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RAPID *IN VITRO* MULTIPLICATION OF *CITRULLUS COLOCYNTHIS* (LINN.) SCHRAD. THROUGH NODAL SEGMENT CULTURE

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A rapid and reproducible regeneration protocol has been established in Bitter apple (*Citrullus colocynthis* (Linn.) Schrad) *via in vitro* nodal segment with axillary bud. Multiple shoots were induced from nodal segment explants cultured on Murashige and Skoog's medium fortified with 3.0 mg/l 6-benzyl aminopurine. An optimum of 18.8 shoots was obtained per nodal segment explant. The shoot primordia developed into well elongated shoots when they were subcultured on MS basal medium with 2.0 mg/l 6-benzyl aminopurine and 2.0 mg/l α-naphthalene acetic acid. The shoots produced were rooted on MS medium fortified with 4.0 mg/l indole-3-butyric acid (IBA). The *in vitro* regenerated plantlests were hardened and subsequently planted in the soil.

Keywords : Bitter apple; *Citrullus colocynthis* (Linn.) Schrad.; Multiple shoots; Nodal segments with axillary bud.

Introduction

Citrullus colocynthis (Linn.) Schrad (Cucurbitaceae) is an important medicinal plant. It is also known as Indrayan or bitter apple. It is a perennial herb and a common desert plant having a wide range growing in the most barren and arid situations. *C. colocynthis* is used in folk medicine by people in rural areas as purgative, antirheumatic, anthelmintic and as remedy for skin infection[1].

In addition to above medicinal properties the root has a beneficial action in opthalia, uterine pains, jaundice[2] and also useful against boils, pimples and enlarged abdomen[3]. Seeds are purgative and leaves are used in migraine and neuralgia. Pulp of fruit has antibacterial activity[4]. The dried and powdered pulp taken orally to cause abortion[5].

The active principles found in this plant are colocynth ($C_{56}H_{84}O_{23}$) and elaterin, where colocynth is a powerful hydragogue. Phytochemical analysis of the fruits and leaves of the plant demonstrated the presence of cucurbitacins A,B, C and D and [infinity] elaterin and other unknown constituents[6,7].

As a result of continuous exploitation of medicinal plants, some plant species have become vulnerable to extinction[8,9]. Due to excessive and destructive exploitation of *C. colocynthis,* it is getting fast depleted. Therefore, it is the time to conserve this plant species by means of micropropagation through tissue culture technique which is the need of the hour, to improve the growing stock.

Materials and Methods

Citrullus colocynthis plants were collected from the Campus of University of Rajasthan, Jaipur. Healthy explants (nodal segment with axillary bud) were collected from these plants, washed with Teepol solution (0.1% $^{v}/_{v}$) for 5 min under running tap water and immersed in 0.1% aqueous mercuric chloride ($HgCl_2$) solution for 2-3 min followed by three washings with sterile distilled water 5 min for each wash.

The surface sterilized explants were cultured on MS[10] medium containing 3% ($^{w}/_{v}$) sucrose and 0.8% ($^{w}/_{v}$) agar-agar. Explants were implanted in combinations and concentrations of auxins and cytokinins. The pH of the medium was adjusted to 5.8 before autoclaving and sterilized at 15 psi for 20 min. All the cultures were incubated at 25±2°C and exposed to 16h photoperiod provided by fluorescent light of about 3000 lux intensity. Each treatment consisted of five replicates and the experiment was repeated thrice.

To elicit morphogenesis in cultured explants, MS medium was supplemented with various growth regulators like 6-benzyl aminopurine (BAP), 6-furfuryl aminopurine/ kinetine (Kn), a-nepthalene acetic acid (NAA). The concentration of these growth regulators varied from 0.5-5 mg/l in different combinations as detailed in the observations. For induction of roots, the elongated shoots that regenerated from multiple shoot clusters, were transferred to MS medium supplemented with different concentrations of IBA (1-5 mg/l). Healthy rooted plantlets were taken from the rooting medium and washed several times with sterile distilled water. Plantlets were potted in sterile sand: soil (1 : 1) mixture, covered with polythene bags to maintain high relative humidity and were kept under controlled temperature (25-32°C) and light (3000 lux)

Table 1. Effect of BAP and NAA on direct shoot multiplication rate and shoot length after 4 weeks of shoot tip culture. [Values are mean ± SE from 5 replications in each treatment]

Conc. of growth regulators		Intensity of callus growth	No. of shoots per explants
BAP	NAA		
Control	-	-	-
0.5	0.0	-	1.4±0.457
0.5	0.5	++	33.6±1.402
1.0	0.0	-	2±0.491
1.0	1.0	++	38.5±0.672
1.5	0.0	+	2.8±0.365
2.0	0.0	+	3.6±0.457
2.0	2.0	+++	43.6±0.457
2.5	2.0	+	7.8±0.335
3.0	0.0	+	18.8±1.663
3.0	3.0	+++	41±0.802
3.5	0.0	-	9.6±0.457
4.0	0.0	-	7.8±0.335
4.5	0.0	-	7.2±0.725
5.0	0.0	-	7.6±0.457

(-) poor, (++) moderate, (+++) massive callus formation, control = without any hormonal supplement.

Table 2 . Effect of auxin (IBA) on root induction from shoots of *C. colycynthis* after 4 weeks of culture. [values are mean ± SE from 5 replications in each treatment]

Auxins	Conc. of growth regulator	% of cutting rooted	No. of roots per cutting	Average length of the root
IBA	Control	-	-	-
	1.0	80	6.4±0.457	2.4±0.16
	2.0	65	2±0.283	2.5±0.18
	3.0	75	3.8±0.335	2.8±0.40
	4.0	95	19±1.678	3.0±0.16
	5.0	85	9.6±0.671	2.6±0.63

(-) no callusing; (+) slight callusing; control = without any hormonal supplement.

conditions in the culture room. The bags were removed periodically for gradual hardening. After 6 weeks, when new leaves emerged from such plantlets, they were taken outside the culture room and kept in a shady place under normal temperature and light.

Results and Discussion

Asceptic cultures were established from the nodal segment explants sterilized with 0.1% $HgCl_2$ solution for 2-3 min. Single nodal segment with axillary bud from young shoots of *C. colocynthis* were cultured on Murashige and Skoog's (MS) agar medium supplemented with 0.5 to 5.0 mg/l BAP. All concentrations of BAP induced varying number and length of multiple shoots. However, MS medium supplemented with 3.0 mg/l BAP supported rapid multiplication and produced highest number of shoots (18.8 ± 1.663) in 4 weeks. For further multiplication, the nodes and shoot tips from *in vitro* derived shoots were recultured on MS medium fortified with BAP (0.5-3.0 mg/l) and NAA (0.5-3.0 mg/l) . The number of shoots having 3-5 nodes in 4 weeks period was maximum (43.6 ± 0.457) at 2. mg/l BAP in combination with 2mg/l NAA (Table 1).

The cultured shoot cuttings produced roots at the cut ends without callus formation at the base in MS medium supplemented with different concentrations of IBA. Best rooting (95%) was observed in MS medium supplemented with 4 mg/l IBA, within 4 weeks whereas in the control medium no rooting was observed. Roots were thick and with white root hairs. *In vitro* grown plantlets

were transplanted to natural environment (Fig. 6). Micropropagation using nodal segment with axillary bud has been preferred in recent times over conventional vegetative propagation because of the rapidity of multiplication within a short period of time.

In *Citrullus colocynthis* multiple shoots were obtained in the presence of BAP and NAA. The optimum concentration was found to be 2.0 mg/l BAP and 2.0 mg/l NAA (Table 1). The dose of cytokinin is critical in shoot organogenesis[11]. The regeneration frequency increased with increase in concentration of cytokinin (2.0 mg/l) which was found to be optimum for maximum shoot multiplication. Concentration above 2.0 mg/l drastically decreased the shoot bud regeneration. Dong and Jia[13] reported an improvement in shoot bud development in *Citrullus vulgaris* (Watermelon) cotyledons, when cytokinin and auxin were combined in the induction media, but Comptom and Gray[14] and Srisvastava *et al*[15]., detected an inhibition of shoot organogenesis, when NAA or indole-3-acetic acid (IAA) was added to the induction medium. However, in *Citrullus colocynthis,* higher concentrations of BAP in the medium enhanced multiple shoot induction. At the same time BAP alone was not satisfactory in inducing shoot multiplication. BAP (2.0 mg/l) in combination with NAA (2.0 mg/l) produced multiple shoots. In *Randia dumetorum* it was found that higher concentrations of BAP and NAA were effective on shoot multiplication but produced fast growing callus[16].

In the present investigation, it was observed that multiple shoot regeneration is possible from the nodal segment with axillary bud of *C. colocynthis*. Plant growth regulators were used either alone or in combination for shoot induction. The combination effect of a cytokinin and an auxin was found to be more striking. During the course of preliminary investigation, it was observed that both cytokinins as well as auxin were essential for increasing the morphogenesis and that the type and rate of plantlet growth was dependent on their relative ratio.

In the present study BAP proved better than *Kn* in evoking shoot multiplication in *C. colocynthis*. BAP along with auxins was found most suitable for shoot multiplication. Various concentrations of BAP (0.5-5.0 mg/l) in combination with IAA, IBA, NAA (0.5-5.0 mg/l) induced varied types of response. IAA alongwith BAP in the medium induced shoot tip elongation. BAP and IBA in combination also induced shoot multiplication. The number of shoots varied from 4-16. BAP in combination with NAA induced shoot formation along with callusing. About 4-9 extensively branched green, normal and healthy shoots were formed on various combinations. Best proliferation of shoots was observed on BAP (2.0 mg/l) and NAA (2.0 mg/l) supplemented medium.

Rooting of the *in vitro* multiplied plantlets were usually achieved in auxin containing medium. The plantlet regeneration protocol developed in this study may be utilized for the cultivation practices of this economically important medicinal plant. This is the first report of its kind applicable to *C. colocynthis*. This work provides primary information and methodology for rapid propagation, *in vitro* and *ex situ* conservation of this threatened and valuable medicinal plant.

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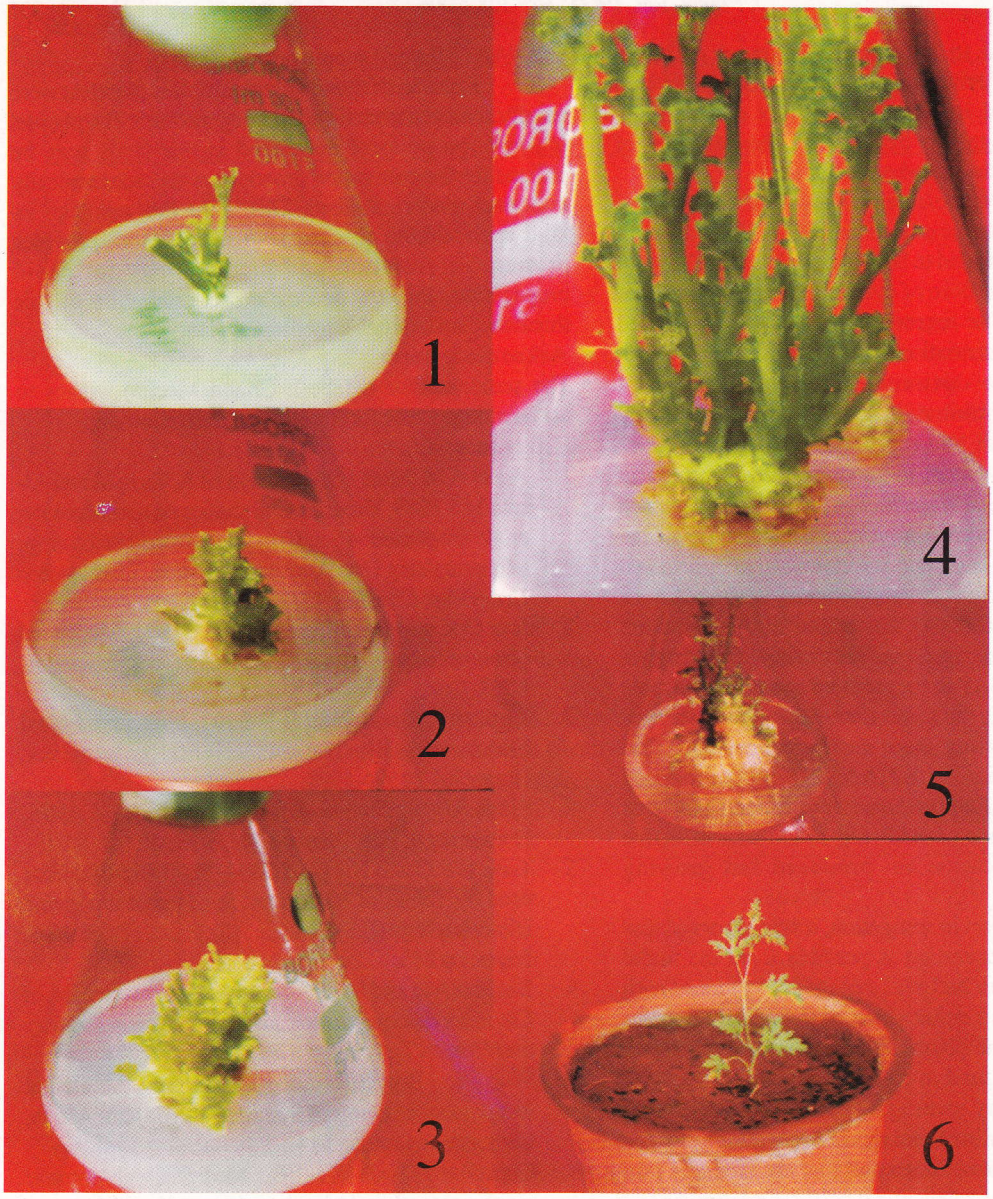

Fig.1-4. Initiation and multiple shoot formation of plant of *Citrullus colocynthis*. 1- Initiation of multiple shoots from nodal segment with axillary bud after 7 days of inoculation. 2-3- Multiple shoot proliferation after 15 days and 30 days of inoculation. 4- Elongation of proliferated shoot. 5- *in vitro* rooting of elongated shoots. 6- Hardening of *in vitro* raised plantlet.

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