



***IN VITRO* PROPAGATION FROM LEAF EXPLANTS OF SPONGE GOURD (*LUFFA AEGYPTIACA* (MILL.) A MEDICINALLY IMPORTANT PLANT**

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Abstract

Luffa aegyptiaca Mill is important plant of family Cucurbitaceae, which have not been much investigated for their *in vitro* cultivation and propagation of multiple shoots. In the present investigation shoots propagation initiation was reported after 21-30 days of leaf culture, In *Vivo* Leaf explants were cultured on MS medium containing various concentrations of cytokinins TDZ/BAP/Kn (0.5-2.5mg/L) alone and also in combination with auxins IAA (0.5 mg/L). Maximum number of shoot bud proliferation was observed at (0.5 mg/L) IAA + (1.5 mg/L).TDZ, compared to all other concentrations of Kn/BAP alone. As the concentration was increased above (2.0mg/L) the shoot bud induction was reduced gradually in both the cytokinins tested. Maximum and High number of shoot bud's proliferation was observed at (1.5 mg/L) TDZ, compared to all other concentrations of BAP/Kn alone. As the concentration was increased above (2.0mg/L) and below (1.5mg/L) the shoot bud induction was reduced gradually in both the cytokinins tested. The concentric effect of cytokinin was found to be effective in inducing maximum number of shoots. Maximum frequency of shoot buds was induced at (0.5 mg/L) IAA (1.5mg/L) TDZ/BAP/Kn. The *in vitro* regenerated shoots produced a greater number of roots on half strength MS medium containing (1.0mg/L) IBA. Thus, the plant developed *in vitro* using leaf explants cultures were established in pots containing garden soil outside under shade in wound temperature and light conditions. These plants flowered after 8 weeks following transfer to pots. The protocol established can be used for rapid multiplication of the specific producing true to type plants.

Key words: *Luffa aegyptiaca* Leaf explants, multiple shoot buds, direct regeneration, Growth hormones

Introduction:

Luffa aegyptiaca (Mill), a synonym of *Luffa cylindrica* (Linn) M. Roem, is a unique vegetable member of the Cucurbitaceae family, native to Asia and widely cultivated in several tropical and sub-tropical regions worldwide for its economical, medicinal and nutritional uses (Lawal *et al.*, 2010). It is commonly named sponge gourd, vegetable sponge, bath sponge, dish cloth gourd and loofa (Oboh and Aluyor, 2009).

. There are two common species in the genus Lufa: Smooth Lufa (*Lufa aegyptiaca* Mill.), Also known as Sponge gourd and Angular Luffa (*Lufa acutangula* (L.) Roxb.). In the 16th century, Vestingius was called Lufa "Egyptian cucumber." Common English terms for angular luffa include Chinese okra, dishcloth flour, ribbed loofah, chopped loaf, sponge flour, silk louk, sink towel sponge (USDA 2016), strainer vine and vegetables.

It is a wild annual climber, which is monoecious and in the raceme inflorescence of the male flower, one female flower exists, which produces a large green cylindrical fruit called gourds, with spongy endocarp and about 30 flat and round black seeds have been used in the treatment of respiratory disorders (Indumathy *et al.*, 2011). Juice extracted from the stem and the seed has emetic action (Bailey, 1989). Ethanol and aqueous extract of different parts of *L. cylindrica* (L.) syn. *Luffa aegyptiaca* Mill., possess anti-inflammatory, analgesic, sedative (Muthumani *et al.*, 2010), antifungal (Parkash *et al.*, 2002), expectorant (Partap *et al.*, 2012) and antimicrobial (Indumathy *et al.*, 2011) properties It has been discovered that sponge gourd can supply some antioxidant constituent to human body (Oboh and Aluyor, 2009) and is a potential source of vegetable protein in human and animals (Dairo *et al.*, 2007). The seeds are used for extraction of industrial oil (Bal *et al.*, 2004), and its use as biodiesel is now gaining wide acceptance because of low CO₂ emission and other considerations (Ajiwe *et al.*, 2005).

Application of plant biotechnology tools for improvement of *L. aegyptiaca* is limited. So far, only two papers have reported micropropagation of *L. aegyptiaca* but number of plantlets produced was very low (Singh *et al.*, 2011; Nahar *et al.*, 2010). Recently, callus induction in leaf explants on BAP (1.5 mg/L) was reported (Shrivastava and Roy, 2012). Preliminary information on *Agrobacterium tumefaciens* mediated genetic transformation of *L. cylindrica* Roem was reported (Oboh and Aluyor, 2009).

The objective of the present study was to develop a rapid *in vitro* clonal propagation from leaf explants on MS + TDZ, BAP or KN and in combination with IAA.

MATERIALS AND METHODS:

Sponge gourd. (*Luffa aegyptiaca* Mill.) was collected from wild, Laxminayak Thanda (Village), Mahabubabad (District) (District-Geographical location, 18.0° N 79.58°E), Telangana (State), India. The seeds in the spongy endocarp were white as opposed to black in *L. aegyptiaca* the plant material was preserved as herbarium specimen in the Department of Botany, Govt Degree College Mahabubabad Affiliated Kakatiya University, Warangal and the authentication the plant material was performed by well-known taxonomist, Prof. V. S. Raju, Plant Systematics Laboratory, Department of Botany, Kakatiya University, Warangal, T. S.

The plant material was identified as *L. aegyptiaca* and an accession number 1868 was given to it. We planted the seeds of *L. cylindrica* (L.) 1868 during July 2011 and the vine, flower and fruits were morphologically similar to *L. cylindrica* (L.) syn. *Luffa aegyptiaca* Mill. We collected gourds with white seeds in January 2021 and used them for Leaf culture, studies (Figure 1).

Leaf (1 cm ca) explants were collected from 3-months old *L. cylindrica* (L.) 1868 and were washed with running tap water for 15 min and surface sterilized with 0.1% (w/v) mercuric chloride (HgCl_2) for 3 min, and finally rinsed with several changes of sterile distilled water. Murashige and Skoog (1962) (MS) medium supplemented with 3% sucrose (w/v) was adjusted to pH 5.8 with 1 N NaOH, solidified with 0.8% agar (w/v) and was autoclaved at 15 lbs for 20 min. Leaf and nodal explants were cultured on MS medium fortified with (0.5) to (2.5 mg/L) Benzylaminopurine (BAP), Kinetin (Kn) and Thidiazuron (TDZ), for shoot bud induction and individual micro-shoots were cultured on MS medium fortified with (1.0 mg/L) indole-3-butyric acid (IBA) for rooting. All cultures were maintained under white fluorescent light (80 μ EM-2 S1) at $25 \pm 2^\circ\text{C}$ under 16 h photoperiod. Complete plantlets were transferred to pots containing sterile soil and compost (1:1) for 2 weeks, and then transplanted to research field.

Data analysis 20 replicates were maintained for each treatment. Each treatment was repeated at least once with similar results. Data were recorded after eight weeks of culture.

RESULTS.

The role of cytokinin and Auxin- cytokinin combinations on direct plant regeneration and adventitious bud induction from leaf explants was studied in order to find out the efficient protocol and potential on MS medium fortified with different concentrations of cytokinins alone and auxin's (0.5mg/L) in combination with various concentrations of cytokinins such as BAP/Kn/TDZ (0.5-2.5 mg/L). These explants were enlarged 3-4 fold within one week of culture initiation. Morphogenic change as were apparent after 6 weeks of culture. The leaf explants developed shoot primordial in large numbers directly from all cut surfaces in contact with medium in all the concentrations and combinations of phytohormones used even in combination with (0.5 mg/L) IAA. The results are presented in Table (1-2).

Leaf explants cultured on MS medium containing various concentrations of cytokinins BAP/Kn/TDZ alone the direct shoot regeneration (Table-1) and (0.5 mg/L) IAA in combination with (1.0-2.50 mg/L) BAP/Kn/TDZ showed variable response in leaf explants culture of *L. aegyptiaca*. These explants were elongated 3-4 folds within one week of culture initiation. Morphogenic changes were apparent after 6 weeks of culture. The results are presented in (Table 1-2) and shown in (Fig-1).

Effect of BAP:

Leaf explants were cultured on MS medium amended with various concentrations of BAP (0.5-2.5 mg/L) as role growth regulators showed the direct organogenesis (Table -1) Maximum number of shoot bud proliferation (4.5 ± 0.43 shoots /explant) was found at (1.5 mg/L) BAP. At 2.0, and 2.5 mg/L BAP induced (2.6 ± 0.35 and 1.8 ± 0.35) shoots/explant with 55 and 50% of cultures responded. When the concentration was increased up to (2.0 mg/L) BAP gradually induction of multiple shoots were reduced. The number of shoot bud induction was

found to be decreased as the concentration of BAP increased. At high concentration of BAP showed less number of shoots per explants.

Effect of Kn:

Leaf explants were cultured on MS medium containing different concentrations of cytokinin Kn (0.5- 2.50 mg/L) as role growth regulators showed the direct organogenesis/ shoot formation (Table-1) to find out the difference between BAP and Kn in inducing the direct plant regeneration from leaf explants in *L. aegyptiaca*.. Maximum number of shoot bud proliferation (5.0 ± 0.65 shoots/ explant) was found at (1.5 mg/L) compared to all other concentrations of Kn at (0.5 and 1.0 mg/L) Induced (2.002 ± 0.25 and 3.0 ± 0.45 mg/L) shoots / explant with 40 and 45 percentage of cultures responded. Percentage of response was gradually increased up to (2.5 mg/L) Kn. At above (2.5 mg/L) concentration less number of shoots was recorded. However high induction of ability was found in all the concentrations of Kn compared to BAP.

Effect of TDZ:

TDZ was more responsive compared to BAP and Kn in inducing shoot buds from the explants (Table-1) with (0.5 mg/L) TDZ the leaf explants produced (4.65 ± 0.35 shoots/ explants and 65% cultures responded (1.5 mg/L) TDZ was more responsive in inducing maximum number of shoots (9.50 ± 0.35 shoots/ explants) with a greater frequency 75% TDZ at (2.0 and 2.5 mg/L) produced (7.50 ± 0.62 and 3.00 ± 0.54) shoots/ explants with 70% and 60% culture responding. As the concentrations of TDZ were increased from (1.5 mg/L to 2.5 mg/L) the number of shoots per explants was considerably reduced (Table-1).

Table – 1 Table 1. Effect of BAP, Kn and TDZ alone on induction of multiple shoot buds from leaf explants of *L. aegyptiaca* after 4 weeks of culture.

Hormone concentration (mg/L)	% of cultures responding	Average No. of shoots / Explants \pm (SE)*	Average length of shoots (cms) \pm (SE)*	
BAP	0.5	45	2.60 ± 0.42	0.80 ± 0.42
	1.0	50	3.40 ± 0.37	1.00 ± 0.54
	1.5	60	4.50 ± 0.43	2.60 ± 0.32
	2.0	55	2.60 ± 0.35	2.20 ± 0.42
	2.5	50	1.80 ± 0.35	1.70 ± 0.65
Kn	0.5	40	2.00 ± 0.25	0.80 ± 0.25
	1.0	45	3.00 ± 0.45	1.00 ± 0.34
	1.5	52	5.00 ± 0.65	2.20 ± 0.33
	2.0	55	2.50 ± 0.42	1.80 ± 0.22
	2.5	50	1.40 ± 0.24	0.95 ± 0.44
TDZ	0.5	65	4.65 ± 0.35	0.50 ± 0.22
	1.0	68	6.40 ± 0.55	0.80 ± 0.43
	1.5	75	9.50 ± 0.35	1.20 ± 0.42
	2.0	70	7.50 ± 0.62	0.90 ± 0.32
	2.5	60	3.00 ± 0.54	0.40 ± 0.25

* Mean \pm Standard Error

Effect of IAA + BAP:

To find out the influence of auxin-cytokinin combination on direct regeneration. The Leaf explants were cultured on MS medium fortified with (0.5 mg/L) IAA and different concentrations of cytokinin such as BAP (0.5 -2.5 mg/L). IAA in combination with various concentrations of BAP showed variable results (Table 2). Highest percentage of response was observed at (0.5 mg/L) IAA + (1.5 mg/L) BAP. The percentage of response and number of shoots proliferation was increased up to (0.5 mg/L) BAP and later gradually decreased at above (1.5 mg/L) BAP. At (0.5, and 1.0 mg/L) BAP with IAA (0.5mg/L) induced (3.20 ± 0 and 4.30 ± 0.27) shoots/explants with 47 and 55 percentage of cultures were recorded. At the concentrations of BAP was gradually increased above (1.5 mg/L) after wards decreased the number of shoots. (2.0 and 2.5 mg/L) BAP+ IAA (0.5 mg/L) induced (3.20 ± 0.25 and 2.60 ± 0.25) shoots/explants with 50 and 45 percentage of response was recorded. (Table 2).

Table –2 Effect of IAA in combination with BAP/Kn/TDZ on induction of shoots proliferation from Leaf explants cultures of *L. aegyptiaca* after 4 weeks of culture.

Hormone concentration (mg/L)		% of cultures responding	Average No. of shoots / Explants \pm (SE)*	Average length of shoots (cms) \pm (SE)*
IAA+ BAP	0.5 +0.5	47	3.20 ± 0.32	0.90 ± 0.42
	0.5 + 1.0	55	4.30 ± 0.27	1.20 ± 0.54
	0.5 + 1.5	58	5.30 ± 0.23	2.40 ± 0.32
	0.5 + 2.0	60	3.20 ± 0.25	2.60 ± 0.42
	0.5 + 2.5	44	2.60 ± 0.25	1.90 ± 0.65
IAA+ Kn	0.5 +0.5	45	2.40 ± 0.35	0.90 ± 0.25
	0.5 + 1.0	48	3.00 ± 0.25	1.20 ± 0.34
	0.5 + 1.5	57	4.00 ± 0.25	2.40 ± 0.33
	0.5 + 2.0	59	3.20 ± 0.42	1.90 ± 0.22
	0.5 + 2.5	55	2.20 ± 0.24	1.65 ± 0.44
IAA+ TDZ	0.5 +0.5	68	5.35 ± 0.35	0.80 ± 0.22
	0.5 + 1.0	70	6.20 ± 0.55	1.40 ± 0.43
	0.5 + 1.5	75	9.80 ± 0.45	1.40 ± 0.42
	0.5 + 2.0	50	6.40 ± 0.32	1.20 ± 0.32
	0.5 + 2.5	45	3.00 ± 0.54	0.50 ± 0.25

* Mean \pm Standard Error

Effect of IAA +Kn :

Leaf explants cultured on MS medium augmented with (0.5 mg/L) IAA and different concentrations of Kn (0.5-2.5 mg/L) Showed variable response was recorded (Table -2). Maximum shoot bud induction was recorded at (1.5 mg/L) (4.00 ± 0.25 shoots/ explants) with 57 percentage of response was recorded. At (0.5 and 1.0 mg/L) Kn + (0.5 mg/L) IAA induced. (2.4 ± 0.35 and 3.0 ± 0.25 shoots/ explants) with 45 and 48 percentage of cultures were recorded. When the Kn concentration was increased from (2.0 to 2.5 mg/L) it was found that the shoot regeneration and percentage of responses was decreased. (Table -2).

Effect of IAA +TDZ:

Leaf explants cultured on MS medium augmented with IAA (0.5 mg/L) and different concentrations of TDZ (0.5-2.5 mg/L) Showed variable response was recorded (Table -2). Maximum shoot bud proliferation was recorded at (1.5 mg/L) (9.80 ± 0.45 shoot buds/ explants) (Fig-b) with 75 percentage of response was recorded. At (0.5 and 1.0 mg/L) TDZ + (0.5 mg/L)



Fig-I In vitro plantlet regeneration in of *L. aegyptiaca* (L.); from Leaf explants a) Induction of multiple shoots on MS + BAP (1.5 mg/L); b) plant let regeneration on MS + IAA(0.5mg/L) + BAP (1.5 mg/L) c) Induction of multiple shoots on MS + TDZ (1.5 mg/L); d) Plant let regeneration on MS + IAA (0.5mg/L) and TDZ (1.0 mg/L).

IAA induced. (5.35 ± 0.35 and 6.20 ± 0.55 shoots/ explants) with 68 and 70 percentage of cultures were recorded. When the concentration of TDZ was increased from (2.0 to 2.5 mg/L) it was found that the shoot regeneration and percentage of responses was decreased (Table -2).

In vitro rooting fully elongated healthy shoots were transferred on to full strength MS root induction medium (RIM) (Murashige and Skoog 1962) fortified with different concentration of IAA (0.5 – 2.0 mg/L) and IBA (0.5 – 2.0 mg/L). Profuse rhizogenesis was observed on (1.5 mg/L IAA), compared to (0.5 -2.0 mg/L) IAA/ IBA on MS medium containing (1.5 mg/L) IBA whereas 96% of plants produced roots with (14.3 ± 0.27 roots/ explant).

Acclimatization Rooted plantlets were removed from the culture medium and the roots were washed under running tap water to remove agar. Then the plantlets were transferred to polypots containing pre- soaked vermiculite and maintained inside a growth chamber set at 28 °C and 70 – 80 % relative humidity. After three weeks they were transplanted to poly bags containing mixture of soil + s and + manure in 1: 1: 1 ratio and kept under shade house for a period of three weeks. The potted plantlets were irrigated with Hogland's solution every 3 days for a period of 3 weeks.

DISCUSSION:

We were successful in direct regenerating plants from leaf explants of *L. aegyptiaca* cultures on MS medium fortified with different concentrations of cytokinins i.e. BAP/Kn/ TDZ (0.5-2.5 mg/L) individually and also in combination with (0.5 mg/L) IAA. Maximum number of shoot buds was induced at (1.5 mg/L) TDZ in comparison to Kn/BAP as role growth regulators. When the low level of auxin (0.5 mg/L) IAA were added to the medium containing BAP/Kn/TDZ. It was interesting find out that the shoots induction was enhanced in all the concentrations of cytokinins. However the shoot bud proliferation was found to more on (0.5 mg/L) IAA in combination with TDZ compared to (0.5 mg/L) in combination with Kn/BAP but the combination of IAA+TDZ induced highest number of plantlet regeneration among all hormonal combinations and concentrations were used in *L. aegyptiaca*.

In our study, TDZ induced more shoots (number) in leaf explants as compared to BAP or Kn. Thidiazuron (TDZ), a substitute of phenyl urea (N-phenyl-1,2,3-thidiazol-5-ylurea) is a potent cytokinin used in *in vitro* shoot induction experiments and its efficiency in inducing more number of *in vitro* shoots than Kn or BAP has been reported in other cucurbits like *Cucurbita pepo* (Pal *et al.*, 2007), *Melothria maderaspatana* (Baskaran *et al.*, 2009) and *Citrullus colosynthis* (Savitha *et al.*, 2010). Its mode of action maybe to counter the action of cytokinin oxidase, which in turn may modulate the level of endogenous cytokinin (Hare and Van Staden, 1994) or varied translocation rates to the meristematic region and metabolic processes, in which cytokinin may be degraded or get conjugated with sugars or amino acids to form biologically inert compounds (Kaminek, 1992). Another observation for TDZ was, the length of shoot was lesser than those induced by BAP or Kn. Inclusion of TDZ reduced shoot length resulting to miniature shoots in red ginger (Hamirah *et al.*, 2010) and Korarima (Tefera and Wannakrairoj, 2006).

For the first time, we used TDZ in tissue culture of *L. aegyptiaca* and observed that it is more efficient than BAP or Kn in inducing more number of shoots. TDZ (0.5 mg/L) induced 12 shoots in leaf explants respectively as compared to 5 shoots on BAP 1.5 mg/L (Nahar *et al.*, 2010).

CONCLUSIONS:

Research work has mainly been focused on the development of regeneration protocol, somaclonal variations and their physiological as well as morphological aspects in *L. aegyptiaca* Plant. An efficient plant regeneration protocol is a pre-requisite for the exploitation of various biotechnological techniques. However, practical utility of the basic protocol is still far away. It can serve as a platform for the transfer of economically important traits through genetic engineering, inducing somaclonal variations, in vitro mutations, double-haploids induction, development and utilization of somatic hybrids, determining herbicide or pesticide tolerance limits in *L. aegyptiaca* Plant. Therefore, a remarkable progress can be made in *L. aegyptiaca* improvement through the combination of conventional and biotechnological approaches.

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