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In Vitro Plant Regeneration of *Luffa acutangula* Roxb. Var *amara* Lin.: An Important Medicinal Plant

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Abstract: ***Luffa acutangula Lin. Var. amara (Roxb) is an important wild medicinal plant belongs to the family Cucurbitaceae. All parts of the plant are used to cure many diseases, especially in crystalline bitter compound with cucurbitacin B and Amarinin. The whole plant is used to cure many diseases such as Anti-Cancer, Anti-Diabetic, Anti-Jaundice and many ailments. The study was conducted to analyze the effect of plant growth regulators on callogenesis, direct and indirect organogenesis of Luffa amara. Callus culture was induced from leaf and stem explants of Luffa amara in a different hormonal (TDZ + 2, 4 – D, IAA + IBA) combination of MS medium at various concentrations. The best callogenesis response was observed in 2, 4 – D + TDZ - 2.0 mg/L. The nature of callus was produced in hard, green and compact. The good response of callus to produced more number of shoot formation through indirect organogenesis at different concentrations (BAP). Best response of shoot formation was observed in 2.5 mg/L of BAP. The shoots were transferred in both full and half strength MS medium supplemented with Auxin at various concentration (IAA + IBA).***

Keywords: *Luffa amara*, Callogenesis, Organogenesis, Cucurbitaceae

1. Introduction

The tissue culture technique is used for propagation, genotype modification, biomass production, germ plasm preservation and scientific investigations. These separate procedures have been collectively called tissue and organ culture (Ammirata, 1987), in vitro culture (Quak, 1997). Micropropagation (Smith and Obeidy, 1991) and most recently biotechnology (Hartmann *et al*., 1997).

The *Luffa acutangula* Lin. Var. *amara*. Roxb. is an important wild medicinal plant. Plants have been used in traditional medicine for several years (Abu Rabia, 2005). This whole plant is used to curemany diseases, especially in Anti-cancer, Anti-diabetic, Anti-jaundice and many ailments. The seed oil is also known for curing serious skin diseases and prevention of other skin ailments. All parts of the plants are a crystalline bitter principal similar compound to Cucurbitacin B and Amarinin (Despande*et al*., 2001). In addition to the Luffin Colocynthin is also present. The plant possesses Laxative and Purgative property and ethno medico survey of hilly areas in Maharashtra revealed that fruits of *L. acutangula* Var. *amara*. (Roxb) are used in protection from Jaundice when taken in the form of very fine powder through nose (Badgujar *et al*., 2008) while the seeds posses emetic, expectorant, and demulcent property (Kirtikar *et al*, 1987).

The fruit shows presence of Cucurbitacin B and E and Oleanalic acid (Rastogi *et al*., 2001). It is tonic to Intestine Cures Vata, Kapha and anemia. In addition, the cucurbitacins have received great deal of attention because of their cytotoxic and Anti-cancer activities (Chan *et al*.2005; Atta-ur-Rahman, 2005). It is also cure Anti-diabetic, Asthma, Jaundice, Leucoderma and also Tumors. Scientifically it is proved as CNS Depressant activity. In India, drops of liquid from the leaves and fruit of *L. acutangula* Var. *amara*. (Roxb) a wild variety, are used to treat Jaundice (Samvatsar and Diwanji, 2000). It is also possess in Anti-Oxidant and larvicidal activity.

The plant contains β-carotenes, Flavonoid acutosides A-G, Oleanane type of Triterpene, Saponins, acutosides H and I, Oleanalic acid and Saponins. In the present study, callus induction of the *Luffa* from the leaf and inter nodal explants has been investigated with different concentrations of cytokinin considering various parameters.

2. Abbreviations of medium:

- **MS -** Murashige and Skoog (1962) basal medium.
- **BAP -** Benzyl Amino Purine.
- **Kin**– Kinetin.
- **NAA –** Naphthalene Acetic acid.
- **IAA –** Indole-3-Acetic acid.
- **IBA -** Indole-3-Butyric acid
- **2, 4 – D –** Dichlorophenoxy acetic acid
- **TDZ –**Thidiazuron

3. Materials and Methods

Young leaf, Petiole, and inter node of *Luffa acutangula* Lin. Var. *amara*. Roxb. were collected from the mature plant growing in the green house at Bharathidasan University campus, Tiruchirappalli, Tamil Nadu, India.

4. Surface Sterilization

Surface sterilization process is to be followed in various steps so as to avoid contamination in the culture condition. Explants were kept under running tap water for 5 min to remove any soil particles adhering. After this method, 1-2 drops of 0.2% Teepol (Central Drug House, India) was added and kept under running water for 10-15 min. to remove the microbial contamination and rinsed three to four

times with distilled water. After washing procedures, explants were surface sterilized with mercuric chloride ($HgCl_2$) aqueous (w/v) for 1-2 min, followed by (0.1%) Bavistein ($C_9H_9N_3O_2$) aqueous solution (w/v) for 1 or 2 min, subsequently with 70% ethanol for 30 sec after rinsing followed by repeated rinsing 3-4 times with sterile distilled water.

The surface sterilized explants were then ready for inoculation on solid MS basal media supplemented with different growth regulators for callus induction. While for direct organogenesis (shooting) leaf and inter nodal explants were placed on different concentration/combinations of BAP, NAA, and Kinetin. Whereas for indirect organogenesis, primary calli were transferred on regeneration medium after four weeks of callus initiation. All the cultures were kept in a cooled incubator with 16 hrs light cycle in every 24 hrs with temperature at 26 ± 1^0 C. Shoots emerged were separated from callus was removed and planted again on full and half MS medium containing different concentrations of cytokinins for shoot initiation.

5. Media and Culture Condition

The Basal medium consist of MS salts with 0.5% of sucrose (w/v) and solidifying agents with 0.8 % (w/v) agar for the standardization of the callus.

The MS medium with 0.5% of sucrose (w/v) and solidifying agents with 0.8 % (w/v) Agar. 2, 4 – D + TDZ 0.5 to 2.5 mg/Lcallus responses.

The MS medium with 0.5% of sucrose (w/v) and solidifying agents with 0.8 % (w/v) Agar. Kin, 2, 4 – D, BAP, TDZ, GA_3 – 0.5 – 3.5 mg/L Shooting responses.

The MS medium with 0.5 % of sucrose (w/v) and solidifying agents with 0.8% (w/v) Agar. BAP + Kin, BAP + TDZ – 0.5 – 3.0 mg/L Multiple Shoot responses.

The MS medium with 0.5 % of sucrose (w/v) and solidifying agents with 0.8% (w/v) Agar. IAA & IBA 2.0 mg/L combination in Root responses.

6. Callus Induction

The leaf and internodal explants measuring 2 - 5 mm were inoculated to the culture tubes containing MS Basal medium supplemented with hormones like BAP, TDZ and 2, 4 - D. Well grown callus induced from the explants were transferred to the MS hormonal media and subculture every 20 days.

7. Data Collection

Data's were taken 10 - 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 colour of the callus to different concentration of plant growth regulators were recorded.

8. Results and Discussion

After Sterilization the explants were cultured on MS basal medium supplemented with 0.8% agar (w/v) and 0.5%Sucrose (w/v). In basal medium, callus growthwas very slow and approximately 20% responsewas observed after **45** days.

9. Callogenesis

Callogenesis response from different explants were is depending upon morphological characteristics of explant and type concentration of PGPR supplemented in MS medium TDZ & 2, 4-D 1.5 mg/L. The callus induction produced maximum callus from all to explants (Leaf & Stem) but failed produced callus at low level (0.1 & 0.5 mg/L), however leaf explant showed maximum callus percentage callogenic response then other two explants leaf stem and cotyledons, when tested MS medium supplemented with BAP, NAA, TDZ & 2, 4 - D.

The callus produced at these PGRs concentrations including TDZ & 2, 4-D 1.5 mg/L were different structure and morphology depending upon nature of calli PGR at TDZ & 2, 4-D 1.5 mg/L all concentration the callus was greenish and yellowish green but compact and friable. While Thiruvengadam *et al* (2006). Started that MS medium containing 1.0 mg/L approximately 90% of leaf explants of *Momordica charantia* L. gave rise to well organized friable calli, at different concentrations of BAPand Kin green, compact and yellowish friable callus produced. These calluses turned to be embriyogenic under the stress of PGR. Reported that Berg *et al* (1997) BAP as whole plant growth regulator, successfully preferred for good structure of callus development. At different concentration of NAA, the calli produced where soft and yellowish green to green in colour. These callus also turned soft after four weeks, best callogenic response was observed at different concentration of TDZ & 2, 4 – D – 1.5 mg/L in combination but callogenic response was good from the explants of the calli produced soft and light green friabledue to effect of cytokinin and auxin cumlilativly. Nabi *et al* (2002). Found that teasel gourd (*Memordica diocia*Roxb) callogenesis combination of TDZ & 2, 4 – D 1.5(mgL^{-1}), BAP & Kinwas most suitable that produced soft, light green and friable calli.

At the other combination and concentration structure, morphology and colour of calluses varied depending on PGR supplemented in MS medium. However any explant failed to show callogenic response at hormone free MS medium.

10. Indirect Shooting Response

Indirect shooting response is depending upon concentration of cytokinin supplemented in the MS medium. Cytokinin work as signaling molecules that activate totipotency cells of callus for shoot organogenesis where as in the case of direct organogenesis. These molecules activate pre exciting, machinery in the case of somatic cells (Stem & Leaf). While in the case shoot apex they stimulate, the growth due to the presence of meristamatic cells at the tip of explants. A few

combinations (BAP & Kin) only leaves were formed that were lush green in colour at all concentration tested and the callus turned soft and embriyogenic but failed to produce shoot. Manye *et al* (2004). Found that process of callus differentiating adventitious buds, the kind of proportion and quantity of phyto hormones and the type of callus may different result in*Memordica diocia* L. The obtained adventitious buds from yellowish green callus on MS medium with BAP and Kinetin. However they observed very low differentiation form yellow or green callus.

11. Indirect Rooting Response

The shoot tip explants shows best shooting responses BAP 2.0 and BAP 2.5 mg/L supplemented in MS medium where average number of shoots per tube **4.85 ± 0.40** in which shoots attained maximum length of shoots **17.42 ± 0.42** cm. BAP & GA_3 is also exhibited good shooting response with the average number of shoots **3.71 ± 0.35** and length **5.00 ± 0.46cm**. however at BAP alone shooting responses was high at BAP some findings Pierik (1887) who started that cytokinin is often used to stimulate growth and development, BAP and GA_3being in common use. They usually promote cell division, especially if added together with an auxin. At higher concentration they can induced adventitious shoot formation by decreasing apical dominance and they retard aging. Cotyledonary node explants showed comparatively low response on GA_3 hormonal combination giving maximum regeneration frequency **65%** that was **80%** in the case shoot tip explants.

Best rooting response from generated shoots were observed at half strength of MS medium supplemented with IAA & IBA 2.0 mg/L^{-1} with **4.28 ± 0.42** cm average number of giving **95%** response and half strength MS hormonal growth regulator 70% rooting response. While at full strength MS medium IAA & IBA mgL^{-1} gave maximum response **1.28 ± 0.18** cm average roots per tube and shoot length was observed in **10.28 ± 1.12 c**m. Some results were also observed by Agarwal and Kamal (2004) on shoot differentiation in *Memordica diocia* L when alone BAP alone was used. They also obtained good results on NAA in combination with IBA. Similar results were observed by Sulthana and Beri (2003).

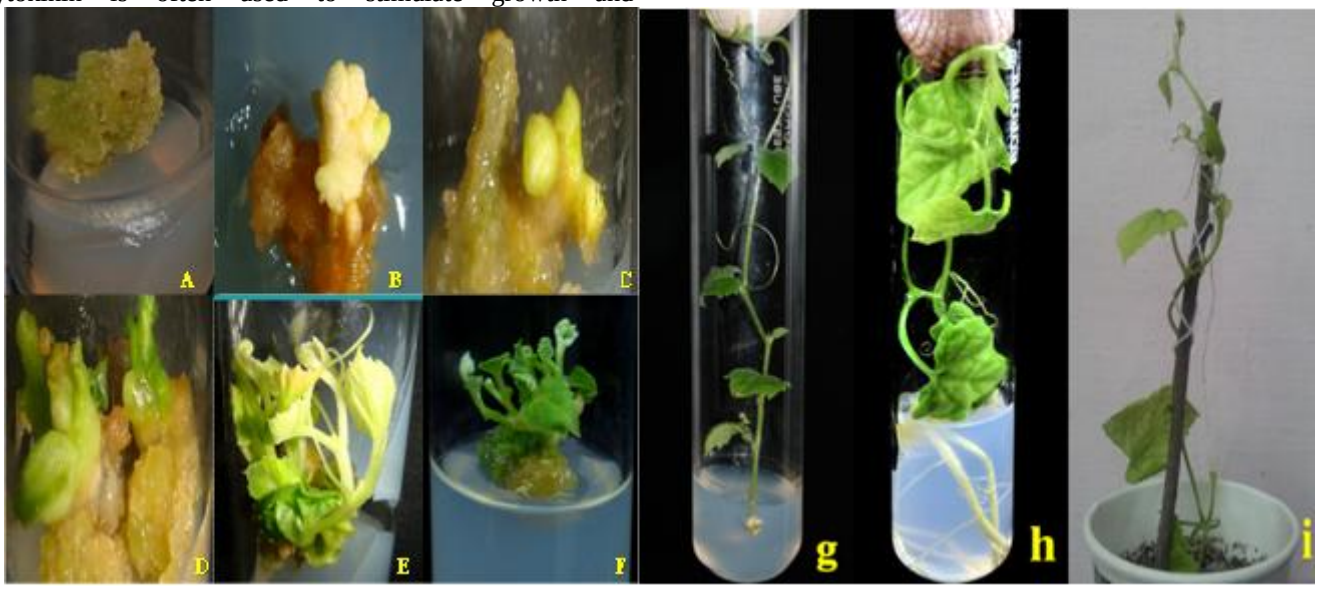

Figure 1: (a) Callus Induction (b) Shoot Initiation from Callus (c) Shoot Formation from Callus (d) Multiple Shoot Initiation (e) Multiple Shoot Formation (f) Multiple Shoot (g) Separated from Shoot (h) Root Formation (i) Hardening Plant

Table 1: Effect of different concentrations and combinations of growth regulators on Callus Induction from inter nodal explants of *Luffa amara* on 2, 4 – D + TDZ Hormones.

	Inter Node		
S. No	**Concentration of Media (MS) Mg/ML**	**Percentage of Callus %**	**Nature of Color's**
1	2, 4 – D + TDZ - 0.5		Whitish Friable Callus
2	2, 4 – D + TDZ - 0.5		Whitish Fragile Callus
3	2, 4 – D + TDZ - 0.5		Whitish yellow Fragile Callus
4	2, 4 – D + TDZ - 0.5	85%	Whitish green Friable Callus
5	2, 4 – D + TDZ - 0.5		Whitish yellow Fragile Callus
6	2, 4 – D + TDZ - 0.5		Whitish Friable Callus
7	2, 4 – D + TDZ – 1.0		Whitish green Friable Callus
8	2, 4 – D + TDZ - 1.0		Whitish green Friable Callus
9	2, 4 – D + TDZ - 1.0		Whitish green Friable Callus

10	2, 4 – D + TDZ - 1.0	90%	Whitish yellow Fragile Callus
11	2, 4 – D + TDZ - 1.0		Whitish yellow Fragile Callus
12	2, 4 – D + TDZ - 1.0		Whitish green Friable Callus
13	2, 4 – D + TDZ – 1.5		Whitish green Friable Callus
14	2, 4 – D + TDZ - 1.5		Whitish green Friable Callus
15	2, 4 – D + TDZ - 1.5		Whitish Fragile Callus
16	2, 4 – D + TDZ - 1.5	100%	Whitish Friable Callus
17	2, 4 – D + TDZ - 1.5		Whitish yellow Fragile Callus
18	2, 4 – D + TDZ – 1.5		Whitish yellow Fragile Callus
19	2, 4 – D + TDZ - 2.0		Whitish green Fragile Callus
20	2, 4 – D + TDZ - 2.0		Whitish green Fragile Callus
21	2, 4 – D + TDZ - 2.0		Whitish yellow Fragile Callus
22	2, 4 – D + TDZ - 2.0		Whitish yellow Fragile Callus
23	2, 4 – D + TDZ – 2.0	100%	Whitish green Friable Callus
24	2, 4 – D + TDZ - 2.0		Whitish green Friable Callus
25	2, 4 – D + TDZ - 2.5		Whitish green Friable Callus
26	2, 4 – D + TDZ - 2.5		Whitish green Fragile Callus
27	2, 4 – D + TDZ - 2.5	100%	Whitish yellow Fragile Callus
28	2, 4 – D + TDZ - 2.5		Whitish yellow Fragile Callus
29	2, 4 – D + TDZ - 2.5		Whitish yellow Fragile Callus
30	2, 4 – D + TDZ - 2.5		Whitish yellow Fragile Callus

Table 2: Effect of different concentrations and combinations of growth regulators on Shoot induction from inter nodal explants of *Luffa amara*.

	Inter Node			
S. No	**Hormone Concentration(µM/L)**	**Percentage of Culture Response**	**Maximum No. of Shoots/Explants(X±S.E)**	**Shoot length in Cm (X±S.E)**
1.	0.5	26 %	0.42±0.20	2.57±0.29
2.	1.0	46 %	0.60±0.20	2.71±0.42
3.	1.5	86 %	0.85±0.14	3.00±0.30
4.	Kin2.0	80 %	1.57±0.20	3.42±0.42
5.	2.5	58 %	1.57±0.20	3.28±0.28
6.	3.0	72 %	1.28±0.18	2.71±0.35
7.	3.5	72 %	1.78±0.28	3.14±0.40
8.	0.5	57 %	1.78±0.28	3.71±0.35
9.	1.0	86 %	1.28±0.28	4.14±0.40
10.	1.5	71 %	1.40±0.20	3.71±0.42
11.	2, 4 – D 2.0	72 %	1.40±0.20	3.57±0.36
12.	2.5	72 %	1.57±0.20	3.57±0.29
13.	3.0	70 %	1.71±0.18	3.57±0.48
14.	3.5	71 %	1.71±0.18	3.28±0.42
15.	0.5	72 %	2.00±0.30	6.00±0.37
16.	1.0	72 %	3.42±0.42	7.57±0.48
17.	1.5	86 %	3.71±0.35	12.28±0.83
18.	BAP 2.0	100 %	4.85±0.40	17.28±0.42
19.	2.5	86 %	1.28±0.18	13.71±0.52
20.	3.0	72 %	1.14±0.14	14.42±0.48
21.	3.5	71 %	1.28±0.18	12.85±0.34
22.	0.5	45 %	1.14±0.14	9.14±0.98
23.	1.0	50 %	1.42±0.20	5.14±0.63
24.	1.5	65 %	1.14±0.14	3.00±0.53
25.	TDZ 2.0	72 %	1.57±0.20	3.57±0.42
26.	2.5	71 %	1.14±0.14	3.57±0.36
27.	3.0	71 %	1.28±0.18	3.42±0.57
28.	3.5	72 %	1.14±0.14	4.14±0.40
29.	0.5	29 %	1.85±0.26	4.71±0.42

30.	1.0	48 %	1.28±0.18	4.71±0.28
31.	1.5	57 %	1.28±0.18	5.00±0.46
32.	GA_3 2.0	68 %	1.14±0.14	4.14±0.34
33.	2.5	70 %	1.14±0.14	4.14±0.40
34.	3.0	72 %	1.28±0.18	3.85±0.73
35.	3.5	71 %	1.14±0.14	3.57±0.36

Table 3: Effect of different concentrations and combinations of growth regulators on Multiple Shoot Induction from inter nodal explants of *Luffa amara*.

Inter Node				
S. No	Hormone Concentration(µM/L)	Maximum No. of Shoots/Explants(x±S.E)	Percentage of Culture Response %	Shoot length in Cm (x±S.E)
1.	0.5	1.57±0.20	31%	1.14±0.14
2.	1.0	2.57±0.20	45%	1.57±0.20
3.	BAP + Kin 1.5	2.42±0.20	52%	1.85±0.26
4.	2.0	5.37±0.32	90%	1.57±0.20
5.	2.5	2.85±0.26	85%	1.85±0.34
6.	3.0	1.85±0.26	70%	1.71±0.28
7.	0.5	2.71±0.28	29%	1.57±0.20
8.	1.0	2.42±0.20	35%	2.28±0.28
9.	BAP + TDZ 1.5	2.42±0.20	48%	2.14±0.34
10.	2.0	2.42±0.20	78%	2.00±0.30
11.	2.5	2.00±0.30	70%	2.00±0.30
12.	3.0	1.85±0.34	70%	2.00±0.30

Table: 4. Effect of different concentrations and combinations of growth regulators on Root Induction from Inter Nodal explants of *Luffa amara*.

	Inter Node			
S. No.	*Hormone Concentration (µM/L)*	*Percentage of Culture Response%*	*Maximum No. of roots/Explants (X±S.E)*	*Root length in Cm (X±S.E)*
1	0.5	43%	1.28±0.18	1.57±0.20
2	1	54%	1.71±0.28	1.85±0.26
3	IAA 1.5	57%	1.57±0.20	2.57±0.20
4	2	82%	2.00±0.30	2.28±0.28
5	2.5	71%	2.00±0.30	2.42±0.29
6	3	72%	2.00±0.37	3.00±0.30
7	0.5	44%	2.28±0.28	2.85±0.26
8	1	60%	2.57±0.29	2.57±0.29
9	IBA 1.5	68%	2.28±0.42	2.85±0.26
10	2	85%	2.42±0.29	2.85±0.26
11	2.5	82%	2.71±0.42	3.00±0.30
12	3	76%	2.71±0.42	2.85±0.34
13	0.5	40%	3.00±0.37	3.00±0.37
14	1	49%	2.71±0.46	2.71±0.42
15	NAA 1.5	56%	2.14±0.34	2.14±0.34
16	2	84%	2.42±0.36	2.42±0.36
17	2.5	78%	2.85±0.34	2.85±0.30
18	3	70%	2.57±0.36	2.57±0.36
19	0.5	54%	2.71±0.28	2.71±0.28
20	1	54%	2.00±0.30	3.00±0.30
21	IAA + IBA 1.5	65%	3.14±0.50	4.28±0.28
22	2	95%	4.28±0.42	10.28±1.12
23	2.5	80%	4.28±0.42	5.00±0.30
24	3	76%	5.00±0.30	3.57±0.36

12. Conclusion

In this present study, an effective *in vitro*shoots and root regeneration protocol was achieved using internodal explants of *L. amara* via indirect organogenesis. The higher percentage callus induction in MS medium was observed in the concentration of TDZ + 2, 4 – D - 1.5 mg/$L^{-1}$and 2.0mg/L. BAP is induced adventitious shoots from organogenic callus. About **4.85 ± 0.40** shoots were produced per tube from internodal explants. Additionally, the effectiveness of a combined rooting and hardening protocol for producing shoots, capable of high survival in field sites has been demonstrated. This protocol proves the targets for conservation strategy and for future phyotomedicine production. In our knowledge, the present study of regeneration system could be used in the production of more plantlets.

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