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In Vitro Root Culture From Roots And Leaf Explants Of Luffa Acutangula(L.) Roxb

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ABSTRACT: An efficient protocol had been developed for the root culture of *Luffa acutangula* (L.) Roxb. The root and leaf explants from 21 day old *in vitro* raised seedlings were cultured on half strength Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of Indole-3-acetic acid(IAA), Indole-3-butyric acid (IBA) and Napthalene acetic acid (NAA). IBA at 1.0 mgl^{-1} combined with NAA (1.0 mgl^{-1}) induced maximum percentage of rooting from leaf explants, under total dark condition. After 3 weeks, well established roots were separated. Fresh root tissue from the leaf explants and root tips from *in vitro* seedlings were subcultured in half strength MS liquid medium supplemented with different concentrations and combinations of IAA, IBA and NAA, under continuous agitation at 80 rpm under total dark condition. The biomass of root culture (derived from leaf explants) in the MS medium fortified with IBA(1.0 mgl^{-1}), NAA(1.0mgl^{-1}) and L-glutamine (20 mgl^{-1}) was increased to 5.38 g FW and the biomass of root culture (derived from root tip) in the MS medium supplemented with IBA (0.5 mgl^{-1}), NAA (1.0 mgl^{-1}) and L- glutamine (20 mgl^{-1}) was increased to 5.82 g FW after 7 weeks of culture.

KEYWORDS: Adventitious root culture, biomass, Subculture, *Luffa acutangula*.

I. INTRODUCTION

Luffa acutangula (L.) Roxb. is commonly called as 'Ridge gourd' or 'angled loofah' for its unripe fruits which are used as vegetable. *Luffa* is nutritionally rich, containing good amount of fibre, amino acids, vitamins and minerals like calcium, potassium, magnesium, zinc and small quantities of iodine (Badgujar and Patil, 2008). GC-MS studies revealed the presence of compounds which are active against inflammation and these compounds may be effective in treating auto immune diseases like Rheumatoid arthritis (Anitha & Miruthula, 2014). Luffin, a ribosome inactivating protein isolated from this plant is used to prepare immunotoxins by conjugation to mono clonal antibodies. It is also reported to possess antiviral properties (Au et al., 2000).Other than these therapeutic values, the plant possess analgesic activity and antioxidant potential (Naresh Singh Gill *et al.*, 2011), hypoglycemic activity (Jural *et al.*, 2008) and hepato protective activity (Jadhav *et al.*, 2010).

The *in vitro* production of medicinal compounds can be possible through plant cell culture under controlled conditions and free from environmental fluctuations (RamaChandraRao and Ravi Shankar, 2002). Even though various organogenic cultures are used to propagate plants, the *in vitro* root culture is highly advantageous method for the production of secondary metabolites of pharmaceutical interest, since it is relatively easy to maintain and manipulate (Sivanandhan et al., 2011).

For adventitious root induction, various plant materials like *in vitro* root, leaf and *in vivo* leaf, root as explants have been studied in the following plants: *In vitro* roots; *Astragalus membranaceus* (Wu *et al.*, 2011); *Stevia rebaundiana* (Reis *et al.*, 2011) and *Eleutherococcus koreanum* (Lee and Pack *et al.*, 2012). *In vitro* leaf explants in; *Chicorium intybus* (Nandagopal and kumari, 2007); *Orthosiphon stamineus* (Ling *et al.*, 2009); *Plumbago zeylanica* (Sivanesan and Jeong, 2009); *Periploca sepium* (Zhang *et al.*, 2011) and *Withania somnifera* (Sivanandhan *et al.*, 2012). In vivo leaf as explants in *Centella asiatica* (Mercy *et al.*, 2011), *Morus alba* (Lee *et al.*,2011) and *Aloe vera* (Lee *et al.*, 2011). *In vivo* roots as explants in *Panax ginseng* (Sivakumar *et al.*, 2005; Paek *et al.*, 2009)

Despite various medicinal uses of *Luffa*, root culture has not been reported previously till date. Therefore the objective of the current study is to develop an efficient and reproducible protocol for *in vitro* regeneration of

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adventitious roots from *in vitro* leaf explants and in vitro root explants for this important plant. To our knowledge this is the first report on influence of exogenous hormones on adventitious root culture of *Luffa acutangula.*

II. LITERATURE SURVEY

Direct regeneration protocols has been reported in *Luffa acutangula (L.)* Roxb. by using nodal explants (Partha sarathi and Biswajit Ghosh, 2014). Efficient regeneration system has been reported in Ridge gourd from immature embryo and cotyledon explants (Zohura *et al.,* 2013). Sujatha *et al.,*2013 reported that indirect organogenesis and genetic transformation has been successful by using leaf and nodal explants. There was no report on invitro adventitious root culture in cucurbitaceae, hence the present study was to establish an efficient root culture protocol from roots and leaf explants of *Luffa acutangula (L.)* Roxb.

III.MATERIALS AND METHODS

3.Explant source and preparation:

3.1) Explants collection from *in vitro* plants

The seeds of *Luffa acutangula* (L).Roxb. procured from Mahyco(Maharashtra Hybrid Seed Company ,Maharashtra,India) were used to obtain the leaf and root explants which were used for the present study. After removal of the seed coat, the seeds were surface sterilized by washing in 3-5 drops of Teepol (commercial bleaching solution) and 2% (w/v) Bavistin (fungal disinfectant) each for 5 minutes, then rinsed with distilled water separately for 3-4 times each. The rinsed seeds were surface sterilized in 0.1% mercuric chloride solution for 3 minutes and rinsed with sterile water 3-4 times in the laminar chamber and it was air dried in sterile tissue paper. Seeds were then placed on basal Murashige Skoog (MS) medium with 3% sucrose and agar (0.8%) in test tubes. The tubes were incubated in dark initially for 48 hours and after transferred to light condition with a 16-h photoperiod (50µmol $m^{-2}s^{-1}$) provided by cool white fluorescent lamp.

The normal root and leaf were excised from 21-day old *in vitro* aseptic seedlings. The root tips (inoculum), (0.5gm) and leaves (1cm, mid nerved portion) detached from the *in vitro* plants using sterile blades, which were used as explants.

3.2 *) Adventitious root induction from root explants:*

Root tip (inoculums, 0.5 gm), explants from 21 day old *in vitro* seedling of *Luffa acutangula(*L.)Roxb. were cultured in 50 ml half strength MS basal liquid medium supplemented with 3% sucrose and various concentrations of auxins namely napthalene acetic acid (NAA), indole butyric acid (IBA) and indole acetic acid (IAA) either separately (each 0.2$mg1^{-1}$ to 2.0$mg1^{-1}$) or in combinations of IBA with NAA (0.5$mgl^{-1}$and 0.2mgl^{-1} to 2.0mgl^{-1} respectively) along with L-Glutamine (20 mgl^{-1}) in 250 ml Erlenmeyer flasks for attaining highest adventitious root number, length and biomass. All the cultures were incubated under total darkness at 25 ± 2^0c with continuous agitation at 80 rpm in rotary shaker. At the end of every 20-25 days, the roots were subcultured into 250 ml flask containing the above mentioned concentrations of NAA and IBA either separately or in combination under total darkness at 80 rpm. Atleast three subcultures were done. Then, adventitious roots were harvested after 7 weeks.

3.3) From leaf explants:

The leaf explants (length, 15-30mm and width, 20-24mm) from 21 day old *in vitro* seedlings were inoculated on MS medium (Murashige and Skoog ,1962) with 3% sucrose (Himedia, India) 0.8%(w/v) agar fortified with NAA (1.0mgl^{-1}) and IBA(0.5mgl^{-1}) separately in test tubes. Leaf explants were placed in the culture tube with their abaxial surface touching the medium. The p^H of the medium was adjusted at 5.8 with 0.1N NaOH or 0.1N HCl before autoclaving and cultured at 25^0C in the dark for adventitious root proliferation. After three weeks, the adventitious roots were individually cut to a length of approximately 2 cm and rinsed in sterile water to remove the sticking agar gel and cultured in 250 ml flask containing 100 ml half strength MS liquid medium supplemented with 3% sucrose and various concentrations of NAA , IBA and IAA either separately (each 0.2 $mgl^{-1}$to 2.0 $mgl^{-1)}$ or in combination of IBA with NAA (0.5 $mgl^{-1}$and 0.2 mgl^{-1} to2.0 mgl^{-1} respectively) along with L-glutamine(20 mgl^{-1}). The culture tubes were maintained at 25 ± 2^0C in the dark on a rotary shaker at 80 rpm. At the end of every two weeks, the roots were

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subcultured to fresh medium containing the same optimal concentration of hormones. Then, adventitious roots were harvested after 7 weeks.

3.4) Determination of Biomass:

The cultured adventitious roots were collected from the media and the fresh weight (FW) was measured after rinsing with sterile water and blotting away surface water. The adventitious root dry weight (DW) was recorded after roots were dried constantly at 60 ^{0}C in oven for two days. The determination of biomass / growth ratio was calculated according to Siva kumar *et al.*, (2005) and Ahmed *et al.*, (2008) as follow:

$$\text{The Growth Rate} = \frac{\text{The harvested dry weight} - \text{The inoculated dry weight}}{\text{The inoculated dry weight}}$$

F/D ratio=Fresh weight/Dry weight.

3.5) Statistical analysis:

The percentage of response, root induction frequency, number of roots, root length, fresh and dry weight of roots from root and leaf explants, were monitored as growth parameters. Data of three independent experiments represented by 10 replicates from each experiment were subjected to statistical analysis (Mean±SE) and Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1976) using SPSS software, version11.5.

IV.RESULTS AND DISCUSSION

4.1 ***Adventitious root induction and proliferation from in vitro root explants:***

a. Effect of individual auxin treatment on in vitro roots on primary adventitious root induction:

Different types and concentrations of auxins along with L-glutamine (20 mgl^{-1}) were tested for primary adventitious root induction. Among the different concentrations of NAA, IBA and IAA tested individually, 1.0 mgl^{-1}NAA and 0.5 mgl^{-1} IBA were most effective for primary adventitious root induction from root explants (Table-1).The percentage of rooting response while using NAA , IBA and IAA individually was 78%, 88% and 60% respectively with the production of 12.40,14.20 and 9.00 roots per explants respectively, after 15-days of culture (initial culture) (Table1). The root induction was visible as thick, greenish yellow long and matty appearance. The explants when cultured continuously (15 days) in the same MS liquid medium containing the growth regulators neither enhanced root number nor resulted in initiation of additional roots. Hence sub culture of explants along with emerging roots was performed in fresh MS liquid medium containing the same growth regulators as in initial culture. After one subculture with a duration of 20 to 25 days in the medium supplemented with NAA (1.0 mgl^{-1}) resulted in 15.00 roots /explant with 5.82 cm mean root length, 3.62 gm FW and 1.08 DW, in the medium supplemented with IBA (0.5 mgl^{-1}) resulted in17.20 roots/explant with 6.04 cm mean root length, 3.88 gm FW and 1.32gm DW and in the medium supplemented with IAA(0.5 mgl^{-1}) resulted in 11.40 roots/explants with 4.32 cm mean root length, 2.84 gm FW and 0.88 gm DW (Table1). Medium containing higher concentration of NAA (2.0 mgl^{-1}) produced 7.60 roots/explant with 3.44 cm length, 1.52 gm FW and 0.48gm DW while medium with higher concentration of IBA (2.0 mgl^{-1}) produced 6.00 roots/explant with 3.61 cm mean root length, 1.46 gm FW and 0.46gm DW and the medium with IAA (2.0 mgl^{-1}) resulted in 7.40 roots/explants with 2.02 cm length, 1.42 gm FW and 0.46 gm DW (Table1).

In the present study, IBA at 0.5 mgl^{-1} was found effective for the induction of maximum rooting percentage (88%) and mean number of roots (17.20). Similarly Mahendranath *et al.*,(2011) reported that in *in vitro* root explants of *Bixa orellana* IBA produced the maximum biomass compared to NAA and IAA. Individually IBA was superior over NAA or IAA in the induction of adventitious roots as reported earlier for *Psoralea corylifolia* (Baskaran and Jayabalan, 2009). The influence of IBA on rooting has been further supported by Neto *et al.*,(2009). IBA , likely played a key role in adventitious root formation by initiating cell division, primordium structure and in inducing cell de-differentiation to form root apical meristem (Berleth and Sachs, 2001). In the present study, prolonged culture of explants in medium with higher concentrations of NAA, IBA and IAA decreased the number of roots, biomass and

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browning of the medium. This could be an indication of osmotic stress (Min *et al.*, 2007). Increasing the concentrations of IBA did not result in root induction in *in vitro* root culture for adventitious root induction in *Periploca sepium*

Concentration of Auxins (mg/l)		Rooting Response (%)	Initial Culture	Sub culture	Mean root length	Fresh weight (gm)	Dry weight (gm)
NAA			**Mean number of roots**				
0.2		29±1.00j	3.62±0.61hi	4.54±0.64kl	1.93±0.32f	0.98±0.12g	0.30±0.07e
0.5		37±1.00i	6.05±0.96fgh	7.21±0.85ghijk	2.51±0.50def	1.78±0.31defg	0.50±0.09de
1.0		**66**±1.09c	**9.47**±0.50cd	**11.13**±0.80ef	**3.46**±0.56bcdef	**2.37**±0.25bcde	**0.87**±0.18abcd
1.5		50±1.41ef	7.82±0.57def	8.95±0.72fghi	2.87±0.53cdef	1.82±0.29defg	0.50±0.12de
2.0		42±1.04h	5.21±0.57fghi	6.32±0.66ijkl	2.09±0.50def	1.14±0.22efg	0.44±0.10e
IBA							
0.2		52±1.37e	6.51±0.92efg	8.71±0.76fghi	2.99±0.70cdef	1.92±0.32cdefg	0.64±0.13bcde
0.5		67±1.14c	9.76±1.01cd	11.35±1.20def	3.88±0.77bcde	2.30±0.30bcdef	0.87±0.10abcd
1.0		**76**±1.70b	**12.32**±1.06b	**15.28**±0.88bc	**5.01**±0.70ab	**3.09**±0.34bc	**1.06**±0.22a
1.5		65±1.51c	8.87±0.78cde	9.96±0.73efg	3.49±0.50bcdef	2.03±0.40bcdefg	0.85±0.11abcd
2.0		44±1.64gh	7.21±0.65def	8.28±0.76ghi	2.08±0.32def	1.08±0.13fg	0.43±0.09e
IAA							
0.2		60±1.30d	5.78±0.64fgh	7.32±0.64ghij	2.68±0.28def	1.76±0.39defg	0.54±0.12de
0.5		**65**±1.92c	**7.21**±0.85def	**9.48**±0.71efgh	**3.02**±0.45cdef	**2.03**±0.44bcdefg	**0.62**±0.08cde
1.0		52±1.67e	4.32±0.63ghi	6.68±0.53hijkl	2.46±0.56def	1.92±0.40cdefg	0.43±0.05e
1.5		50±1.73ef	3.84±0.57hi	5.42±0.68jkl	1.98±0.45ef	1.64±0.38defg	0.38±0.05e
2.0		48±1.70efg	3.02±0.43i	4.08±0.65l	1.82±0.36f	0.95±0.17g	0.29±-0.07e
IBA	NAA						
1.0	0.2	50±1.89ef	9.35±0.82cd	12.12±1.03de	3.67±0.64bcdef	2.22±0.57bcdef	0.87±0.18abcd
1.0	0.5	75±2.09b	13.01±1.14b	16.82±0.96b	4.72±0.78abc	3.19±0.37b	1.07±0.11a
1.0	**1.0**	**92**±1.30a	**17.83**±0.83a	**21.75**±1.66a	**6.12**±0.74a	**5.38**±0.65a	**1.02**±0.10ab
1.0	1.5	68±1.30c	11.35±1.09bc	13.91±1.14cd	3.98±0.70bcd	2.67±0.40bcd	0.95±0.11abc
1.0	2.0	47±1.18fg	7.74±0.87def	8.35±0.58ghi	2.79±0.40def	1.25±0.31efg	0.45±0.10e
Control		9±0.57	2±0.00	2±0.00	1±0.11	0.4±0.00	0.15±0.02

Table 1.Effect of NAA and IBA fortified with L-glutamine(20 mg/l)on adventitious root induction from leaf explants of 21 day in vitro seedlings of Luffa acutangula(L) Roxb.

Control: without growth regulator. Data presented as means ± SE from 20 explants for each treatment and repeated three times. Means followed by same letters within a column are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ level.

4.2) ***Combined effect of NAA and IBA on primary adventitious root induction and proliferation***

IBA at its optimal concentration (0.5 mgl^{-1}) was combined with the different concentrations of NAA (0.2 to2.0 mgl^{-1}) along with L- glutamine (20 mgl^{-1}) to study the synergetic role of IBA and NAA. Among the different combinations used, NAA at 1.0 mgl^{-1} with IBA at 0.5 mgl^{-1} was effective, showing maximum rooting percentage (98%), which is greater than when NAA (1.0 mgl^{-1}), IBA (0.5 mgl^{-1}) and IAA (0.5 mgl^{-1}) were used individually. (Table 1). After subculturing at 20 to 25 days interval resulted in 24.40 roots/explant with 7.86 cm mean root length, 5.82gm FW and 1.314 DW in the combination of NAA (1.0 mgl^{-1}) and IBA (0.5 mgl^{-1}) (TABLE1). During the combined effect of IBA (0.5 mgl^{-1}) and NAA at its highest concentration (2.0 mgl^{-1}) produced 11.00 roots/explants with 4.92 cm mean root length, 2.78gm FW and 1.00 gm DW.

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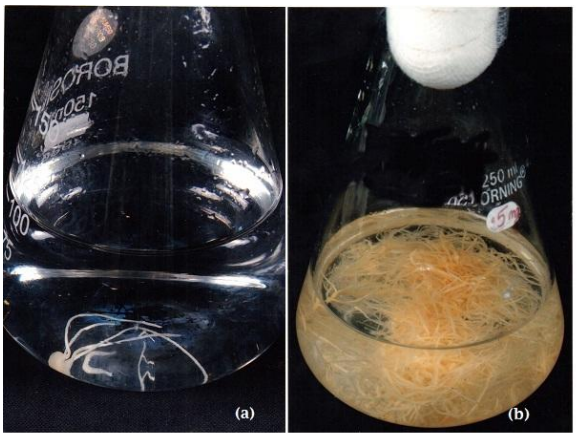

Fig 1: adventitious root culture from *in vitro root* explant
(a) Root explant (b) Root culture after six weeks.

In contrary to the present study Mahendranath *et al.*, (2011) reported that when the combination of NAA with IBA was used there was no significant improvement in root biomass compared to individual treatment of respective auxins in *Bixa orellana*. In another study, MS liquid medium supplemented with IBA and IAA combination led to one and half fold increase of root production from cotyledon derived calli of *Withania somnifera* (Sivanandhan *et al.*, 2012). Earlier studies also reported the effective role of auxin combination on the induction of adventitious roots as in *P.ginseng* (Choi *et al* ., 2000). Maximum percentage of root induction (98.26) was observed in adventitious root culture of *Trichosanthes anguina* when combination of NAA (1.0 mgl^{-1}) and IBA (0.5 mgl^{-1}) was used (Ambethkar, 2012). This is in accordance with our study. Individual treatment of NAA promoted adventitious root proliferation in *Stevia rebaudiana* (Reis *et al.*, 2011) and in *Aloe vera* (Lee *et al.*, 2011). Even though in adventitious root culture of *Aloe vera* , NAA(0.5 mgl^{-1}) enhanced root proliferation there was no significant increase in biomass and dry weight increased only when IBA get combined with NAA. It was noted in the adventitious root culture of *Decalepis arayalpathra* (Sudha and Seeni, 2001) and in the adventitious root culture of *Aloe vera* (Lee *et al.*, 2011) callusing of roots in the presence of NAA and less number of lateral roots decreased the biomass, but IBA combination resulted in increased biomass accumulation. Prolonged cultures of explants in NAA and IBA with higher level of concentrations did not result in root production as observed in *Bixa orellana* (Mahendranath *et al.*, 2011) and in *Periploca sepium* (Zhang *et al.*, 2011) from *in vitro* leaf derived roots.

4.3) *Adventitious root induction and proliferation from in vitro leaf explants*

After 2-3 days of culture, green leaf explants turned pale, began to bulge and bent slightly in basal medium. Different types and concentrations of auxins were tested for adventitious root induction at dark (Data not shown). Among the different concentrations and combinations used in MS medium combination of IBA at 1.0 mgl^{-1} along with NAA (1.0 mgl^{-1}) was most effective and showed maximum adventitious root induction (Fig-1).The root induction was visible compact, stout and stunted.

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Table 2.Effect of NAA and IBA fortified with L-glutamine(20mg/l)on adventitious root induction from root explants of 21 day in vitro seedlings of Luffa acutangula (L)Roxb. on MS liquid medium.

Concentration of Auxins (mg/l)		Rooting response (%)	Initial culture	Subculture	Mean root length(cm)	Fresh weight (gm)	Dry weight (gm)
NAA			*Mean* number of roots				
0.2		33±1.41i	5.56±0.75g	6.80±1.06h	2.82±0.44fg	1.30±0.20e	0.46±0.08e
0.5		58±1.14e	8.20±1.15defg	9.40±1.20efgh	3.80±0.58cdefg	2.50±0.40cde	0.96±0.16abcde
1.0		**78**±1.58c	**12.40**±1.28bc	**15.00**±1.14bc	**5.82**±0.59b	**3.02**±0.32bc	**1.08**±0.22abc
1.5		61±1.41de	9.20±1.11cdef	11.80±1.35cdef	4.70±0.80bcde	2.86±0.34bc	0.98±0.16abcd
2.0		45±1.92gh	6.40±0.81fg	7.60±0.92gh	3.44±0.51cdefg	1.52±0.31de	0.48±0.08de
IBA							
0.2		78±1.87c	13.40±1.20b	15.00±1.30bc	5.94±0.48b	3.02±0.35bc	0.96±0.16abcde
0.5		**88**±1.51b	**14.20**±1.52b	**17.20**±1.65b	**6.04**±0.47b	**3.88**±0.44b	**1.32**±0.22ab
1.0		64±1.70d	11.00±1.30bcd	13.00±1.41cde	4.82±0.37bcd	2.98±0.45bc	1.02±0.11abc
1.5		49±1.30fg	9.20±0.86cdef	11.80±1.77cdef	4.52±0.57bcdef	2.74±0.44bcd	0.96±0.13abcde
2.0		32±1.30i	5.20±0.96g	6.00±0.70h	3.62±0.60cdefg	1.46±0.23e	0.46±0.06e
IAA							
0.2		48±1.41fg	7.20±0.58efg	9.80±1.24defgh	3.02±0.39defg	2.00±0.14cde	0.96±0.16abcde
0.5		**60**±1.92de	**9.00**±0.70cdef	**11.40**±1.16cdefg	**4.32**±0.58bcdef	**2.84**±0.35bc	**0.88**±0.13bcde
1.0		52±1.84f	7.80±0.86defg	10.00±1.22defgh	3.56±0.43cdefg	2.24±0.34cde	0.76±0.11cde
1.5		45±1.78gh	6.00±0.94fg	8.20±1.15fgh	2.98±0.56efg	1.88±0.26cde	0.64±0.12cde
2.0		41±1.58h	5.40±0.67g	7.40±0.92gh	2.02±0.26g	1.42±0.28e	0.46±0.07e
IBA	**NAA**						
0.5	0.2	48±1.76fg	8.20±1.06defg	9.00±1.00efgh	3.96±0.62cdef	2.18±0.40cde	0.88±0.13bcde
0.5	0.5	65±2.91d	10.00±1.00cde	12.40±1.53cde	4.32±0.78bcdef	2.98±0.49bc	1.02±0.15abc
0.5	**1.0**	**98**±1.22a	**20.00**±1.58a	**24.40**±1.07a	**7.86**±0.61a	**5.82**±0.65a	**1.46**±0.21a
0.5	1.5	75±2.60c	12.40±1.12bc	13.80±1.28bcd	5.02±0.45bc	3.04±0.55bc	1.06±0.18abc
0.5	2.0	58±1.41e	9.20±0.80cdef	11.00±0.89cdefg	4.92±0.44bc	2.78±0.38bcd	1.00±0.18abc
Control		11±0.57j	2.2±0.11h	2.0±0.57i	0.3±0.057h	0.3±0.03f	0.06±0.01f

Control: without growth regulator. Data presented as means ± SE from 20 explants for each treatment and repeated three times. Means followed by same letters within a column are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ level.

4.4) ***Effect of individual auxin treatment on in vitro leaf- derived adventitious roots on primary adventitious root induction and proliferation***

Different types and concentrations of auxins along with L-glutamine (20 mgl^{-1}) were tested for primary adventitious root induction. Among the different concentrations of NAA (0.2 $mgl^{-1}$to 2.0 mgl^{-1}), IBA (0.2 mgl^{-1}to2.0 mgl^{-1}) and IAA (0.2 mgl^{-1} to 2.0 mgl^{-1}) individually in MS liquid medium with L-glutamine tested , at the concentration of IBA (1.0 mgl^{-1}) maximum rooting response percentage (76%) was observed with the production of 12.32roots/explant after 15-days of culture followed by NAA (1.0 mgl^{-1}) with 66% rooting response and 9.47 roots/ explants after 15-days of culture (initial culture). In the medium supplemented with IAA at the concentration of 0.5 mgl^{-1} 65% rooting response was observed with 7.21 roots/ explants (initial culture) (Table, 2).The root induction was visible as thick, greenish and wiry in all the above mentioned concentrations.

In dark when the explants cultured continuously (upto 10-15 days) in the same MS liquid medium containing growth regulators neither enhanced root number nor resulted in initiation of additional roots. Hence, subculture of explants along with emerged roots was performed in fresh MS liquid medium containing NAA and IBA as in initial culture. After one sub culture in medium supplemented separately with NAA, IBA and IAA new roots were started to emerge from the explants. At the end of subculture with a duration of 20-25 days, in IBA (1.0 mgl^{-1}) the

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explants produced maximum number of roots (15.28/explant) with 5.01 cm mean root length,3.09gm FW and 1.06 gm DW followed by NAA (1.0 mgl^{-1}) where the explants produced, 11.13 roots/explants with 3.46 cm mean root length, 2.37 gm FW and 0.87 gm DW. In IAA (0.5 mgl^{-1}) 9.48 root/explant was produced with 3.02 cm mean root length, 2.03 gm FW, and 0.62 gm DW(Table,2).

Medium containing higher concentrations of IBA (2.0 mgl^{-1}) produced 8.28 roots/explant with 2.08 cm mean root length, 1.08 gm FW and 0.43gm DW while the medium with higher concentration of NAA (2.0 mgl^{-1}) produced 6.32 roots/explant with 2.09 cm mean root length, 1.14 gm FW and 0.44gm DW and the higher concentration of IAA (2.0 mgl^{-1}) resulted in 4.08 roots/explants with 1.82 cm mean root length, 0.95 gm FW and 0.29 gm DW at the end of single subculture (Table,2). Primary adventitious roots derived from *in vitro* leaf showed long and greenish yellow adventitious roots at all optimal concentrations of NAA(1.0 mgl^{-1}) , IBA (1.0 mgl^{-1}) and IAA (0.5mgl^{-1}). Maximum number of root induction was obtained in the medium supplemented with IBA (1.0 mgl^{-1}) (15.25roots/explant) followed by NAA (1.0 mgl^{-1}) (11.13roots/explant) and IAA (0.5 mgl^{-1}) (9.48 roots/explant) (Table, 2).

In the present investigation, IBA at 1.0 mgl^{-1} was effective for the induction of maximum rooting percentage and mean number of roots. IBA as an exogenous supply induced adventitious roots (Davis and Haissing, 1994). Moreover of the various auxins (IAA, IBA and NAA) tested, IBA was found to be the best growth regulator for the adventitious root induction of *Vernonia amygdalina*, producing the highest root number/explants (38.3) in the basal medium supplemented with IBA at 2.0 mgl-1(Khalafalla *et al.*, 2009). Root induction was suppressed markedly with higher concentration of auxins (Jenifer *et al.*, 2012; Choffe *et al.*, 2000; Khalafalla *et al.*, 2009; Nandagopal and Ranjitha Kumari 2007; Ambethkar, 2012).

4.5) ***Combined effect of auxins on in vitro leaf derived adventitious roots on primary adventitious root induction and proliferation***

When compared to the individual treatment of IBA (1.0 mgl-1), NAA (1.0mgl^{-1}) and IAA (0.5mgl^{-1}) fortified with L-glutamine (20 mgl^{-1}), combination of both NAA (1.0 mgl^{-1}) and IBA (1.0 mgl^{-1}) fortified with L-glutamine (20 mgl^{-1}) was comparatively better in inducing more adventitious roots per explants in dark. Among the different combinations (1.0 mgl^{-1} IBA with 0.2 mgl^{-1} to 2.0 mgl^{-1} NAA fortified with20 mgl^{-1} L-glutamine) used, IBA at 1.0 mgl^{-1} and NAA at 1.0 mgl^{-1} fortified with L-glutamine 20 mgl^{-1} was most effective and showed the maximum production of roots (17.83roots/explant) at the end of initial culture(after 15-days). A maximum number of 21.75 roots/explants,6.12 cm mean root length,5.38 gm FW and 1.02gm DW was produced at IBA (1.0 mgl^{-1}) and NAA (1.0 mgl^{-1}) fortified with L-glutamine (20mgl^{-1})at the end of a single subculture spanning over 20-25 days (Table,2).At their higher concentration (IBA 1.0 mgl^{-1} and NAA 2.0 mgl^{-1} with L-glutamine 20 mgl^{-1}) produced 8.35 roots/explant, 2.79 cm mean root length,1.25 gm FW and 0.45gm DW.

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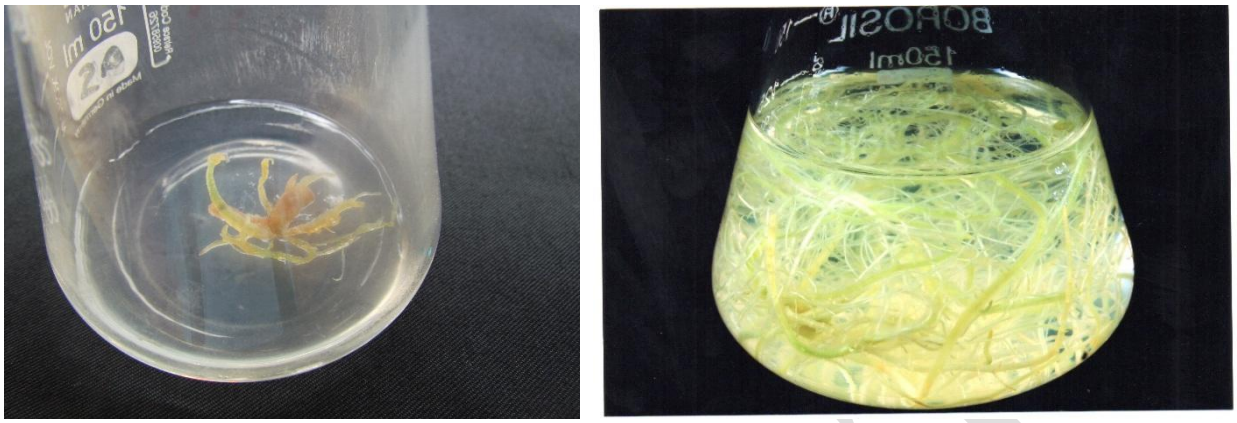

Fig 2: Adventititous root culture from *in vitro* leaf (a) explants.
Induction of roots from leaf explant (b) Root culture after six weeks.

In support to the present study, combined effect of NAA(0.2 mgl^{-1}) and IBA (0.5 mgl^{-1}) in half strength MS medium showed maximum (89.1%) rooting response with 5-roots/explants in adventitious root culture from *in vitro* leaf explants of *Chicorium intybus* (Nandagopal and Ranjitha Kumari, 2007). In *Trichosanthes anguina* also NAA (1.0 mg^{-1}) combined with IBA (0.5 mgl^{-1}) fortified with L-glutamine (20 mgl^{-1}) resulted in maximum rooting response (95.45%) with 25.61 roots/explant in adventitious root culture from *in vitro* leaf explants (Ambethkar, 2012). In another study , leaf derived callus was cultured in MS medium supplementd with 1.0 mgl^{-1} BAP and 2.0 mgl^{-1} NAA induced 2-5 roots in *Bryonopsis laciniosa* (Caroline and Mallaiah, 2011).

4.6) Root Biomass accumulation:

4.6a) Combined effect of NAA and IBA on in vitro root derived primary adventitious roots and their biomass accumulation;

When compared to the individual treatment of IBA (1.0mgl^{-1}),NAA (1.o mgl^{-1}) and IAA (0.5mgl^{-1}), the combination of NAA (1.0 mgl^{-1}) with IBA (0.5 mgl^{-1}), was more effective in inducing biomass accumulation (Fresh weight and Dry weight) of primary adventitious roots in dark. Among the different combinations of NAA(0.2 $mgl^{-1}$to 2.0 mgl^{-}) with IBA (0.5 mgl^{-1}) fortified with L-glutamine used, NAA (1.0 mgl^{-1}) with IBA(0.5 mgl^{-1}) was most effective and showed the maximum production of 5.82 gm FW and 2.2937 gm DW at the end of subculture spanning over 5-6 weeks (Table,2).At its highest concentration (2.0mgl^{-1}) produced 1.46 gm FW and 0.475 g DW at the end of subculture.

Contrary findings were reported by Mahendranath et al., (2011) in *in vitro* root derived adventitious roots of *Bixa orellana*. They reported 0.1mgl^{-1} IBA produced maximum biomass compared to NAA and IAA. In support to this study, NAA (1.0 mgl^{-1}) in combination with IBA (0.5 mgl^{-1}) resulted in maximum biomass accumulation in *in vitro* root derived adventitious roots of *Trichosanthes anguina* (Ambethkar,2012) and similar findings were reported by Lee et al.,(2012) in *in vitro* root-derived adventitious roots of *Eleutherococcus koreanum.*

4.6b)Combined effect of NAA and IBA on in vitro leaf derived primary adventitious roots and their biomass accumulation:.

Among the different combinations of NAA(0.2 mgl^{-1} to 2.0 mgl^{-1}) with IBA(0.5 mgl^{-1}) fortified with L-glutamine tested, NAA, at 1.0 mgl^{-1} concentration with IBA(0.5 mgl^{-1}) was the most effective and showed the maximum production of 5.38gm FW and 1.02 gm DW at the end of subculture with a duration of 5-6 weeks (Table,2) At its higher concentration of NAA(2.0 mgl^{-1}) with 0.5 mgl^{-1} concentration of IBA produced 1.25 gm FW,0.45 gm DW at the end of subculture. Comparatively, the biomass accumulation obtained from leaf derived adventitious culture (5.38 gm FW) was lesser than that obtained from root derived adventitious culture (5.82 gm FW). This is in accordance with the study on adventitious root culture of *Periploca sepium* where it was reported that adventitious root proliferation and biomass accumulation were higher than those derived from leaf explants (Zhang et al.,2011). In another study ,on induction of adventitious roots from *Aloe vera* leaf tissues, increasing NAA concentration resulted in callus like

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adventitious root (Lee et al.,2011).In the present study, no callusing of roots was seen and maximum biomass production (5.38 gm FW) was observed.

V. CONCLUSION

In the present study, maximum number of adventitious roots (24.40 roots/explant) was regenerated from *in vitro* root explants derived from 21 day-old *in vitro* grown seedlings. Combined treatment of IBA at 0.5 mgl^{-1} with NAA (1.0 mgl^{-1}) showed the maximum root induction with 5.82 gm (FW) biomass accumulation from *in vitro* root explants followed by 5.38 gm (FW) biomass accumulation with maximum number of adventitious roots (21.75roots/explant)from the *in vitro* leaf explants in the culture where IBA at 1.0 mgl-1 concentration, combined with NAA (1.0 mgl^{-1}). To our knowledge this is the first report on influence of exogenous hormones on adventitious root culture of *Luffa acutangula*. This simple yet repetable protocol would be useful for *in vitro* production of medicinally important compounds.

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