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Regenerative Effect of L-Ascorbic Acid on the *In vitro* Grown Plants

S. N. Sunitha[1*]

[1]*Department of Biotechnology, Mount Carmel College, Bangalore, India.*

Author's contribution

This work was carried out by the author SNS. The author SNS, designed the study, wrote the protocol, conducted research work, managed the literature search, and wrote the first draft of manuscript. Author SNS has read and approved the final manuscript.

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ABSTRACT

Aim: In the present work, we have studied the effect of L ascorbic acid (LAA) on the regeneration of plants from different families cultured *in vitro*.
Study Design: Plants belonging to three different families are cultured in Murashige and Skoog (MS) medium with and without 1µg/ml L ascorbic acid (LAA), in the absence of any other growth regulators. Thus the study brings out the effect of LAA on plant regeneration. In addition regeneration capacity of LAA involving other growth regulators was also studied.
Place and Duration of Study: Department of Biotechnology, Mount Carmel College, Bangalore, India, between August 2012-August 2013.
Methodology: The work was conducted on *Centella asiatica, Santalum album,* and *Trigonella foenumgraecum. C. asiatica* and *T. foenumgraecum* are herbaceous whereas *S. album* is a tropical woody plant. Stem explants of *C. asiatica* and *S. album* and the

**Corresponding author: Email: sunitha.shroff@gmail.com;*

seed explants of *T. foenumgraecum* were used for the *in vitro* culture and chlorophyll content in thus obtained leaflets was measured. Further, growth related parameters such as shoot/root length, leaf areas were measured.

Results: LAA aided the shoot regeneration in all the three plants cultured *in vitro*. In *C. asiatica* and *T. foenumgraecum* it resulted in the regeneration of plantlets with shoots and roots, however in the case of *S. album* only shoot regeneration occurred. Chlorophyll content was found to be higher in the *in vitro* plants grown in the presence of LAA. Shoot/root lengths and area of leaves were more in LAA grown plants as compared to control plants.

Conclusion: *In vitro* culture of stem explants of *C. asiatica* and seed explants of *T. foenumgraecum* revealed that supplementing LAA aided in the whole plant regeneration, whereas in the case of *S. album* supplementing LAA only resulted in the shoot regeneration, but no root formation. Shoot/root lengths, area of leaves and chlorophyll content was found to be higher in the *in vitro* grown plants with LAA as compared to those grown without LAA, suggesting that LAA is mitigating the function of both auxin and cytokinin. Enhanced chlorophyll production in *in-vitro* grown plants with LAA is suggestive of involvement of LAA in chlorophyll biosynthesis/protection from degradation and hence the regeneration. Through our results, we show that using LAA in the culture medium can result in regeneration of whole plants. The effect was observed in plants belonging to different families indicating LAA could be used as a general growth enhancer and adding LAA would be beneficial in the regeneration of whole plants.

Keywords: In vitro culture; Centella asiatica; Santalum album; Trigonella foenumgraecum; vitamin C or L ascorbic acid (LAA); regeneration.

1. INTRODUCTION

Vitamins are the organic substances required for the overall health of organisms, of which Vitamin C, commonly known as L-ascorbic acid (LAA) has been proven to be highly beneficial. It is established that Vitamin C, is an abundant antioxidant in plants. In *in vitro* cultures LAA primarily used as an antioxidant, to prevent browning of tissues [1,2]. LAA helps plants to deal with stresses including those due to drought, ozone and UV radiation. LAA is an enzyme co-factor that has multiple proposed functions in plants [3-6]. Many citrus plants are rich sources of LAA. In plants apart from its role as an abundant antioxidant, in few cases LAA is currently been considered as a regulator of cell division and differentiation and involved in a wide range of important functions as antioxidant defense, photo-protection, regulation of photosynthesis and growth [7]. Recent evidence suggests that it may also play a role in floral induction [6]. Recently, John Dowdle et al. [8] have identified that, mutants that lack ascorbate biosynthesis results in seedling inviability. In *Cupressus sempervirens*, foliar application of LAA resulted in pronounced vegetative growth and chemical constituents [9].

Vitamin C is not an essential component of the Murashige and Skoog (MS) medium (plant tissue culture medium), which contains other vitamins, including riboflavin, nicotinic acid and thiamine. Few studies have indicated the effect of LAA on *in vitro* cultures. For example Joy et al. [10], have shown that it is possible to enhance shoot formation in young callus tissue of *Nicotiana tabaccum* by exogenous application of vitamin C to a shoot forming medium. Thus earlier studies only indicated that LAA as an additional supplement in *in vitro* culture in the presence of other growth regulators. Despite its widespread role as an antioxidant, a cofactor for many enzymes, a regulator of proliferation and differentiation, the use of

ascorbic acid in tissue culture for the regeneration purpose is minimal. We here explore advantages of incorporating Vitamin C as growth component in the medium to obtain regeneration. Therefore in our current study we have focused on the elucidation of effect of LAA on the *in vitro* growth and regeneration of plants of diverse nature and belonging to diverse families. For the current study, *Centella asiatica, Trigonella foenumgraecum* and *Santalum album* belonging to the families mackinlayaceae, fabaceae and santalaceae respectively, were considered. Other rationale behind the selection of these plants is, their medicinal use and also *Santalum album* has been listed in the 'vulnerable category' in the IUCN red list of threatened species. Apart from elucidating the role of LAA in regeneration, the study is also aimed at the rapid mass production of medicinal plants/rare plants by plant tissue culture techniques. This would help in propagating them at a rapid rate on a larger scale to meet the demands of the public and help in conserving biodiversity.

2. MATERIALS AND METHODS

2.1 Materials

Synthetic MS medium, L-ascorbic acid, Indole acetic acid, Naphthalene acetic acid, 2,4-Dichlorophenoxy acetic acid, kinetin and Benzyl adenine were obtained from Himedia and Nice chemicals. Stem explants of *Centella asiatica* and *Santalum album* were obtained from college botanical garden, Bangalore. The seeds of *Trigonella foenumgraecum* were purchased.

2.2 Methods

2.2.1 Preparation of explants

For the sterilization, explants were first washed with running tap water to remove dirt and other solid particles, treated with surfactant 2% tween 20 (in distilled water) to remove, loose spores and bacteria and other debris that might be harboring microbes. Then explants were rinsed thoroughly with sterile distilled water to remove traces of surfactants. Further sterilization procedures were carried out under aseptic conditions in laminar air flow cabinet by washing with sterile double distilled water, followed by treating with 0.1% $HgCl_2$ for 2 minutes. To remove traces of mercuric chloride, we further washed them several times with double distilled water. Then the explants were cut using sterile blade, dried on sterile filter paper and inoculated on the MS medium supplemented with or without LAA in the absence/presence of other growth regulators.

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2.2.2a Preparation of medium

Murashige and Skoog (MS) medium [11] was prepared as per the manufacturer's instructions (HiMedia®). In brief, 42 gms of HiMedia MS medium (with sucrose and agar) was weighed and dissolved in 800ml of double distilled water, pH was adjusted to 5.6 to 5.8 using 1N HCl or 1N KOH, heated to melt the agar, volume was made up to 1L and autoclaved. 20 ml of the medium was poured into sterile plant tissue culture tubes, allowed to cool to ~50℃ followed by the addition of respective growth regulators and LAA.

2.2.2b Preparation of growth regulators and LAA

All the growth regulators and LAA are prepared at the stock concentration of 1mg/ml and working concentration used was 1µg/ml. For the preparation of 2,4-D, 50mg of the chemical

was dissolved in 1ml/few drops of ethanol, volume was made up to 50ml with sterile distilled water, filtered using 0.2 µM membrane, aliquoted and refrigerated till the use. Similar procedure was followed for other growth regulators except for the solvent used to dissolve is 1N NaOH. LAA is prepared in sterile distilled water, filtered using 0.2 µM membrane, aliquoted, refrigerated till use.

2.2.3 Inoculation of explants

Approximately 5mm-8mm of the surface sterilized stem or seed explants were inoculated into the tubes and baby jar bottles containing the MS medium with sterile forceps. Part of the stem is inserted vertically into the medium, whereas the seed is placed on the medium. The mouths of tubes and baby jar bottles were then flamed with the help of sprit lamp and the caps were closed. The inoculated explants were incubated in the incubation chamber at temperature of 25℃, under natural light and dark conditions for their growth.

2.2.4 Estimation of Chlorophyll

The chlorophyll content was estimated by the method of Witham et al. [12]. Briefly, chlorophyll was extracted from 1g of the sample using 20ml of 80% prechilled acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for 5-13 minutes. The volume in the flask was made up to 100ml with 80% acetone. The absorbance of the extract was read in a spectrophotometer at 645 and 663nm against 80% acetone as blank. The amount of total chlorophyll in the sample was estimated using the following formula.

$$\text{Total chlorophyll} = 20.2\ A_{645} + \quad 8.02\ A_{663}\ \text{x}\ \frac{\text{V}}{1000\ \text{W}}$$

where,

V = final volume of the extract
W = fresh weight of the leaves

Absorbance of Chlorophyll-A and Chlorophyll-B respectively are A_{663} and A_{645}

The values are expressed as mg chlorophyll/g sample.

2.2.5 Estimation of growth related observations

After specified duration of growth *in vitro,* the plantlets were removed from the culture medium without damaging the roots. Lengths of shoots and roots were measured and noted the number of secondary roots, if any. Leaves were separated from the shoot. They were placed on a white paper next to a marked line of 1cm length. Images of the leaves along with the marked line were captured using Dino® digital microscope. Leaf size is first determined in terms of number of pixels by counting number of pixels for each leaf-image and then knowing the area/pixel (through the image of the marked line), areas of leaves were determined.

2.2.6 Hardening the plants

Hardening was done with respect to *Centella asiatica* and *Trigonella foenumgraecum*, where there was root formation. The tissue culture medium was washed off with sterile water, in

order to avoid the molds growing on the remaining gel media. Sterile soil was used for sowing the plantlet. A humidity tent was made with a plastic bag which aids the acclimatization of the plant. The plants were kept sealed for a week. Gradually the bag is opened to get the plants used to lower humidity. Direct sunlight was avoided for two weeks.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of LAA on regeneration of *Centella asiatica* using stem as an explant

Centella asiatica stem explants were inoculated on plain MS medium (without LAA as the control) and in the MS medium supplemented with 1µg/ml LAA alone, in order to test the role of LAA in the plant regeneration. The stem explant on plain MS medium (control) did not show any growth, later turned brown and perished. Whereas the explant in LAA supplemented MS medium showed small green shoot regeneration after one week of inoculation, the size and the number of shootlets increased subsequently. After three weeks, we observed root regeneration in the same medium (MS with LAA) without transferring to rooting medium. The regenerated plantlets transferred to the soil and within few days the hardened plantlet developed like a normal plant (Figs. 1a to 1f).

3.1.2 Effect of LAA on regeneration of *Santalum album* using stem as an explant

Similar results were obtained with *Santalum album*, using the stem explants. Without the addition of any other growth regulator, explants on LAA supplemented MS medium gave raise to shoot and leaflets as shown in the figure (Figs. 2a to 2e). The control explants without LAA in the medium did not grow and they became brown. With respect to the explants grown on medium with LAA, though profound shoot regeneration with many leaflets was observed, there was no root growth. The reason behind this is not very clear, however owing to the woody nature of the plant, different concentration of LAA may be required. Nevertheless the shoot and leaflet regeneration indicated the positive effect of LAA in the regeneration of *S. album*. The similar results obtained in both the cases indicate that LAA was functioning in different types of plants belonging to different species, is suggestive of its broad spectrum effectiveness.

3.1.3 Effect of LAA on the regeneration of *T. foenumgraecum* with seed explants

Nodal, stem and leaf explants did not show any growth in the absence of LAA. Hence in the third case, in order to have plantlet in the untreated control, we used the seed explants. The seeds have the growth components and the nutrients to grow and these explants can be grown even in the absence of LAA/growth regulators. Supplementing LAA in the MS medium thus brings out enhancing effect of LAA, if any, on the regeneration of plantlet. For this experiment, we chose seeds of *T. foenumgraecum.* Surface sterilized seeds of *T. foenumgraecum* were inoculated on MS medium without-LAA supplement (control) and also MS medium supplemented with LAA (1µg/ml). After a week we observed shoot regeneration in both the cases. In the case of control, seeds although showed shoot formation within a week of inoculation, root formation was not observed (Fig. 3a). Whereas, in medium supplemented with LAA both shoot and root formation was observed within a week. The size of the plantlet which grew in this case (Fig. 3b) clearly indicated that the growth of shoots was more profound in LAA substituted explants as compared to the control. Measurement of lengths of stem and roots indicated that the lengths of shoots/roots was

more in the seeds grown in presence of LAA as compared to the control seeds (Figs. 5 and 6). The leaf area also found to be higher in LAA supplemented plants (as tabulated in Table 1), indicating the probable effect of LAA on cell division and proliferation [13].

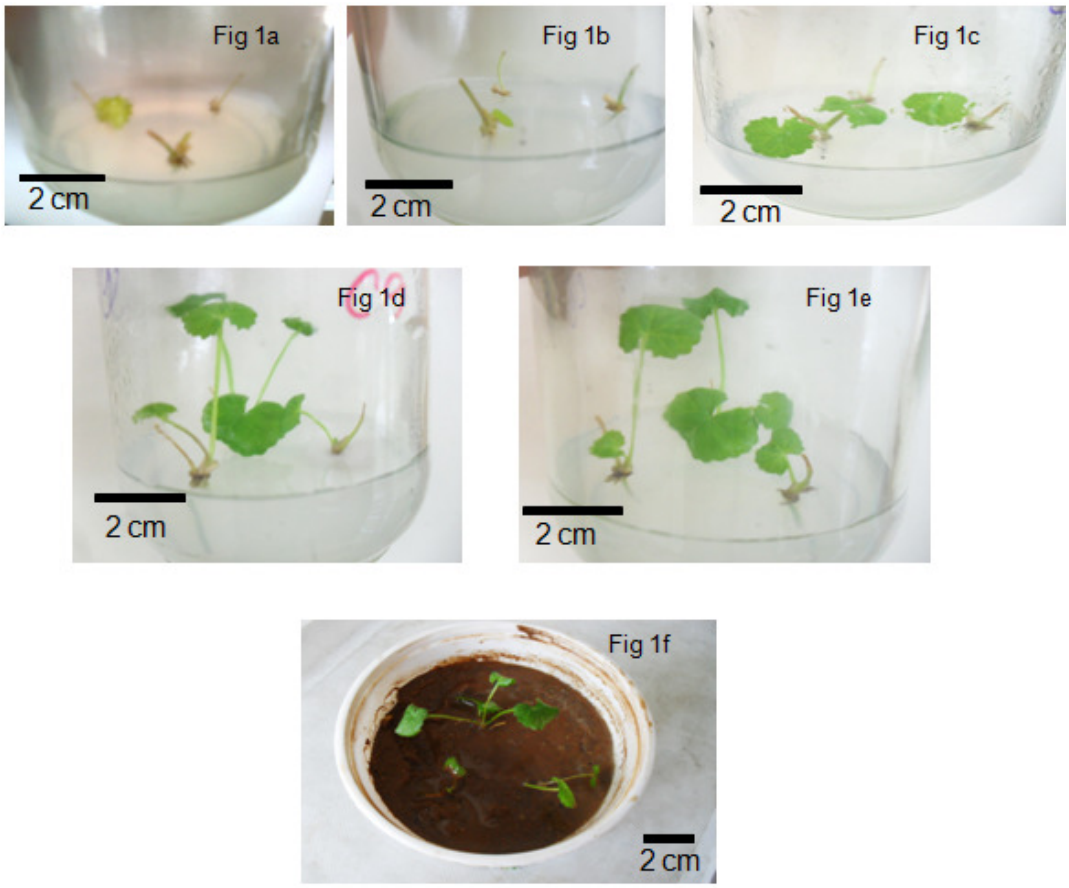


Fig. 1. Showing the shoot regeneration from *Centalla asiatica* stem explants obtained without any growth hormones but with L-Ascorbic acid (1µg/ml). Pictures were taken every week after inoculation. Fig. 1a shows a small green shoot regenerated from the stem explants after one week of inoculation, Fig. 1b shows increase in shoot size, which developed leaflets by 3 weeks as shown in Fig. 1c. Fig. 1d and 1e show increased height of the plantlets along with root formation by 3-4 weeks. Fig. 1f shows the hardened plantlets after 40 days of inoculation

3.1.4 Estimation of Chlorophyll content in *In vitro* plants grown with and without LAA in the medium

In all three plants that we have studied, irrespective of type of explants, we observed that shoot regeneration was profound upon the usage of LAA. In case of seed explants of *T. foenumgraecum*, supplementing Vitamin C, resulted in longer and better shoots, which were lush green as compared to the control. It is known that chlorophyll is vital for formation of green tissues and it is an obligate component of photosynthesis. Hence we tested the chlorophyll content in the leaflets of *in vitro* grown plants in medium supplemented with or

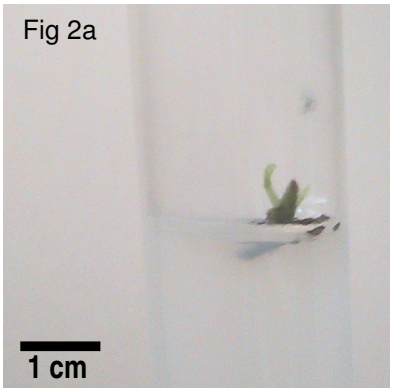
without 1µg/ml LAA. Chlorophyll estimation has showed (Fig. 4) that, leaflets of LAA supplemented *in vitro* plant has almost two fold (0.257mg/g tissue) more chlorophyll content as compared to those grown without LAA (0.125mg/g tissue). Though in earlier literature and in our own results it is been evident that Vitamin C promotes the regeneration of plantlets, its mechanism is not understood. However, there are evidences to show that LAA acts in the protection of degradation of chlorophyll [17], probably due to this increase in chlorophyll content in LAA treated plants was observed. At this juncture we can conclude that LAA could be used as a growth regulator in tissue culture medium and it promotes the growth possibly through enhancement of chlorophyll biosynthesis and protection of chlorophyll from degradation.

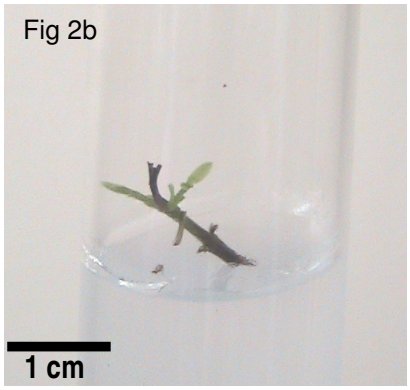
3.1.5 Usage of BAP/Kinetin and NAA/IAA along with or without LAA in the medium

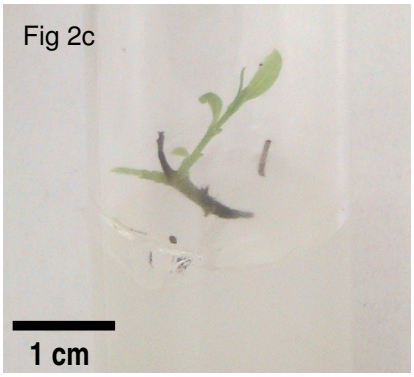
Kinetin and BAP are cytokinins which aid the regeneration of shoots whereas NAA and IAA are auxins. Experiments were performed using BAP/Kinetin and NAA/IAA at the concentration of 1µg/ml along with or without LAA in order to compare regeneration capacity of LAA involving treatment with growth regulators. Four different combinations of growth regulators were used. Kinetin+NAA, Kinetin+IAA, BAP+NAA and BAP+IAA with and without LAA. Results were tabulated after 10 days of inoculation. Combinations of NAA along with cytokinins did not show regeneration, but turned into callus like structures, which in presence of LAA showed somewhat leaf like structures. Experiments with Kin+IAA showed shoot formation, which along with LAA resulted in longer shoot, and also root formation. Combination of BAP+IAA also showed similar results, however there was no root formation. The data of the above, which was collected after 10 days of inoculation are shown in Fig. 7 and the growth parameters are tabulated in the Table 2. Through this experiment two points were observed. First one is, the use of LAA has resulted in differentiated tissue, which is evident from Kin+NAA/BAP+NAA combination which resulted in larger callus without LAA, however when LAA was used along with these combinations it resulted in small leaf like structures. Secondly in combinations (Kin+IAA and BAP+IAA) where shoot regeneration was observed, inclusion of LAA enhanced shoot length and formation of root by 10 days.

Table 1. Showing the growth related parameters in *In vitro* T. *foenumgraecum* plants grown without/with LAA (data is collected 23 days after inoculation)

	Stem length, in cms (Avg)		**Root length, in cms**	**Leaf nature**	**Leaf area/plant in mm² (Avg)**	
Control:						
1	3.0		-	Unopened	64.5	
2	7.0	**(4.2)**	8	No leaf	-	**(21.5)**
3	2.8		-	-	-	
LAA treated:						
1	7.5		5.0 & 9 Sec. roots	Opened-2 leaves	72	
2	7.0	**(7.2)**	11.5	3 leaves: 2+1 small	68	(**75.3)**
3	7.0		8.5	3 leaves: 2+1 small	86	







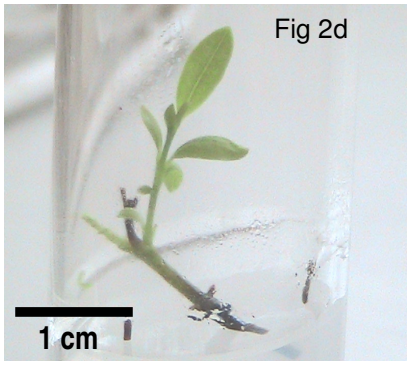


Fig. 2. 2a to 2e show the growth of *Santalum album* stem explants in MS medium containing 1µg/ml LAA. The pictures were taken every week to assess the growth. The shoot size and number of leaflets increased with time

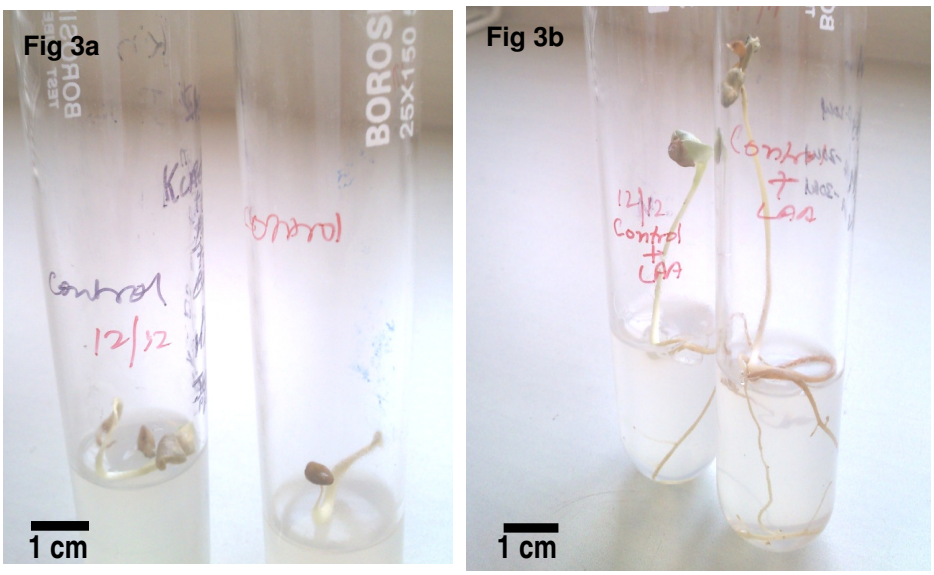


Fig. 3. Indicates the growth of *Trigonella foenumgraecum* seed explants. Figure 3a shows growth of control seed without any growth regulators or LAA and 3b shows growth of seed explants in LAA supplemented MS medium (1µg/ml). Pictures were taken 3 weeks after inoculation

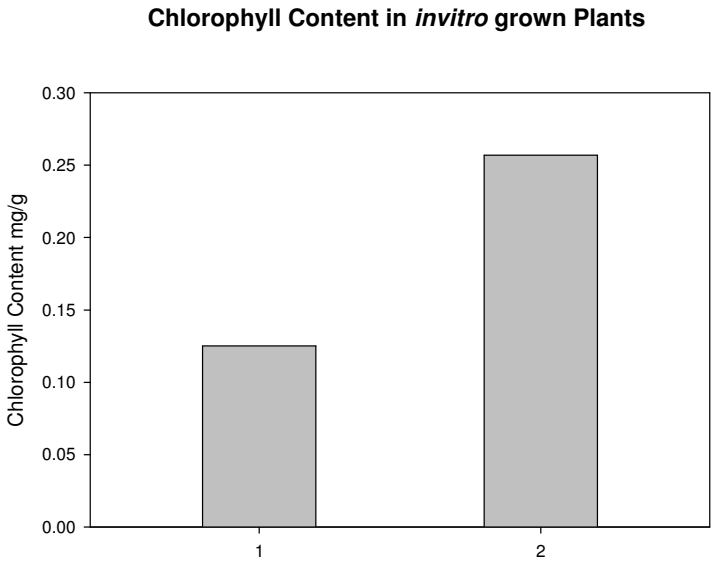


Fig. 4. Shows bar diagrams indicating chlorophyll content in the leaflets obtained from *In vitro* plants grown without or with LAA (1µg/ml). Bar 1 shows chlorophyll content in *In vitro* leaflets of plants grown without LAA and Bar 2 indicates chlorophyll content in *In vitro* leaflets of plants grown in presence of 1µg/ml LAA

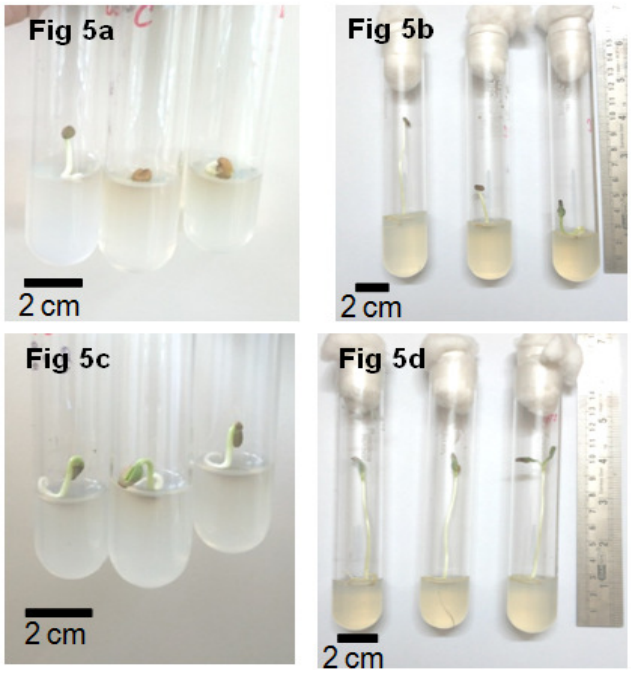


Fig. 5. 5a and 5b indicate the control seeds of *T. foenumgraecum* inoculated on MS medium without LAA after three days and twenty one days of inoculation respectively. Figs. 5c and 5d indicate the seeds inoculated on MS medium supplemented with LAA (1µg/ml) after three and twenty one days of inoculation respectively. Culture tube used here is of 25mm in diameter

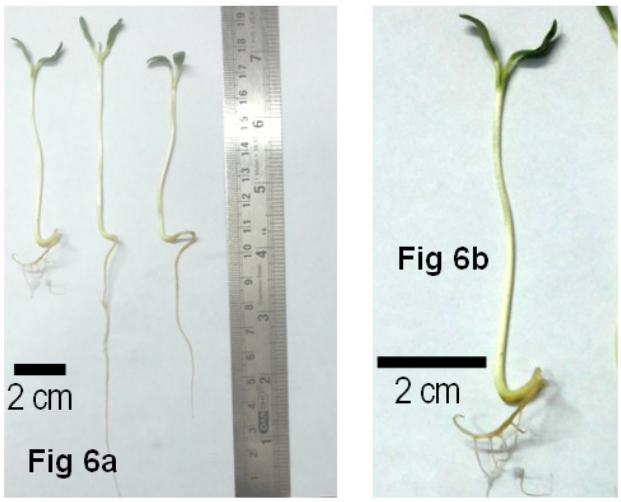


Fig. 6. 6a showing seeds of *T. foenumgraecum* grown in LAA (1µg/ml) supplemented MS medium, removed from the tubes twenty three days after inoculation to show the root formation and to measure growth related parameters. Photograph is taken according the scale. 6b showing the enlarged plantlet from 6a in order to indicate the secondary roots

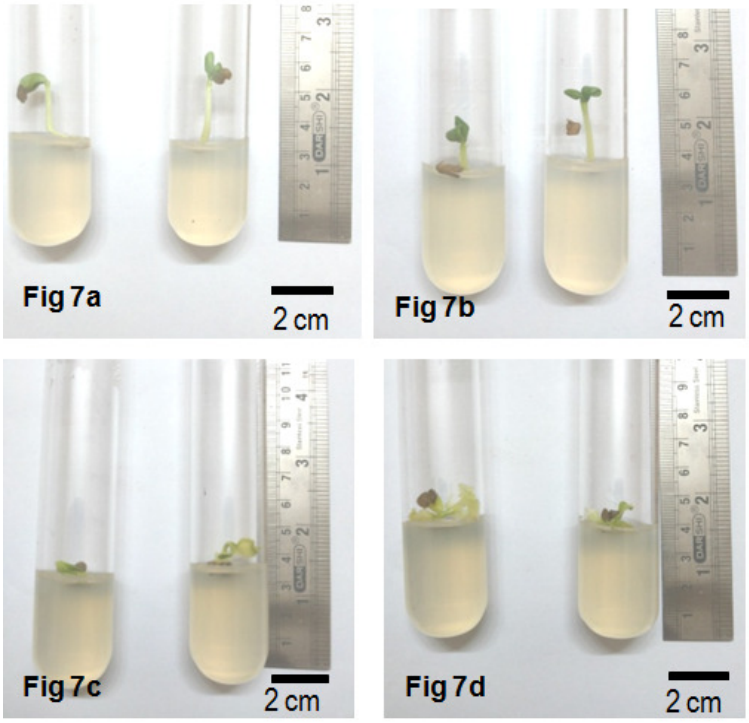


Fig. 7. Shows the *T. foenumgraecum* seeds grown in MS medium supplemented with different growth regulators with and without LAA. Photos taken 10 days after inoculation. a) Kin+IAA, Kin+IAA+LAA, b) BAP+IAA, BAP+IAA+LAA, c) Kin+NAA, Kin+NAA+LAA, d) BAP+NAA, BAP+NAA+LAA. (All growth regulators and LAA are used at 1µg/ml concentration)

Table 2. Showing the growth related parameters of *in vitro* grown *T. foenumgraecum* plants in presence of BAP/Kinetin and NAA/IAA combinations along with or without LAA (time period 10 days)

Growth regulators	Size of shoot (in cm)	Size of root (in cm)	Nature of leaf	Leaf area (in mm²)
BAP+NAA	No shoot(callus)	No root	No leaf (only callus)	
BAP+NAA+LAA	No shoot(callus)	-	One leaf	
BAP+IAA	1.0	-	Two leaves	~60
BAP+IAA+LAA	2.5	-	Two leaves	~64
Kin+NAA	No shoot	-	Small leaf	~20
Kin+NAA+LAA	No shoot	-	Two leaf like, pale green structures	~43
Kin+IAA	2.0	-	Two leaves	~65
Kin+IAA+LAA	3.0	1.0	Two leaves	~68

3.2 Discussion

Effect of LAA on the growth and regeneration of three different plants cultured *in vitro* was studied. The three plants selected have diverse habits and belong to entirely different

families. Such a diverse plant variety is chosen in order to understand whether the effect of LAA observed was plant/species specific or could be used as a general medium supplement to achieve the regeneration. The Murashige and Skoog (MS) medium is a regular medium used universally for the *in vitro* plant growth/regeneration, which contains thiamin, riboflavin and niacin, and these are water soluble B group vitamins. Vitamin C is a well known antioxidant in organisms including both in plants and animals to ward off free radicals. In animals there are umpteen reports indicating usage of LAA in healthcare [14] and there are evidences to indicate the effect of LAA in wound healing/regeneration as reviewed in BMC, Nutrition journal, 2003 [14]. In plants, it has been shown that applying LAA would enhance the flowering [6]. With respect to *in vitro* cultures there are few reports showing better shooting from the callus [10], aiding proliferation of tobacco cells [15] in the presence of Vitamin C. However, there are no reports till date to our knowledge, that LAA could be used as a sole source of growth regulator in the medium for the whole plant regeneration using any type of explant. There is no comprehensive work showing the generalized effect of LAA in shoot regeneration with respect to plants belonging to the different families and having different habits. Hence we chose herbs (*Centella asiatica, Trigonella foenumgraecum*) and an woody plant (*Santalum album*) belonging to the different families. The rationale behind such a selection is not only an aim to select different plant species but also that all these are medicinally important and two of them are listed in IUCN red list book. As we all know that due to human invasion, there is a threat to the biodiversity and species are getting extinct at much faster rate than in past. Hence it is imperative to conserve the biodiversity and one such tool for conservation is tissue culture. There is always a search for more effective supplements which will aid in the positive, faster, more pronounced regeneration. Our results indicate that one such answer may be LAA, as it is known for its various beneficial activities, including its antioxidant activity, acting as a cofactor for various enzymes, and as a regulator in proliferation and differentiation. The antioxidant nature of LAA also is responsible for protecting the cut explant from deleterious oxidation of phenols which might have liberated from the explant in the culture medium. Taking together the beneficial properties of LAA one cannot ignore advantage of using the same in tissue culture medium to achieve *in vitro* growth. Our result showed stem explants of *C. asiatica* and *S. album* grew to give shoots, and roots in *C. asiatica*. The control plants without LAA did not show any growth, became brown and perished in the medium in both the cases. Lack of root formation in case of *S. album* may be due to its woody nature and may be they require different concentration of LAA. The seed explant of *T. foenumgraecum* grown in LAA supplemented medium showed longer, straighter shoots with lush green leaflets and roots, as compared to the seeds grown on the medium without LAA. This result clearly indicated the positive role played by the LAA in plant regeneration *in vitro*. In a review Nicholas Smirnoff [16] has given an account on implication of ascorbic acid in various physiological functions, including cell wall expansion, regulation of cell division by influencing progression from G1 to S phase [13] and studies have indicated involvement of ascorbic acid in cell division and elongation [15]. It is also known that ascorbic acid acts as a cofactor for various enzymes involved in different pathways (3). Hence it is possible that ascorbic acid may be aiding/accelerating the roots and shoots formation owing to its combined activities of cell expansion, division and differentiation. However more work is needed to decipher the exact pathway of LAA action.

The profound shoot regeneration was an indication of enhanced photosynthetic pigments, which was further confirmed by chlorophyll content estimation in control and LAA supplemented plants. Chlorophyll estimation indicated almost two fold increase of Chlorophyll content in LAA supplemented medium as compared to control plants without LAA. Though the direct evidence for chlorophyll biosynthesis by LAA is not known yet, it's role in photosynthesis has been investigated which is been described in detail in review by

Nicholas Smirnoff [16]. LAA has a central role in photosynthesis through, 1) scavenging reactive oxygen species such as hydrogen peroxide [17] as chloroplasts lack catalase, thus protecting chlorophyll degradation, 2) acts as a direct electron acceptor [17,3] it is a cofactor in a reaction required for the formation zeaxanthin, which acts as a photoprotectant [18,19]. It has been shown in barley leaves that ascorbate pool is correlated with photosynthetic capacity and with the supply of carbohydrates [20]. Thus the supplemented LAA may be aiding in protection of chlorophyll and therefore increase in chlorophyll content was observed.

The larger amount of chlorophyll presence may further aid in better shoot regeneration and formation of leaflets. Through this study we have found that Vitamin C has the regeneration capacity to induce both shoots and roots in different plants grown *in vitro* belonging to different families and having diverse habits. The use of LAA in the tissue culture medium is helpful in many ways, as an antioxidant and also as a growth regulator. Presence of LAA alone can mitigate the functions of supplemented cytokinin and an auxin as shown by our experiments, though the mechanism and pathways behind this function is unclear at present.

4. CONCLUSION

In vitro culture of stem explants of *C. asiatica* and seed explants of *T. foenumgraecum* revealed that supplementing LAA aids in the whole plant regeneration, whereas in the case of *S. album* supplementing LAA only resulted in the shoot regeneration, but no root formation. Results obtained owe to its divergent properties including, antioxidant nature which is important to protect the explants from harmful oxidation of phenols, its role as a cofactor for many enzymes involved in key pathways, its effect on cell proliferation and cell division. Chlorophyll content was found to be higher in the *in vitro* grown plants with LAA as compared to those grown without LAA, suggesting that LAA is mitigating the function of both auxin and cytokinin. Our results indicate that LAA can be used as an useful component in the tissue culture medium for the purpose of regeneration to micropropagate plants including rare, endangered, economically and medicinally important plants to protect the germplasm and to have a continuous supply of the plant material. However the mechanism and pathways behind the growth promoting effect of LAA has to be understood for its better application.

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COMPETING INTERESTS

Author has declared that no competing interest exists

REFERENCES

1. Horton RH. Coenzymes and Vitamins. Principles of Biochemistry. Pearson Education International: Upper Saddle River; 2006.

2. George EF, Hall MA, De Klerk GJ. The Components of Plant Tissue Culture Media II. Plant Propagation by Tissue Culture. Springer. Dordrecht. 2008;115-119
3. Arrigoni, De Tullio. Ascorbic acid: much more than just an antioxidant. Biochimica et Biophysica Acta. 2002;1569:1-9
4. Smirnoff, Wheeler. Ascorbic acid in plants: biosynthesis and function. Critical Reviews in Biochemistry and Molecular Biology. 2000;35:291-314
5. Conklin PL. Recent advances in the role and biosynthesis of ascorbic acid in plants. Planta, Cell and Environment. 2001;24:384-394
6. Conklin PL, Barth C. Ascorbic acid, a familiar small molecule intertwined in the response of ozone, pathogens and the onset of senescence. Plant, Cell and Environment. 2004;27:959-971
7. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidant, oxidative damage and oxygen deprivations stress. A Review Ann. Bot. 2003;91:179-194.
8. John Dowdle, Takahiro Ishikawa, Stephen Gatzek, Susanne Rolinski, Nicholas Smirnoff. Two genes in *Arabidopsis thaliana* encoding GDP-L-galactose phosphorylase are required for ascorbate biosynthesis and seedling viability. The Plant Journal. 2007;52:673-689. Doi: 10.1111/j.1365-313X.2007.03266.x
9. Farahat MM, Soud Ibrahim MM, Lobna SL, Taha, Fatma El-Quesni EM. Response of vegetative growth and some chemical constituents of *Cupressus sempervirens* L. to foliar application of ascorbic acid and zinc at Nubaria. World J. of Agric. Sci. 2007;3(3):282-288.
10. Joy RW, Patel KR, Thorpe TA. Ascorbic Acid Enhancement of Organogenesis in Tobacco Callus. Plant Cell, Tissue and Organ Culture. 1988;13(3):219-228. Doi:10.1007/BF00043670
11. Murashige T, Skoog F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiologica Plantarum. 1962;15(3):473-497. Doi:10.1111/j.1399-3054.1962.tb08052.x
12. Witham FH, Blaydes DF, Devlin RM. Experiments in Plant Physiology. Van Nostrand Reinhold Company. New York. 1971;55-56.
13. Arrigoni O. Ascorbate system in plant development. Journal of Bioenergetics and Biomembranes. 1994;26:407-419
14. Akhilender Naidu K. Vitamin C in human health and disease is still a mystery ? An Overview. BMC, Nutrition Journal. 2003;2:7
15. De Pinto MC, Francis D, Gara L. The Redox State of the Ascorbate-dehydroascorbate Pair as a Specific Sensor of Cell Division in Tobacco BY-2 Cells. Protoplasma. 1999;209(1-2):90-97
16. Nicholas Smirnoff. The Function and Metabolism of Ascorbic Acid in Plants. Annals of Botany. 1996;78:661-669.
17. Miyake C, Asada K. Thylakoid bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product, monodehydroxyascorbate radicals in the thylakoids. Plant and Cell Physiology. 1992;33:541-553.
18. Neubauer C, Yamamoto HY. The role of ascorbate in the related ascorbate peroxidase, violaxanthin de-epoxidase and non-photochemical fluorescence-quencing activities. In: Yamamoto HY, Smith CM, eds. Photosynthetic responses to the environment. Rockville. Maryland: American Society of Plant Physiologists.1993;166-171

19. Neubauer C, Yamamoto HY. Mehler-peroxidase reaction mediates zeaxanthin formation and zeaxanthin-related fluorescence quenching in intact chloroplasts. Plant Physiology. 1992;99:1354-1361.
20. Smirnoff N, Pallanca JE. Ascorbate metabolism in relation to oxidative stress. Biochemical Society Transactions. 1996;24:472-478.

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