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Micropropagation of *Coccinia indica* Wight & Arn. - A medicinal plant

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Abstract

Nodal segments of *Coccinia indica* Wight & Arn were cultured on MS supplemented with various concentration and combination of 6-BAP in combination with Kn stimulated shootlets formation with varied percentage. Highest percentage (71.3 ± 0.69) of shootlets formation was achieved on MS supplemented with 1.5 mg/l of BAP in combination with 0.5 mg/l Kn. Highest percentage of rootlets formation was observed in half strength of IAA combined with 0.5 mg/l of Kn. In addition, Callus formation at the base was observed *in vitro* derived shootlets cultured on 2,4-D supplemented medium. Sixty eight per cent of plants were established in the field.

Key words: *In vitro* propagation, medicinal plant, nodal segments.

Introduction

Coccinia indica Wight & Arn., (Cucurbitaceae) commonly known as Kovai" as a valuable medicinal plant distributed in Africa, Arabia, Asia (Pakistan, Malayusia and India) and Australia. In India it is widely distributed in Tamil Nadu Andhra Pradesh, Kerala, Karnataha etc. The plant is useful to treat cathartic, antispasmodic, glycosuria, pityriasis and anthelmitic etc. (Noorulla *et al.,* 2009). Chemical constituents of its roots contain resins, certain alkaloids, starch, glucose, gum, fatty acids, carbonic acid and ash that constitute about 16%. Besides these it contains minerals like calcium, iron and phosphorus. Pharmacologically this plant is used as kapha pitta suppressant. It is a good wound healer and reduces any kind of inflammation occurring in body. It is good appetizer and helps in improving digestion. It is a good laxative and stimulates liver for proper secretion of bile juices. It is also used in wormal infestation particularly in amoebiasis caused by *Entamoeba hystolitica*. It also purifies blood. It is also helpful in expelling the extra amount of mucus accumulated in the respiratory tract. It also controls the frequency of micturation and widely used in diabetes as it controls the glucose level in the blood. It also helps in opening the pores in the skin so as to facilitate the easy secretion of sweat thus expelling out the toxin in the body. Powder is used in the gastrointestinal disturbances, liver weakness, vomiting and dysentery. It also relieves from mouth ulcers and stomatitis. It purifies blood and curbs any infection happening in the body. It is also effective in chronic cough and cold and gives good results in bronchitis and asthma. Juice extracted from the roots is effective in diabetic conditions and is also helpful in urinary tract infections and other related troubles. Leaves are being used in painful conditions and injuries when it is applied locally (Noorulla *et al.,* 2009). Although *C. indica* can be propagated through seeds and vegetative cuttings, seed viability is poor and rooting behaviors of the stem cuttings is not satisfactory. Besides, such conventional propagation processes are season dependent and most of the propagation achieved only during the monsoon period. The application of tissue culture techniques might be of great value an alternative method to achieve large scale propagation independent of season (Fay 1994). De novo regeneration *in vitro* has been reported for a growing list of medicinal, aromatic, economically important plants (Siva Subramanian *et al*., 2002; Senthilkumar *et al.,* 2007and Karrupusamy *et al*., 2006.

The present study was aimed at developing a large scale multiplication protocol for *C. indica* using nodal culture. The morphological growth and floral features of *in vitro* raised plants were also examined.

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Materials and Methods

Plant *Coccinia indica* was collected from Thathanuthu, Tirunelveli, Tamilnadu, India and established in the greenhouse and herbal garden attached with Centre for Biodiversity and Biotechnology, St. Xavier's College (Autonomous). Young shoots were washed with running tap water for 10 min and surface sterilized in 0.1 (w/v) $HgCl_2$ solutions for three minutes. After rinsing three- four times with sterile distilled water, leaves, stem nodes, internodes were cut into smaller segments (1cm) used as the explants. The nodal explants were placed vertically on solid basal MS medium in test tubes (150 x 25 mm) containing 15 ml medium supplemented with 3% sucrose, 0.6%(w/v) agar (HIMEDIA, Mumbai) and different concentration and combination of BAP, Kn, NAA, IAA and 2, 4-D. For rooting, the *in vitro* raised shootlets were recultured on to half strength MS supplemented with various concentrations and combinations of auxins. The pH of the medium was adjusted to 5.8 before autoclaving at 121^{o}C for 15 min. The cultures were incubated at 25$\pm$2^{o}C under cool fluorescent light (2000 lux 14 hr photoperiod). For hardening, the *in vitro* raised plantlets were removed from culture, washed thoroughly with tap water planted in small polycups filled with sterile garden soil and sand (3:1), covered by unperforated polybags and hardened for four weeks in a mist chamber before transfer to field.

Results and Discussion

In many cases, explants determined the relative success of in vitro culture. In the present study also the young explants showed maximum frequency of regeneration compared to the matured ones. Three minutes sterilized nodal segments with 0.1% $HgCl_2$ treed contaminants and showed shoots initiations and elongation of axillary buds within four days (Fig. 1- A). Less or more than three minutes failed to regenerate, less than three minutes sterilized nodal segments were contaminated by fungal and bacterial colonies, more than three minutes sterilized cultures showed the browning on the wound surface and showed high percentage of mortality. Shoot initiation from nodal segments was mainly a cytokinin effect, because the explants in cytokinin free medium did not respond. The role of BAP in bud breaking has been recorded in many medicinal plants such as *Wadelia calendulacea* (Emmanual *et al*., 2000), *Vitex trifolia* (John Peter Arulanandam & Ghanthikumar 2011), *Wattakaka volubilis* (John Peter Arulanandam *et al*., 2011). Reports of the previous workers agree with present reports. Effect of cytokinin and auxin on shoot multiplication from nodal segments is shown in table – 1. MS augmented with 1.0 mg/l of BAP in combination with 0.5 mg/l of Kn showed maximum percentage (71.3 ± 0.69) shoot formation. Callus induction was observed on the cut surface of nodal segments on MS augmented with BAP in combination with Kn. The degree of callus production varied with reference to the supplementation of the plant growth regulators on the medium. Highest degree of callus induction was observed on MS augmented with 2.5 mg/l of BAP in combination with 1.0 mg/l of Kn. Literatures suggest that BAP is most active at combination of 1.0 mg/l to 2.0 mg/l in many plant systems (Senthilkumar *et al*.,2007, Karrupusamy *et al*., 2006, John Peter Arulanandam & Ghanthikumar 2011, John Peter Arulanandam *et al*., 2011).

Table -1: Effect of BAP and IAA on multiple shoot proliferation from nodal explants of *C. indica*

MS + Plant growth regulator in mg/l		% of shoot response ± S.E	Degree of callus formation*
BAP	Kn		
0.5	-	20.8 ± 0.34	NIL
1.0	-	24.2 ± 0.61	NIL
1.0	0.5	31.3 ± 0.32	+
1.5	-	64.8 ± 0.48	NIL
1.5	1.0	71.3 ± 0.67	++
2.0	1.0	70.8 ± 0.54	+++
2.5	1.0	50.4 ± 0.34	++++

* + : Low callus; ++ : Average/ medium; +++: High.

Table -2: Rooting response of excised shoots of *C. indica*

MS + Plant growth regulator in mg/l		% of shoot response	Degree of Callus produced
IAA	Kn		
0.5	-	-	NIL
1.0	-	30.3 ± 0.64	NIL
1.0	0.5	80.3 ± 0.53	++
1.5	0.5	78.4 ± 0.48	++
2.0	0.5	64.3 ± 0.54	++

* + : Low callus; ++: Average/ medium; +++ : High.

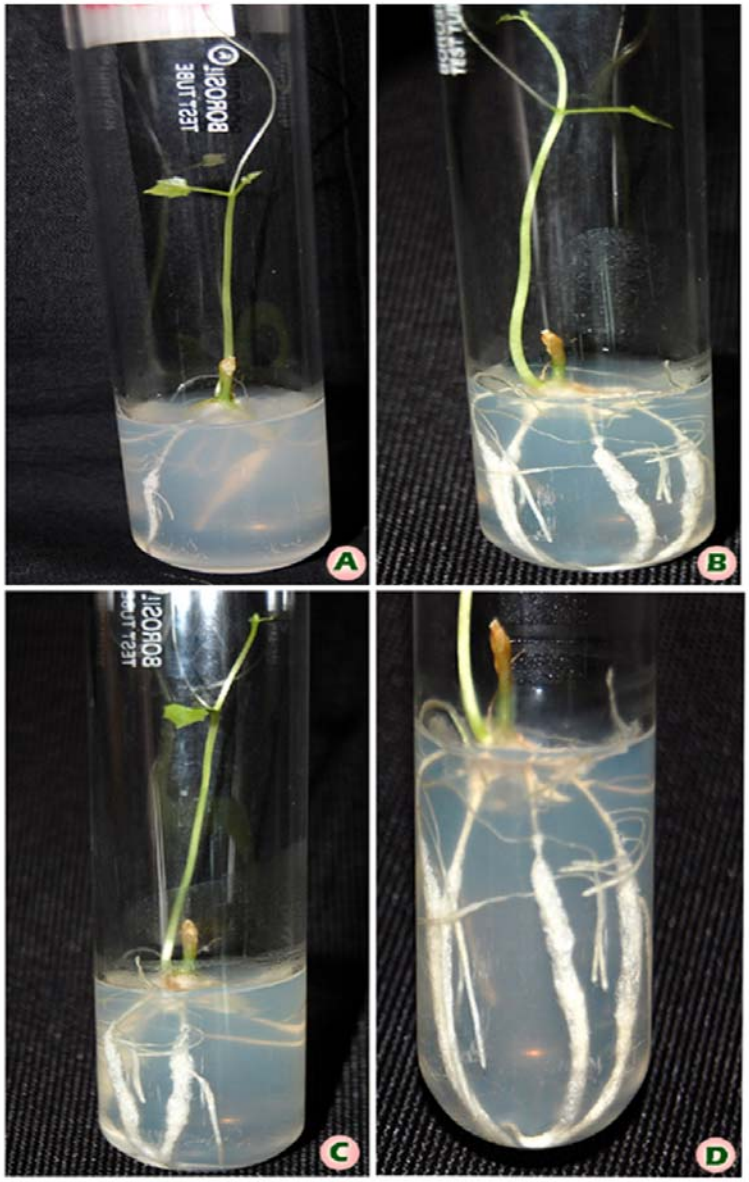

Fig.1: Micropropagation of *C.indica*

The *in vitro* raised shoots were inoculated on to half strength MS supplemented with various combinations of auxin and cytokinin for root initiation. Highest frequency (80.30 ± 0.53) of rooting were observed on half MS fortified with 1.0 mg/l of IAA in combination with 0.5 mg/l of Kn (Fig.1 B-D). However, at lower combination of IAA 0.5 mg/l produced very less percentage of roots (30.3 ± 0.34). The reduced rooting may be due to the imbalance between the endogenous auxin and exogenous auxin, IAA. Thirty five plants were transferred to the hardening in polycups, of which 30 were established in the polycups and they were transferred to herbal garden for reestablishment. Twenty one (68%) plants were established in the field. Cent per cent of similarities were observed *in vitro* raised plantlets, were true to type to this mother plants. As compared to the primary cultures there was no significant improvement in the rate of multiplication during subsequent subcultures. In the present study, initially the rooting percentage was very low, these results are in accordance with those on *Withania somnifera* (Manickam *et al*., 2000), when IAA was tested for rooting, there was not only decrease in the rooting response but also enhanced basal callusing from the *in vitro* raised shoots were observed. These results conform to those of Anand *et al*., (1997) in *Kaempfera rotunda*, John Peter Arulanandam & Ghanthikumar (2011) in *Vitex trifolia* John Peter Arulanandam *et al*., (2011) in *Wattakaka volubilis.*

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