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# Rapid *in vitro* adventitious rooting and proliferation by leaf and nodal cultures of *Momordica cymbalaria* Fenzl.

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**ABSTRACT**

An effective approach for rapid *in vitro* rooting and proliferation of leaf and nodal cultures of *Momordica cymbalaria* has been developed. To the ability of induction of rhizogenesis, both leaf and nodal explants were used in culture on Murashige and Skoog (MS) medium. The effects of auxins such as α-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA) at different concentrations have been studied. The maximum number of roots was produced from nodal explants containing 1.5 mg/L of NAA (9.3 ± 0.61), 1.0 mg/L of IBA (6.5 ± 0.41), and 1.0 mg/L of IAA (3.5 ± 0.66), and in leaf explants containing 1.0 mg/L of NAA (5.7 ± 0.56), 1.0 mg/L of IBA (6.9 ± 0.61), and 1.5 mg/L of IAA (5.0 ± 0.73) on the half-strength MS medium. For the root induction, NAA is the very effective auxin in node explants of *M. cymbalaria.* Moreover, a large amount of quercetin bioactive compound is presented in the roots, which is used in anticancer drugs, and we have described an effective method for the *in vitro* rhizogenesis of the *M. cymbalaria.*

## 1. INTRODUCTION

*Momordica cymbalaria* Fenzl. (Cucurbitaceae) is a climber and perpetual herb. It is also called as athalakkai. It climbs on the ground surface and supports by the help of tendrils. *Momordica cymbalaria* fruits resembled a small variety of *Momordica charantia*. The plant is available with fruits in various states of India such as Telangana, Madhya Pradesh, Karnataka, and Tamil Nadu states. During the rainy season, it is around the fences of farms [1]. The plants die at the end of the season, but the underground tuberous roots were remained and emerge in the next season to maintain its perennial habits. This plant is not very popular because of its bitter taste and lack of understanding of its nutrient content [2].

Recent research has revealed that methanol extraction of *M. cymbalaria* has anticancer properties in aerial and underground parts compared to standard cyclophosphamide against ehrlich ascites carcinoma-induced cancer in rats [3]. Fruit of *M. cymbalaria* consists of a high amount of fiber along with calcium, potassium, and C vitamin [4]. According to the previous studies, fruit and root extracts of athalakkai were very useful in various treatments like diabetes, hypolipidemia [5,6], diarrhea [7], and ulcer [8]. For menstrual irregularities, antifertility, antiovulatory, abortifacient, cardioprotective properties, and hepatoprotective activity, roots of *M. cymbalaria* are used [9].

Rhizogenesis is the process of root formation in plants. The initiation of roots is one type of organogenesis [10]. It is essential to have successful root growth, although root growth does not occur in the initial stage. After micropropagation, root formation is essential for the plant growth, although it does not occur in the initial stage.

The ability of the shoots to initiate root or plant to survive acclimatization on the concentration of cytokinins and auxins in the Murashige and Skoog (MS) medium is required.There are three stages such as induction, initiation, and elongation in the *in vitro* root development. The MS medium that contains auxins such as 2,4-D, α-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA) is a very suitable medium for the root and shoot proliferation. Cytokinins and auxins at high concentrations are favorable for shoot formation, but it restricts root formation. The MS medium without adding any plant growth

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regulators and less amount of auxin containing medium gives much rooting in many cucurbits [11]. The combination of NAA and IBA is a very suitable combination than 2,4-D for the callus induction and direct rhizogenesis from leaf and stem explants of *Heliotropium indicum* [12]. High proportion of rooting was recorded in *Tricosanthes dioica* by the combination of 0.5 mg/L of IBA and 2.0 mg/L of NAA [13]. In *Erythrina variegata*, without any involvement of callus formation, high percentage of rooting was observed by using lower concentrations of NAA and 2,4-D [14].

The main objective of this experiment is the optimization of *in vitro* rooting and plant growth regulator conditions in different parts of *M. cymbalaria*. Our investigation of *in vitro* adventitious rhizogenesis from leaf and node explants of *M. cymbalaria* was not done until now. In this study, the protocol that is very useful for the extraction of bioactive compounds from the roots of medicinally valuable plants is also discussed.

## 2. MATERIALS AND METHODS

The tubular roots of *M. cymbalaria* Fenzl were collected in the rainy season, in the Jammikunta, Kamalapur Crop Farms, Warangal District, Telangana, India. The collected plants were maintained in the Departmental Greenhouse. Young, healthy plants were raised under *in vivo* condition and different explants like a leaf and nodes were washed in running tap water for half an hour and rinsed with labolene detergent for 3–4 times and again washed with running tap water. The rinsed explants were surface sterilized in 0.1% mercuric chloride for 3–5 minutes and rinsed with double sterilized distilled water for 3–4 times in the laminar airflow chamber and then left for air dry in a sterile environment.

The sterilized leaf and node explants were inoculated into the test tubes containing the MS medium with 0.8% agar-agar, 3% sucrose, and various concentrations of NAA, IBA, 6-Benzylaminopurine (BAP), and IAA at 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L individually. The pH of the medium is regulated between 5.6 and 5.8 by the addition of 0.1 N NaOH or 0.1% HCl, then agar (0.8%) is added to the medium and heated for dissolving. The medium was sterilized in the autoclave at 121°C for 15 minutes below 15 psi. The culture tubes were maintained at temperature between 25°C ± 2°C for 16/8 hours with light and dark cycle. Fluorescent tubes (Philips, India) were used for regulating photoperiod.

## 3. RHIZOGENESIS

Young healthy leaf and node explants selected from *in vivo* grown plants were inoculated on medium containing various concentrations of NAA, IAA, and IBA (0.5, 1.0, 1.5, 2.0, and 2.5 mg/L) (Tables 1 and 2). The NAA is a more suitable plant growth regulator for the induction of callus, stimulation of the cluster, and multiple roots than IBA and IAA. The percentage of induction of callus and proliferation of roots per explants was recorded after 4 weeks of culture. Rooted shoots should be carefully taken from the medium and thoroughly cleaned with distilled water. The obtained roots were stored in the shade for 15–30 days and then dried and preserved in a polyethylene cover for biological activity and phytochemical analyzes in future studies.

## 4. RESULTS AND DISCUSSION

When node and leaf explants were grown on MS basal media without hormones, no morphogenetic response was observed, whereas induction of callus was observed within one week of culturing on the MS medium enriched with various concentrations

**Table 1:** Rhizogenesis from nodal explants of *M. cymbalaria* Fenzl.

| Growth Harmones mg/L | Callus Morphology | | No of inoculated calli | No of calli forming roots | Time taken to initiate roots from callus (days) | Mean number of roots ± standard error (SE) | Mean number of root length ± SE |
|---|---|---|---|---|---|---|---|
| Node | Color | Texture | | | | | |
| **NAA** | | | | | | | |
| 0.5 | Green | Nodular | 20 | 17 | 13–14 | 5.8 ± 0.71 | 2.0 ± 0.70 |
| 1 | green | Compact | 20 | 19 | 13–14 | 7.3 ± 0.68 | 3.1 ± 0.55 |
| 1.5 | green | Compact | 20 | 14 | 10–12 | **9.3 ± 0.61** | 2.4 ± 0.62 |
| 2 | brown | Friable | 20 | 11 | 10–12 | 6.2 ± 0.78 | 2.8 ± 0.68 |
| 2.5 | brown | Friable | 20 | 10 | 10–12 | 5.7 + 0.63 | **3.2 ± 0.86** |
| **IBA** | | | | | | | |
| 0.5 | White | Puff shaped | 20 | 13 | 8–10 | 4.5 + 0.59 | 3.10 ± 0.47 |
| 1 | white | Puff shaped | 20 | 17 | 8–10 | **6.5 + 0.41** | 1.4 ± 0.32 |
| 1.5 | green | Compact | 20 | 16 | 8–10 | 4.9 ± 0.35 | 3.0 ± 0.62 |
| 2 | green | Compact | 20 | 11 | 10–12 | 3.4 ± 0.40 | 2.5 ± 0.38 |
| 2.5 | green | Nodular | 20 | 12 | 10–12 | 2.4 ± 0.33 | **5.0 ± 0.77** |
| **IAA** | | | | | | | |
| 0.5 | Half white | Friable | 20 | 20 | 12–14 | 2.9 ± 0.53 | 2.4 ± 0.58 |
| 1 | white | Friable | 20 | 19 | 12–14 | **3.5 ± 0.66** | 1.5 ± 0.31 |
| 1.5 | Light green | Nodular | 20 | 13 | 18–20 | 3.2 ± 0.46 | 2.5 ± 0.38 |
| 2 | green | Compact | 20 | 15 | 18–20 | 2.3 ± 0.42 | 2.0 ± 0.43 |
| 2.5 | green | Compact | 20 | 11 | 18–20 | 2.2 ± 0.51 | **3.18 ± 0.47** |

**Table 2:** Rhizogenesis from leaf explants of *M. cymbalaria* Fenzl.

| Growth Harmones mg/L | Callus Morphology | | No of inoculated calli | No of calli forming roots | Time taken to initiate roots from callus (days) | Mean number of roots ± SE | Mean number of root length ± SE |
|---|---|---|---|---|---|---|---|
| Leaf | Color | Texture | | | | | |
| **NAA** | | | | | | | |
| 0.5 | light green | nodular callus | 20 | 18 | 10–12 | 5.4 ± 0.59 | 2.9 ± 0.63 |
| 1 | green | Compact callus | 20 | 20 | 8–10 | **5.7 ± 0.56** | 1.8 ± 0.36 |
| 1.5 | Dark green | Compact callus | 20 | 16 | 8–10 | 4.7 ± 0.57 | 2.7 ± 0.53 |
| 2 | light brown | friable callus | 20 | 15 | 14–15 | 3.4 ± 0.51 | 2.5 ± 0.47 |
| 2.5 | Dark brown | Compact callus | 20 | 12 | 14–15 | 2.8 ± 0.52 | **3.9 ± 0.66** |
| **IBA** | | | | | | | |
| 0.5 | Brown | friable callus | 20 | 12 | 10–12 | 5.4 ± 0.59 | 3.4 ± 0.57 |
| 1 | light green | Compact callus | 20 | 14 | 8–10 | **6.9 ± 0.61** | 1.7 ± 0.40 |
| 1.5 | Dark green | compact callus | 20 | 11 | 8–10 | 4.0 ± 0.58 | 2.5 ± 0.42 |
| 2 | Light brown | Nodular callus | 20 | 10 | 13–14 | 2.69 ± 0.47 | 2.0 ± 0.43 |
| 2.5 | brown | nodular callus | 20 | 10 | 10–12 | 2.8 ± 0.38 | **4.1 ± 0.66** |
| **IAA** | | | | | | | |
| 0.5 | Half white | friable callus | 20 | 19 | 10–12 | 4.1 ± 0.72 | 2.8 ± 0.68 |
| 1 | green | Compact callus | 20 | 18 | 14–15 | 3.9 ± 0.67 | 2.5 ± 0.68 |
| 1.5 | green | Compact callus | 20 | 14 | 14–15 | **5.0 ± 0.73** | 1.3 ± 0.28 |
| 2 | light brown | puff shaped | 20 | 11 | 12–14 | 4.5 ± 0.64 | 2.3 ± 0.34 |
| 2.5 | brown | Nodular callus | 20 | 10 | 12–14 | 2.0 ± 0.35 | **3.2 ± 0.65** |

of auxins such as IBA, IAA, and NAA of 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L individually (Tables 1 and 2, Fig. 1a, Fig 2a–c). The same callus was subcultured on the half-strength MS medium, as a result rhizogenesis was occurred (Fig. 1c–f, Fig. 2d–f).

Direct rhizogenesis also appeared in some node explants before callus induced completely on 0.5 mg/L of NAA alone with the half-strength MS medium (Fig. 1a). Different types of colors and textures of calli were produced in both node and leaf explants containing MS medium with various concentrations of NAA, IBA, and IAA (Tables 1 and 2). Green nodular callus formation has taken place in node explants on MS medium fortified with 0.5 mg/L of NAA alone (Fig. 1a). Brown and green colored callus was produced in leaf explants containing 0.5 and 1.0 mg/L of IBA, respectively (Fig. 2b and c).

The highest percentage of rhizogenesis was obtained from green compact callus on the half-strength MS medium containing 1.5 mg/L of NAA in node explants, but in the leaf explants, light green compact callus was produced a maximum percentage of roots containing a medium at 1.0 mg/L of IBA.

In this study, various concentrations of auxins, that is, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/L were analyzed for their consequence on rhizogenesis. In node explants, a number of adventitious roots were initiated at 0.5, 1.0, and 1.5 mg/L of IAA, IBA, and NAA, respectively (Table 1), whereas in leaf explants, numerous root induction takes place at 1.0, 1.0, and 0.5 mg/L of NAA, IBA, and IAA, respectively (Table 2).

The percentage of rhizogenesis was decreased with an increase in the concentration of auxins NAA, IBA, and IAA alone on node and leaf explants. The same tendency is seen in all cultures, but in node explants, rhizogenesis capacity is somewhat different, which is 1.5 mg/L of NAA produced when compared with low concentration (Tables 1 and 2). Among the different concentrations of auxins, the highest mean number of root length was recorded at 1.5 mg/L of NAA alone in the node explant (Tables 1 and 2).

In general, auxins (IBA, NAA, and IAA) have been promoted a maximum percentage of rooting in plants. However, in this study, NAA is the most successful auxin for the induction of rooting in node explants. Similar findings were reported in tomato [15].

It was observed that both node and leaf explants do not have an equal potential to regenerate roots. Node explants have shown a higher percentage of rhizogenesis than leaf explants. Previous studies were also similar to our result, that is, various growth regulators influenced the induction of roots as well as their elongation [16,17]. In *Citrullus colocynthis*, 2.0 mg/L of IAA and 1.5 mg/L of IBA were more suitable for the formation of cluster roots in stem explants and also 2.0 mg/L of 2,4-D, 1.5 mg/L of IBA, and 2.0 mg/ L of IAA are the best for the production of multiple root production in leaf explants [18]. The discrepancy in rooting response may be a result of genotype or cultural conditions or other factors in plants.

On the other hand, Mahendranath et al. [19] reported that IBA has produced the maximum biomass when compared to IAA and also individually superior over IAA or NAA in the induction of rooting

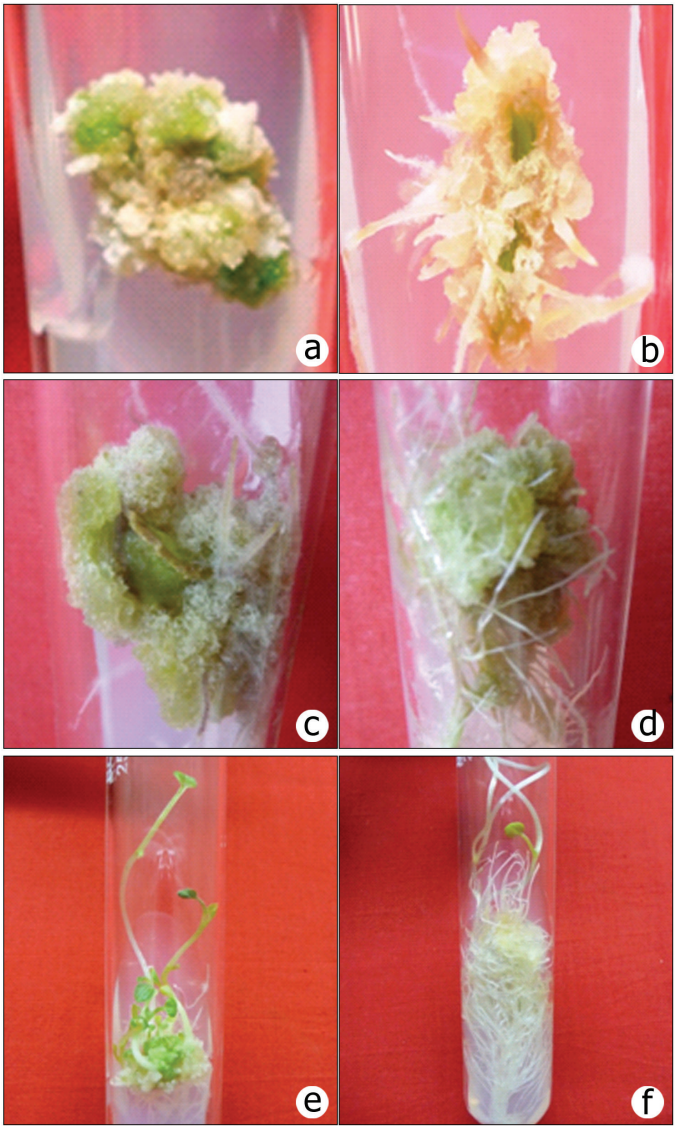


**Figure 1:** Rhizogenesis from nodal explants of *M. cymbalaria* Fenzl. (a) Green nodular callus formation of node explants on the MS medium fortified with 0.5 mg/L of NAA. (b) Roots initiation from cut margins of node explant on the MS medium fortified with 0.5 mg/L of NAA. (c) Roots initiation from green compact callus on the MS medium fortified with 1.0 mg/L of NAA. (d) Elongation of roots after three weeks of culture on the half-strength MS medium fortified with 1.0 mg/L of NAA. (e) Formation of shoots and roots on the MS medium fortified with 1.5 mg/L of NAA. (f) Maximum number of roots after three weeks of culture on the half-strength MS medium fortified with 1.5 mg/L of NAA..

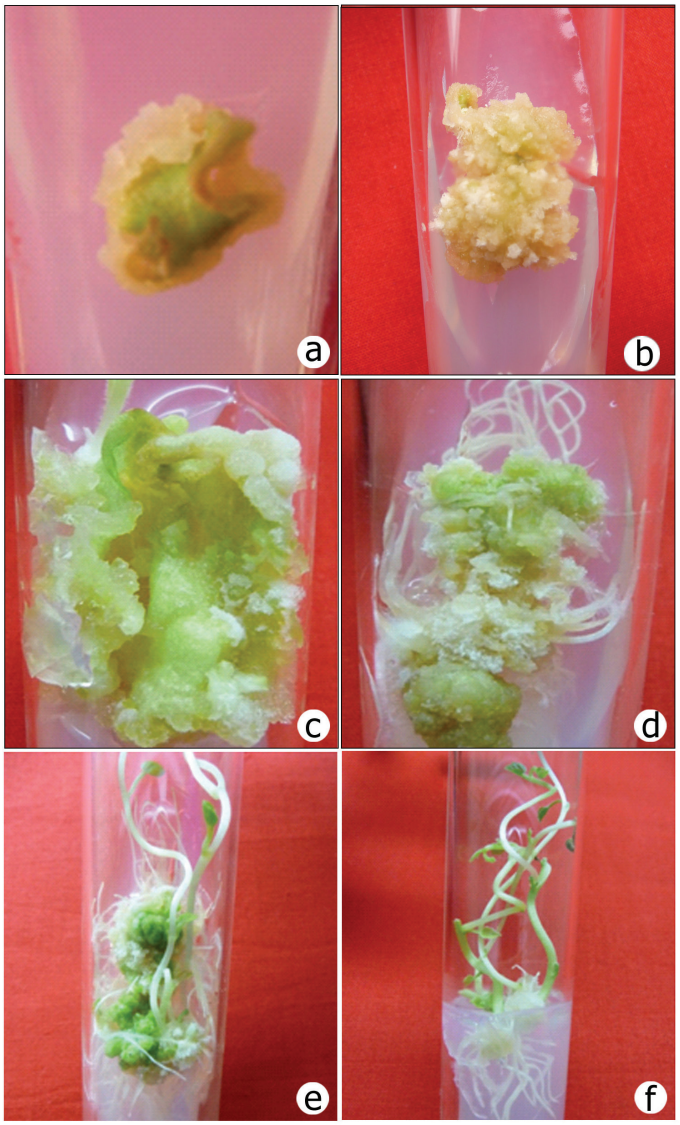


**Figure 2:** Rhizogenesis from leaf explants of M. cymbalaria Fenzl. (a) Callus initiation from cut margins of leaf explant on the MS medium fortified with 0.5 mg/L of IBA. (b) Light brown callus production on the MS medium fortified with 0.5 mg/L of IBA after 10–12 days of inoculation. (c) Induction of green callus on the MS medium fortified with 1.0 mg/L of IBA. (d) Initiation of roots from leaf callus cultures on 1.0 mg/L of IBA after four weeks of culture. (e) Formation of shoots and roots after subculture for two weeks on the MS medium fortified with 1.0 mg/L of IBA. (f) Maximum number of roots after three weeks of culture on the half-strength MS medium fortified with 1.0 mg/L of IBA.

has been reported earlier in *Psoralea corylifolia* [20]. A similar study was also observed in *Withania somnifera* [21], *Morinda citrifolia* [22], and *Periploca sepium* [23].

In this study, we have been observed that the IBA was found effective for the induction of maximum rooting in leaf explants. The influence of IBA on rhizogenesis has also been supported by Neto et al. [24].

Of the three auxins, NAA, IBA, and IAA, tested NAA is the most effective for the induction of roots in node explants (Table 1), whereas IBA is the most effective for rhizogenesis in leaf explants (Table 2).

## 5. CONCLUSION

The present study revealed that efficient rhizogenesis was achieved in *M. cymbalaria* Fenzl. Both leaf and nodal explants were responded for the rhizogenesis at different concentrations of auxins. Compared with leaf explants, nodal explants induced more number of roots (9.3 ± 0.61) by using NAA at 1.5 mg/L concentration. Depending on genotype and culture conditions, variation in rhizogenesis response may occur. In addition, this protocol is useful for the production of large amounts of bioactive compounds in certain medicinal plants. Therefore, the roots of *M. cymbalaria* contain a quercetin bioactive compound that is used

in pharmacy for the design of anticancer. A unique characteristic of this study is the *in vitro* adventitious rooting and proliferation of leaf and node explants of *M. cymbalaria,* which have not been previously reported.

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## REFERENCES

1. Devi T, Rajasree V, Premalakshmi V, Hemaprabha K, Praneetha S. *In vitro* protocol for direct organogenesis in *Momordica cymbalaria* Fenzl. Int J Curr Microbiol App Sci 2017; 6(4):2392–402.
2. Nikam TD, Ghane SG, Nehul JN, Barmukh RB. Induction of morphogenic callus and multiple shoot regeneration in *Momordica cymbalaria* Fenzl. Indian J Biotechnol 2009; 8:442–7.
3. Chittapur R. *Momordica cymbalaria* nutritious underutilized vegetable taxonomy, nutritional ,medicinal, propagation, hybridization and cytological aspects. Int J Argicul Sci Res 2015; 5(4):2250–7.
4. Rao BK, Kesavulu MM, Giri R, Appa Rao C. Antidiabetic and hypolipidemic effect of *Momordica cymbalaria* Hook fruit powder in alloxan-diabetic rats. J Ethano Pharmacol 1999; 67:103–9.
5. Grover JK, Yadav S, Vats V. Medicinal plants of India with antidiabetic potential. J Ethano Pharmacol 2002; 81:81–100.
6. Vrushabendra swamy B, Jayaveera K, Raveendra reddy K, Bharathi T. Anti-diarrhoeal activity of fruit extract of *Momordica cymbalaria* Hook. F. Internet J Nut Wellness 2008; 5(2).
7. Bharathi Dhasan P, Jegadeesan M, Kavimani S. Antiulcer activity of aqueous extract of fruits of *Momordica cymbalaria* Hook in wistar rats. Phcog Res 2010; 2(1):58–61.
8. Raju K, Saraswati CD, Balaraman R, Ajeesha EA. Anti Implantation activity of the ethanolics extract of *Momordica cymbalaria* Fenzl in rats. Indian J Pharmacology 2007; 39(2):90–6.
9. Raju K, Balaraman R, Firdous KMW, Vinoth Kumar M. Hepatoprotective effects of *Momordica cymbalaria* Fenzl against CCl4 induced hepatic injury in rats. Pharmacologyonline 2008; 1:365–74.
10. Tabei Y, Kanno T, Nishio T. Regulation of organogenesis and somatic embryogenesis by auxin in melon, *Cucumis melo* L. Plant Cell Rep 1991; 10(5):225–9.
11. Mythili JB, Thomas P. Micropropagation of pointed guard (*Trichosanthes dioica* Roxb.). Scientia Horticulture 1999; 79 (1–2):87–90.
12. Bagadekar AN, Jayaraj M. Invitro rhizogenesis from leaf and stem callus of *Heliotropium indicum* L. Medicinal herb. Int J Plant Anim Environ Sci 2011; 1(2):1–5.
13. Kumar S, Singh M, Singh AK, Srivastava K, Banerjee MK. Invitro propagation of pointed guard (*Trichosanthes dioica* Roxb.) Indian Institute Veg Res 2003; 2(6):74–5.
14. Shasthree T, Imran MA, Mallaiah B. *In vitro* rooting from callus cultures derived from seedling explants of *Erythrina variegata* L. Current Trend Biotechnol Pharm 2009; 3(4):447–52.
15. Taylor JLS, Van Staden J. Plant-derived smoke solutions stimulate the growth of *Lycopersicon esculentum* roots *in vitro*. Plant Growth Reg 1998; 26:77–83.
16. Balvanyos I, Kursinszki L, Szoke E. The effect of plant growth regulators on biomass formation and lobeline production of *Lobelia inflata* L. hairy root cultures. Plant Growth Reg 2001; 34:339–45.
17. Balestri E, Bertini S. Growth and development of *Posidonia oceanica* seedlings treated with plant growth regulators: Possible implications for meadow restoration. Aquat Bot 2003; 76:291–7.
18. Ramakrishna D, Shasthree T. Adventitious rooting and proliferation from different explants of *Citrullus colocynthis* (L.) Schard an endangered medicinally important cucurbit. Asian J Biotechnol 2015; 7(2):88–95.
19. Mahendranath G, Venugopalan A, Parimalan R, Giridhar P, Ravishankar GA. Annatto pigment production in root cultures of Achiote (*Bixa orellana* L.). Plant Cell Tiss Organ Cult 2011; 16:517–22.
20. Baskaran P, Jayabalan N. Psoralen production in hairy roots and adventitious root cultures of *Psoralae corylifolia*. Biotechnol Lett 2009; 31:1073–7.
21. Sivanandhan G, Arun M, Mayavan S, Rajesh M, Mariashibu TS, Manickavasagam M, Selvaraj N, Ganapathi A. Chitosan enhances withanolides production in adventitious root cultures of *Withania somnifera* (L.) Dunal. Industrial Crops and Products 2012; 37:124–9.
22. Baque MA, Lee EJ, Paek KY. Medium salt strength induced changes in growth, physiology and secondary metabolite content in adventitious roots of *Morinda citrifolia*: the role of antioxidant enzymes and phenylalanine ammonia lyase. Plant Cell Rep 2010; 29:685–94.
23. Zhang J, Gao WY, Wan J, Li XL. Effects of sucrose concentration and exogenous hormones on growth and periplocin accumulation in adventitious roots of *Periploca sepium* Bunge. Acta Physiologiae Plantarum 2012; 34:1345–51.
24. Neto VBP, Res LB, Finger FL, Barros RS, Carvalho CR, Otoni WC. Involvement of ethylene in the rooting of seedling shoot cultures of *Bixa orellana* L. *In vitro* Cell Dev Biol Plant 2009; 45:693–700.

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