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CALLUS MULTIPLICATION OF A MEDICINALLY IMPORTANT VEGETABLE- *LUFFA CYLINDRICA*

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ABSTRACT

Luffa cylindrica belongs to the family cucurbitaceae is an economically important plant widely grown for its vegetable purpose,but the plant as a whole is able to cure many of the skin diseases,thus possess medicinal importance also. In the present study callus multiplication of the luffa from the leaf explant have been investigated with different concentrations of auxin (NAA) with cytokinine (BAP) considering various parameters. The best result were obtained at BAP(1.5mg/L) and in combination with NAA at BAP(1.5mg/L) and NAA (1.0mg/L with maximum percent of callus which also initiated in short period of time of 10 to 12 days.

Key words: **Callus, differentiation, *Luffa cylindrica,* cucurbitaceae**.

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ABBREVIATIONS

BAP Benzyl amino purine; 2,4-D 2,4-Dichlorophenoxyacetic acid; MS Murashige and Skoog (1962) basal medium; NAA α-naphthalene acetic acid; IBA Indole-3-butyric acid; IAA Indole acetic acid

INTRODUCTION

Plants have been used in traditional medicine for several thousand years (Abu-Rabia 2005). The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani, Siddha. Medicinal plants continue to be an important resource material for therapeutic agents both in developed and developing countries (Kumar 2000).

The process of growth and morphophysiological specialization of cells from unorganized mass of callus cells i.e. differentiation, is a prerequisite for the application of biotechnology for crop improvement. Differentiation of organized structures in tissue culture is controlled by growth regulators such as cytokinins and auxins along with other components of the culture medium. Differentiation through callus cultures involves changes in some of the biochemicals (Kumar *et al*., 2010).

Cucurbits are vegetable crops belonging to family Cucurbitaceae, which primarily comprises of spices consumed as food worldwide.It is typically distributed in the tropical countries and poorly represented in temprate regions. Metcalfe and Chalk (1950) pointed out that the family of cucurbitaceae is noted for its rapid vegetative growth. Cucurbits are excellent fruit in nature having composition of all the essential constituents required for good health of humans (Rahman, 2003; Duke, 1999 and Chopera *et al*., 1992).

Luffa [*Luffa cylindrica* (L.) Roem syn *L. aegyptiaca* Mill] commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd. Some cultures have used it as a natural cure for skin diseases like leprosy..The seed oil is also known for curing serious skin diseases and prevention of other skin ailments. Because the chemical composition does not allow synthesis of certain proteins, the skin cancer cell toxicity makes it a natural anti-cancer essential. Uses such as healing of skin boils and shingles, comes from the anti-fungal and anti-inflammatory elements of the *luffa cylindrica* seed oil.

Thus present investigation is under taken to enhance the callus production of a medicinally important vegetable.

MATERIAL AND METHODS

Plant material:

Young leaves of *Luffa cylindrica* were collected from mature plant growing in the potted plants of nursery of Rajasthan University, Jaipur, India.

Surface sterilization:

Surface sterilization process is to be followed in various steps so as to avoid contamination in the culture conditions. Explants were kept under running water for 15-20 min to remove any soil particles adhering. They were then washed with regular mild detergent Teepol (0.2%) (Central Drug House, India) and rinsed three- four times with distilled water. After washing procedures, explants were surface sterilized with 70% alcohol and subsequently with (0.1%) mercuric chloride ($HgCl_2$) aqueous solution (w/v) for 1-2 min, followed by repeated rinsing (3-4 times) with sterile distilled water. The surface sterilized explants were then ready for inoculation on solid nutrient media.

Media and culture conditions

The basal medium consisted of MS salts with 3% sucrose (w/v) and solidified with 0.8% (w/v) agar, BAP (0.5-2.5mg/l) and NAA (0.5-2.5mg/l). The media were adjusted to pH 5.7 with 1.0 N NaOH or HCl before adding agar, and then sterilized by autoclaving at 121°C for 20 min.

Callus induction

To study the callus induction from the leaf, the explants measuring 2-3mm were inoculated to the culture flasks containing MS medium supplemented with BAP and NAA added signally as well as in combination (Table 1). Provided with the incubation at 20^0 C under 16h light/8h dark photoperiod. Well grown callus induced from explants were transferred to the original media and sub-cultured every 20 days.

Data collection

Data were taken after 5-45 days by visual observation of the culture. At the end of the observation period the percentage of response, the day of callus initiation and the nature as well as color of the callus to different concentrations of plant growth regulators were recorded.

RESULT AND DISCUSSION

Surface sterilized leaf explants cultured on MS medium supplemented with different concentration and combination of BAP and NAA showed callusing from leaf edges (Fig 1). Similar results but with callus from nodal explants, were reported in *Stevia rebaudiana* where also maximum callus production was obtained from nodal explants culture in MS medium with 13.56µM 2,4-D (Uddin *et al*., 2006). Results showed that BAP alone was also capable to initiate a good amount of callus from the cut ends of the explants, while combination of NAA (1.0mg/l) and BAP (1.5mg/l) showed maximum response (Table 1). But the rate of callus production was decreased at lower and higher concentrations. The leaf explants cultured on higher concentration of BAP 2.5mg/l with NAA 1.5mg/l showed only 35% response (Fig.2), after 16 days of callusing, Gupta *et al*., 2010, reported the same results where leaf explants showed 100% response at lower concentration of NAA and 2-4,D (0.75mg/l NAA + 1.0mg/l 2-4,D), while at higher concentrations of NAA the callus induced response was only 33% .

Figure1
Callus initiation from the edges

Figure 2
Less % response on higher concentration of BAP (2.5mg/l)

Table 1
Effect of auxin and cytokinin on leaf explants if Luffa cylindrica for callus development.

S.No.	Explant used	BAP	NAA	% of callusing	% of response	Callus initiate in days	Callus Type
1	Leaf	0.5	-	Average	15	18	Fragile soft
2		1.0		Good	25	14	Fragile soft
3		1.5		Maximum	60.36	10	Fragile soft
4		2.0		Good	48.21	16	Fragile soft
5		2.5		Good	30.17	10	Fragile soft
6		-	0.5	No Response	-	-	-
7		-	1.0	Average	20	21	Fragile soft
8		-	1.5	Good	40.00	11	Fragile soft
9		-	2.0	Good	35.7	16	Fragile soft
10		-	2.5	Good	30.1	12	Fragile soft
11		1.0	0.5	Average	20	20	Fragile soft
12		1.5	1.0	Maximum	72.4	12	Fragile soft
13		2.0	1.0	Good	40.6	18	Fragile soft
14		2.5	1.5	Good	35	16	Fragile soft

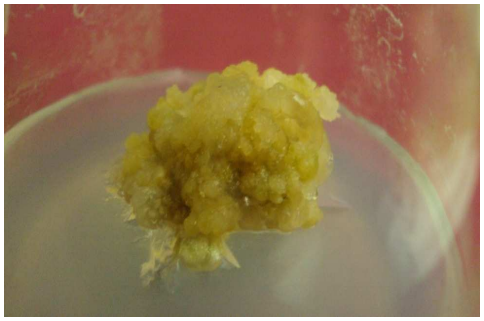

Figure 3
Creamy callus

Figure 4
Maximum callus response (72%)

The callus obtained from the explants was creamy white in color and soft in nature (Fig 3). NAA at lower concentration (0.5mg/l) was not able to induce any callus when maintained for three weeks also.. The growth of the callus increased significantly with the incubation period from 4-6 weeks in leaf explants. After the initiation, green fragile callus gradually covered the entire surface of the explants. The most widely used cytokinins are BAP and Kinetin. Krens and Jamar, 1989 reported that auxins were more effective when combined with low BAP levels for callus production. Auxins and cytokinins are the most widely used plant growth regulators in plant tissue culture and usually used together (Gang et al. 2003). It was revealed that auxins played an important role in the callus induction and different types of auxins had various effects (Skoog and Armstrong 1970; Baskaran et al., 2006), and the cytokinins facilitated the effect of auxin in callus induction (Rao et al., 2006; Yang et al., 2008). The role of auxins and cytokinins in callus induction was also advocated by Kumar and Singh (2009a) in S*tevia rebaudiana,* Goel and Singh (2009) in *Peganum harmala*, Kumar and Singh (2009b) in *Prosopis cineraria*, Lal and Singh (2010) in *Celastrus paniculatus* and Yadav and Singh (2010) in *Spilanthes acmella*. The study showed that the most favorable medium for callus production was MS medium supplemented with 1.5mg/l BAP and .10mg/l NAA with an average of 72.4% response (Table 1, Fig 4).

CONCLUSION

It is important to develop an efficient protocol for callus proliferation in order to start *in vitro* selection to broaden the opportunities for genetic manipulation of *Luffa* through tissue culture, using various explants and media having different composition of growth hormones

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