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# *IN VITRO* MORPHOGENIC STUDY OF LUFFA CYLINDRICA AND CITRULLUS COLOCYNTHIS

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## ABSTRACT

Luffa cylindrica Linn. and Citrullus colocynthis Linn. are two important plants of family cucurbitaceae. which have not been much investigated for their in vitro morphogenic studies. In the present investigation shoot iniation was reported after 12-15 days of sub-culturing of callus cultures on MS medium Supplemented with different growth hormones in both the plants. The shoot initiation in L. cylindrica was observed from callus on MS medium supplemented with combination of auxin and cytokinin. The maximum number of shoot production (70.8%) was observed when media was supplemented with Kn + BA **(70.8%**), and length of shoot was approximately 2.5 cm. Whereas the shoot initiation in C. colocynthis was observed from callus on MS medium supplemented with cytokinin combination. Shooting was reported on BAP+Kn. However, the maximum number of shoot production (75.6%) was observed when media was supplemented with BAP+ Kn, ( **75.6%)** length of shoot was approximately 2.6 cm.

**KEY WORDS**: Luffa cylindrica, Citrullus colocynthis, Multiple shoots, Callus cultures, Growth hormones.

## INTRODUCTION

Luffa cylindrica Linn. and Citrullus colocynthis Linn. are two important plants of family cucurbitaceae which have not been much investigated for their in vitro morphogenic studies. An important milestone in the study of morphogenesis in tissue culture is the role of auxin and cytokinin interaction. Auxins are concerned with the physiological activity leading to cell enlargement. A number of plant tissues in vitro synthesize growth regulators in sub-optimal

amounts and therefore, require addition of external phytohormones (Reinert and White, 1956; Gamborg et al., 1968). Cytokinins are involved indirectly in a wide variety of biochemical activities of physiological functions leading to most heterogeneous, histological and morphological results. They play an important role in inducing chlorophyll formation and shoot induction in plant tissue culture (Murashige, 1974). Skoog and Miller (1957) reported that high levels of auxins promoted callus initiation and establishment in tobacco plant. The observations show that high auxins : low cytokinin ratio favors callus and root induction. Therein it has been shown, that as we cannot gauge the endogenous levels of these two compounds, the relative concentration for obtaining the desired end results is largely a matter of trial and error in each case as has been reported by many workers (Lee and Rao, 1980; Kong and Rao, 1981a, b; Gharyal and Maheshwari, 1982, 1983; Datta et al., 1983; Singh, 1985; Tulecke, 1989). The present investigation on morphogenesis of callus cultures of Luffa cylindrica and Citrullus colocynthis (Samariya and Sarin, 2014a, b) was carried out in vitro keeping in view of their callus cultures raised from different explants under defined nutritional, hormonal and cultural conditions responded differently.

## MATERIAL AND METHODS

Seeds, leaves and nodal segments of healthy plants of Luffa cylindrica and Citrullus colocynthis were collected from Rajasthan university campus as explants for the tissue culture studies. All explants were washed under running tap water followed by several rinses in sterile distilled water. The excised explants were then inoculated in culture flasks containing 30-35 ml MS (Murashige and Skoog, 1962) medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar (Qualigens, India). Auxins (Indole 3- acetic acid, 2,4-Dichlorophenoxy acetic acid, and Napthalene acetic acid,), cytokinin (6- benzylaminopurine, kinetin) were incorporated into basal medium in varying concentrations and combinations as indicated in the results. The pH of all the media combinations was adjusted to 5.8± 0.1 using 0.1 N NaOH or 0.1 N HCl before autoclaving. Autoclaving was done at 1.06 kg $cm^{-2}$ at 121° C for 25 minutes. Cultures were incubated at 24° ± 2° C temperature and 55 ± 5% relative humidity with 16 hours photoperiod. All treatments had 4 to 8 replicates. Each culture flask contained 30 ml of culture medium and 4 explants were inoculated per flask. All experiments were repeated thrice For each treatment, 25 flasks were inoculated with the desired explant and incubated under optimal conditions as defined above. The inoculated culture flasks/tubes were kept in culture room under controlled conditions. The cultures were maintained at a temperature of 30±2°C under 55±5% relative humidity in the culture room. A rhythmic cycle

of 16 hours light followed by 8 hours darkness was given to the cultures. Light was provided by a combination of cool white fluorescent tubes and incandescent bulbs in the ratio of 3:1. The light intensity as irradiance varied according to experimentation in with range of 2500 lux to 3000 lux. The temperature light and relative humidity conditions varied according to the experiment to get optimal regenerations.

Callus cultures of each plant raised on MS-medium in vitro was maintained for one year by frequent subculturings on fresh medium after every 3-4 weeks, for its biomass production. Callus was also subcultured on fresh medium in order to evaluate whether the callus showed any morphogenic response or continued to proliferate as such. Fresh weight of callus was recorded by weighing the callus tissue after separating it from the plant.

**Multiple shoot proliferation**

In order to induce shoot buds and subsequent shoot differentiation, the callus mass of L. cylindrica and C.colocynthis from stock callus was used. For the induction of shoot in the callus tissue, the MS media supplemented with individually and/ or in combination of auxin or cytokinin was used. Callus tissue transfered in to shoot bud induction media in L.cylindrica (MS+Kn+BA+NAA) and C.colocynthis (MS+BAP+Kn+NAA) supplemented with different homones (Tables 1 and 2).

**RESULTS AND DISCUSSION**

The result obtained in both plants viz. L. cyindrica and C. colocynthis are as follow:-

**L. cylindrica**

The shoot initiation in L. cylindrica was observed from callus on MS medium supplemented with cytokinin individually and in combination of Kn+ BA Shooting was reported on Kn, BA, (Kn + BA), (Kn+NAA), Shoot, iniation was reported after 12-15 days of sub-culturing. However, the maximum number of shoot production (70.8%) was observed when media was supplemented with (Kn + BA), shoot induction **(70.8±0.25**), number of shoots less then approximately 4.2±1.62 and length of shoot was approximately 2.5±1.21 cm. (Table 1 Figs.1 and 2).

**C. colocynthis**

The shoot initiation in C. colocynthis was observed from callus on MS medium supplemented with cytokinin individually and in combination of BAP + Kn. Shooting was reported on

BAP, Kn**, (**BAP+Kn), (BAP+NAA), Shoot, iniation was reported after 12-15 days of sub-culturing. However, the maximum number of shoot production (75.6%) was observed when media was supplemented with (BAP+Kn), shoot induction (**75.6±0.21**), number of shoots less then approximately 6.5±1.51 and length of shoot was approximately 2.6± 0.85 cm.( Table 2 and Fig 3A and B).

**Table 1. Morphogenic responces (Shooting formation) of callus of L. cylindrica to different concentrations of cytokinin and auxin.**

| Sr. No. | Hormones | Conc. (mg/L) | Shoot induction (%) | No. of shoots (cm.) | Length of shoot (cm.) |
|---|---|---|---|---|---|
| 1. | **Kn** | 0.1 | 58.5±0.26 | 1.0±0.02 | 0.8±0.21 |
| | | 0.3 | 60.1±0.45 | 1.2±0.05 | 0.9±0.42 |
| | | 0.5 | 63.3±0.68 | 1.4±0.82 | 1.0±0.52 |
| 2. | **BA** | 0.1 | 43.2±0.67 | 2.1±0.84 | 1.2±0.28 |
| | | 0.3 | 45.2±1.31 | 2.5±0.81 | 1.3±0.67 |
| | | 0.5 | 52.2±0.25 | 2.7±1.62 | 1.4±0.54 |
| 3. | **Kn+BA** | 0.1+0.1 | 64.8±0.03 | 2.9±0.21 | 1.6±0.11 |
| | | 0.3+0.3 | 69.4±1.20 | 3.1±0.11 | 2.2±0.55 |
| | | **0.5+0.5** | **70.8±0.25** | **4.2±1.62** | **2.5±1.21** |
| 4. | **Kn+NAA** | 0.1+0.1 | 58.1±0.81 | 3.1+0.26 | 1.9±0.63 |
| | | 0.3+0.3 | 62.2±1.22 | 3.6+0.33 | 1.8±0.76 |
| | | 0.5+0.5 | 64.1±0.23 | 3.8+1.54 | 1.7±0.55 |

Value represent treatment of three replicates ±SE

**Table 2. Morphogenic responces (Shooting formation) of callus of C. colocynthis to different concentrations of cytokinin and auxin.**

| Sr. No. | Hormones | Conc. (mg/L) | Shoot induction (%) | No. of shoots (cm.) | Length of shoot (cm.) |
|---|---|---|---|---|---|
| 1. | **BAP** | 1.0 | 54.1±0.25 | 1.2±0.02 | 1.0±0.22 |
| | | 2.0 | 56.2±0.45 | 2.1±0.54 | 1.1±0.52 |
| | | 3.0 | 61.7±0.68 | 2.8±0.80 | 1.2±0.41 |
| 2. | **Kn** | 0.1 | 41.2±0.65 | 1.1±0.74 | 1.2±0.28 |
| | | 0.3 | 44.2±0.67 | 2.5±0.81 | 1.3±0.57 |
| | | 0.5 | 50.0±1.34 | 3.5±0.36 | 1.4±0.27 |
| 3. | **BAP+Kn** | 1.0+0.1 | 65.6±0.46 | 5.8±0.41 | 2.1±0.11 |
| | | 2.0+0.3 | 68.7±0.51 | 6.2±0.98 | 2.4±0.23 |
| | | **3.0+0.5** | **75.6±0.21** | **6.5±1.51** | **2.6±0.85** |
| 4. | **BAP+NAA** | 1.0+0.1 | 58.1±0.83 | 3.0±0.45 | 2.3±0.84 |
| | | 2.0+0.3 | 60.4±0.32 | 4.1±0.68 | 2.2±0.65 |
| | | 3.0+0.5 | 62.8±1.23 | 4.5±0.47 | 2.3±0.78 |

Value represent treatment of three replicates ±SE

**Fig. 1. Shooting formation in L. cylindrica from callus on MS (1962) medium supplemented with (Kn + BA) (0.5 + 0.5) mg/l**

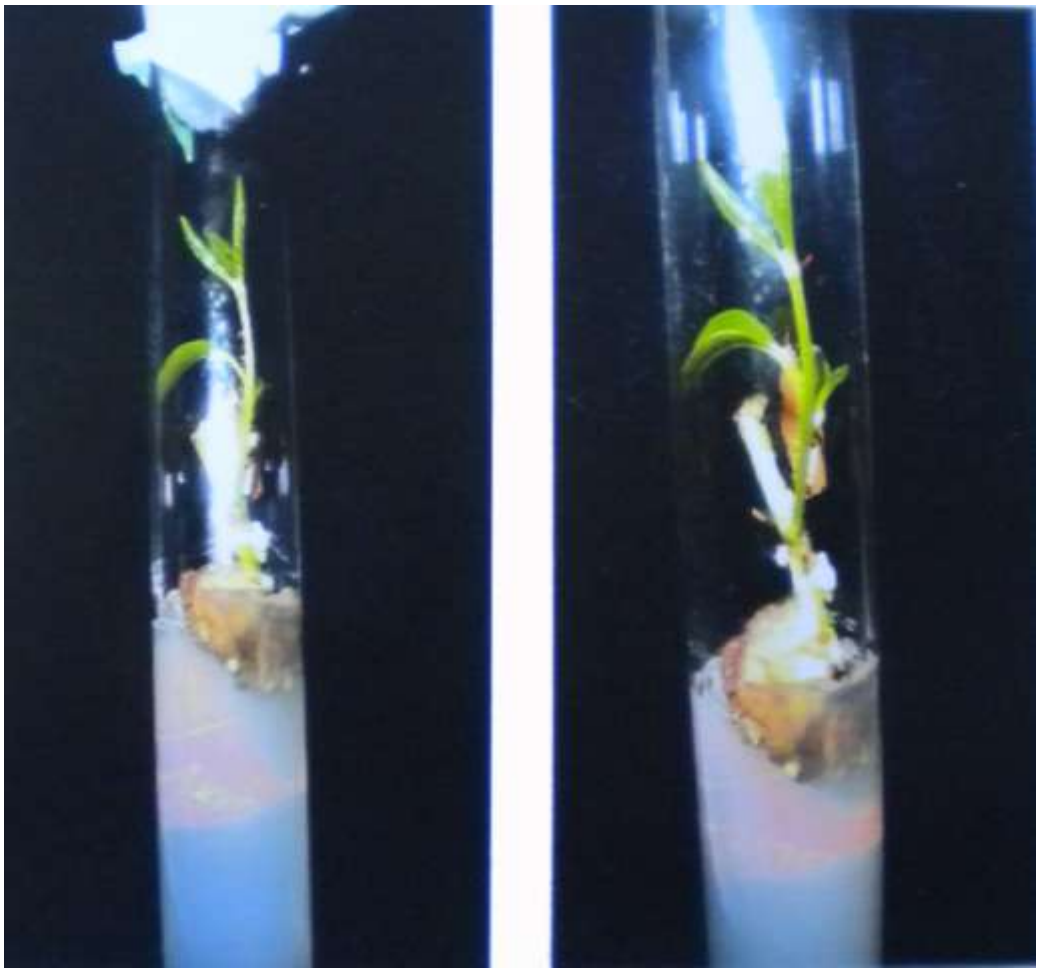

**Fig. 2. In vitro regenerated plantlets of L. cylindrica on MS (1962) medium supplemented with (Kn + BA) (0.5 + 0.5) mg/l**

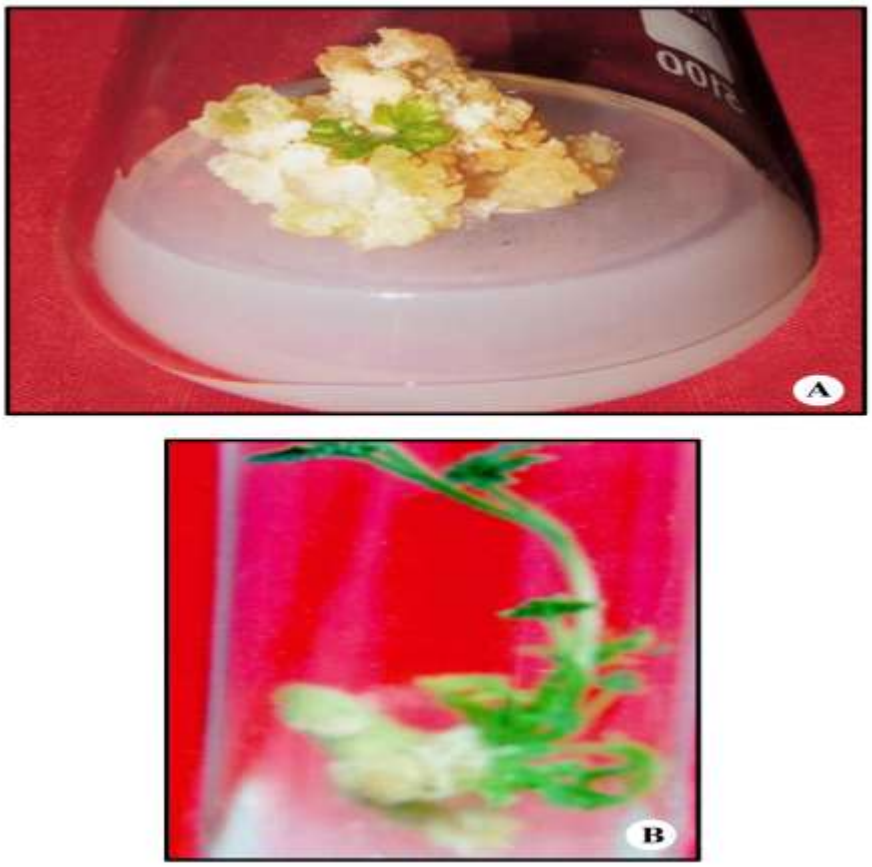


**Fig. 3. A. Shooting formation in C. colocynthis from callus on MS (1962) medium supplemented with (BAP + Kn) (3.0 + 0.5) mg/l**
**B. In vitro regenerated plantlets of C. colocynthis from callus on MS (1962) medium supplemented with (BAP + Kn) (3.0 + 0.5) mg/l**

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