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PLANT REGENERATION FROM THE NODAL PART OF *CITRULLUS COLOCYNTHIS* (L.) SCHARD.

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Abbreviations:

MS medium- Murashige and Skoog medium, BAP- 6- Benzylamino purine, IBA- Indole 3-butyric acid, Kn- 6-furfurylamino purine, IAA- Indol Acetic Acid

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ABSTRACT

Citrullus colocynthis, belonging to the family of cucurbitaceae. It is a xerophytic desert plant, widely used for its medicinal purpose. In tribal areas it is used for the treatment of constipation, oedema, bacterial infection, cancer, diabetes and abortifacient. Present study is based on developing a protocol for plantlet regeneration from the nodal segment. The sterilized explants were inoculated in different concentrations of cytokinins and auxins. Among the various treatments, MS (1962) medium supplemented with BAP (2.0mg/l) gave the best shooting culture establishment. For shoot elongation MS medium supplemented with IBA (0.1 mg/l) was found to be best. *In vitro* elongated shoots rooted on MS medium supplemented with IBA (0.1 mg/l). The plantlets were hardened, acclimatized and transplanted in the field, where they showed 60% survival rate.

INTRODUCTION: Cucurbitaceae family contains about 90 genera and over 700 species of economic importance [1]. Cucurbits are dietary vegetable, which is primarily comprised species consumed as food worldwide. Cucurbitis are excellent fruit in nature that have composition of all the essential constituents required for good health to humans [2, 3]. It is typically distributed in the tropical countries poorly represented in temperate regions.

It is a wide spread annual uncultivated, procumbent herb having small yellow flowers. The fruit is very bitter. The bitter taste is caused by high concentration of Cucurbitacin E. glycoside or colocynthine [4]. It grows fast in the sandy soils and widespread in different parts of Saudi Arabia. This plant is used as anticancer agent in many drugs. It is also used as anti-pyometra in animals [5]. Organogenesis, micropropagation and chemical analysis also performed earlier by various workers [6, 7, 8].

The present study deals with the establishment of protocol for plant regeneration from the nodal segment of *Citrullus colocynthis*.

MATERIAL AND METHOD:

Plant Material: Nodal segments of the *Citrullus colocynthis* were collected from mature plant growing in the campus of Rajasthan University, Jaipur, India. The nodal parts collected were ranging from 2 to 3 cm in size.

Surface Sterilization: Surface sterilization process is to be followed in various steps so as to avoid contamination in the culture conditions. Nodal segments were then excised and washed thoroughly in the running tap water for 5 to 10 min under aseptic conditions. Explants were surface sterilized with 70% of ethyl alcohol (v/v) for 1to 5 min. Followed by dipping in 0.1% fresh aqueous mercuric chloride (w/v) solution for 1 to 2 min, subsequently washed

thoroughly with double distilled water to remove the traces of mercuric chloride.

Culture Media and Culture Conditions: Explants were then transferred to shoot induction media consisted with basal medium of MS [9], supplemented with 3% sucrose and 0.8% agar-agar (w/v), BAP (0.5-2.5 mg/l) and Kn (0.5-2.5 mg/l). The pH was adjusted to 5.7 prior to autoclaving for 20 min at 121^0 C for 15 min. The experiments were repeated three times. All cultures were grown under 16/8 (light/dark) hr photo period, provided by cool white fluorescent light.

Shoot Regeneration: Nodal segments inoculated on the MS medium supplemented with BAP (2.0mg/l) resulted in better results as multiple shoot were observed with in the inoculation of two to three weeks. The multiple shoots obtained were regularly subculture on fresh medium at 2 to 3 weeks intervals and observations were recorded.

***In vitro* Morphogenesis:** Elongated and healthy shoots were then transferred aseptically to rooting medium containing IBA (0.05-0.15mg/l), IAA (0.05-0.15 mg/l). After *in vitro* rooting the regenerated plantlets were taken out and were washed carefully to remove agar and then transferred to pots containing sterile soil and vermiculite (3:1). After 10-15 days the plantlets were kept or placed in green house; they were then transferred in the normal environmental conditions, for normal growth.

RESULT: The nodal explant, cultured on MS medium supplemented with BAP and IBA generated best response in terms of regeneration of complete plantlet. The shoots aseptically transferred to shoot proliferating medium having treatment of cytokinine, BAP (0.5mg/l - 2.5mg/l) and kinetin (0.5mg/l - 2.5mg/l). Among different concentration used, best, maximum multiple shoots (12.1±0.5) were obtained on MS medium supplemented with BAP (2.0mg/l). From the results (**Table 1**), it is clear that BAP (2.0mg/l) and kinetin (2.0mg/l) (Table 1) at higher concentration were suitable for shoot multiplication as well as shoot elongation.

The rooting of the developed shoots was usually achieved in auxin containing medium [9]. The rooting response differed according to different concentration of auxins used (**Table 2**).

In vitro grown healthy micro-shoots (1-2 cm) were excised and cultured on MS medium supplemented with different concentrations of IBA (0.05mg/l-0.15mg/l) and NAA (0.05mg/l-0.15mg/l) (table 2).

IBA alone is most potential in inducing high percentage (70-80-%), of rooting (51.4±0.1). NAA (0.1mg/l) induced, (60-70%) of rooting (46.0±0.4) per shoot (Table 2). The numbers of induced roots were less in NAA in compared to IBA. Most of the shoots had produced roots with in 2 to 3 weeks after placing on rooting medium (**Figure 3**).

Low concentration of auxin facilitated better rooting. When medium supplemented with higher concentration of auxin(s) IBA or IAA, culture resulted in white callus instead of rooting, but when the same cultures were maintained for longer period rooting initiated in them.

TABLE: 1 EFFECT OF CYTOKININ (S) ON SHOOT PROLIFERATION OF *C. COLOCYNTHIS*

Treatment	Concentration	No. of shoots per explant Mean ± S.E.
BAP	0.5	3.2±1.1
	1.0	5.7±0.9
	1.5	6.1±0.7
	2.0	12.1±0.5
	2.5	7.6±0.7
Kn	0.5	1.0±0.3
	1.0	1.3±0.3
	1.5	2.0±0.7
	2.0	3.7±0.8
	2.5	2.5±0.8

TABLE: 2 EFFECT OF AUXIN(S) FOR ROOT INITATION AND ELONGATION

Treatment	Concentration (mg/l)	Response (%)	Roots/explant Mean ± S.E.
IBA	0.05	50-60	40.2 ± 0.2
	0.1	70-80	51.4 ± 0.1
	0.15	50-55	38.2 ± 0.3
NAA	0.05	30-40	33.1 ± 0.1
	0.1	60-70	46.0 ±0.4
	0.15	45-50	35.4 ± 0.2

DISCUSSION: The plant showed good response towards the growth hormones, especially towards the cytokinine as it can be noted that the percent of shoot length were higher in BAP as compared to that of kinetin. Thus, when shoots were transferred to medium containing BAP (2.0 mg/l), resulted in best

percentage of proliferation. The earlier study reported that the low concentration of BAP (1mg/l) in *Impatients balsamina,* induces highest rate of shoots [10.] The number of shoots per explant was also registered highest for the above treatment; similarly maximum number of shoot regeneration has been achieved by supplementing MS medium with 4.4-20µM BAP as the only growth regulator [11, 12, 13, 14, 15].

Combinations of different concentration of NAA with 8.8 µM BAP induced shoot buds in all treatments but the response percentage and the number of shoots per regenerating leaf segments of *Achras sapota* were very low as compared to BAP used alone [16], while BAP in combination to NAA with the same concentration (0.5mg/l) reported for the highest number of shoots of *Citrullus colocynthis* [17].

MS medium containing IBA (0.1mg/l) recorded highest (70-80%) response, similar results were reported in *Citrullus laratus* [18]. In *Daphne* L. species, root development was best obtained on medium containing either IBA and NAA as compared to IAA [19].

The present study reveals that, IBA is better than NAA in inducing rooting. Among all plant growth regulators IBA is widely used for root induction in cucurbits [20] while NAA is also used [21]. Efficient rooting was achived in *Trichosanthes dioica* at different concentration with IBA (0.5mg/l) and NAA (2.0mg/l) [22]. Variation in rooting response may be a result of genotype or culture conditions. Subsequently, the rooted plantlets were removed from agar medium, washed thoroughly and placed in soil pots after 2 weeks for acclimatization and initial hardening under culture room conditions. Almost 60% of these regenerants survived and developed new branches and were ready for planting in the field for further growth.

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