.5mg/l) at lower concentration was suitable for shoot multiplication as well as shoot elongation. The interacting influence of BAP and Kn was significant in our investigation as has also been reported by some earlier workers[14-17]. During the present investigation different plant growth regulators were used eiher alone or in combination for shoot induction. The combined effect of BAP and Kn was found to be more striking. When various

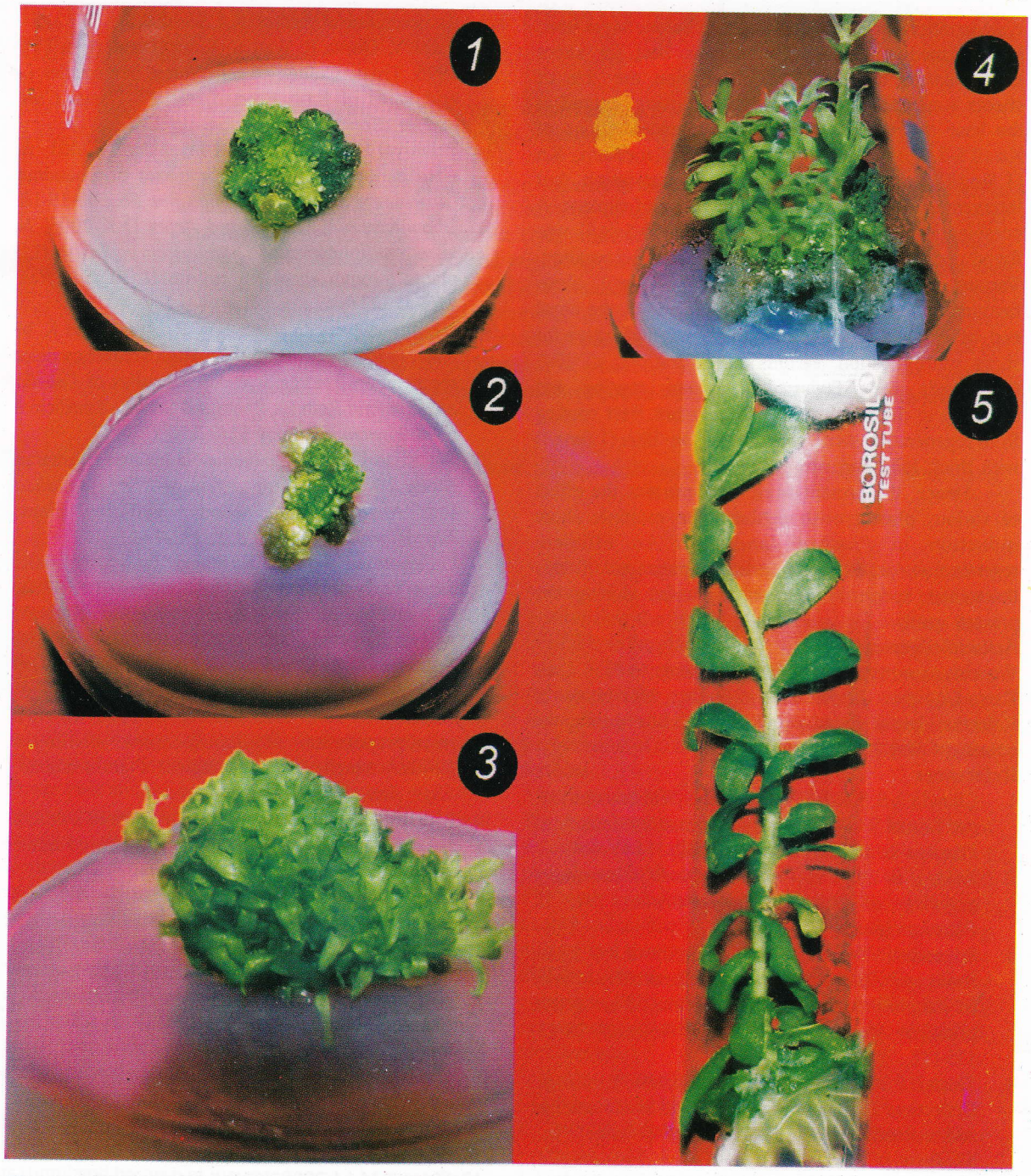

Fig. 1-5. Multiple shoot formation from cultured nodal explant in *Pluchea lanceolata.*
1. Shoot bud proliferation on MS+1.0 mg/l BAP; 2. Shoot bud proliferation on MS+1.5 mg/l Kn; 3. Shoot bud proliferation on MS+0.25 mg/l BAP + 0.5 mg/l Kn; 4. Elongation of multiple shoot on MS+0.25 mg/l BAP+0.5 mg/l Kn; 5. Elongation and root induction in regenerated shoot on MS+1.0 mg/l IBA.

concentrations of BAP(0.25-2.0 mg/l) alone were used in MS medium, BAP(0.1mg/l) was best for shoot multiplication, but at this concentration the size of shoots was very small and slight callusing occured at the base of explant. When various concentrations of Kn (0.5-2.0mg/l) were used in growth medium, Kn (1.5mg/l) was best for shoot multiplication but intervening callus was found at the base of explant. A combination of BAP (0.25mg/l) and Kn (0.5mg/l) was found optimum for shoot multiplication and elongation. Various concentrations of NAA (0.5-2mg/l) alone favoured elongation, rooting and callusing at the basal end of explant. NAA alone was found ineffective for shoot multiplication. Various concentrations of IBA (0.5-2mg/l) alone favoured elongation, rooting and callusing at the basal end of the explant. Best rooting from regenerated shoot was found on IBA (1.0mg/l) with thick, long and white root hairs.

Hence, the present work demonstrates the effect of various plant growth regulators on multiple shoot induction in *Pluchea lanceolata*. An efficient regeneration protocol for micropropagation of this threatened species has been developed during the present study.

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