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# *In vitro* Clonal Propagation of *Physalis minima* L. - A Medicinal Herb Using Nodal Explants

**Kamrun Nahar, Md. Abul Kashem*, Tapash Kumar Bhowmik and Md. Mahbubur Rahman**

*Laboratory of Plant Tissue Culture and Biotechnology, Department of Botany, University of Chittagong, Chattogram-4331, Bangladesh*

*Key words:* Organogenesis, Medicinal herb, Callus induction, *In vitro* rooting

## Abstract

A highly efficient and repeatable *in vitro* protocol has been developed for rapid and mass scale propagation of *Physalis minima* L. Fresh nodal segments from field grown plants were aseptically cultured on MS medium alone or in combination with different PGRs**.** The nodal segments showed direct organogenesis and produced the highest number of multiple shoot buds (MSBs) (4.87 ± 0.12) per explant when cultured on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA. Nodal and leaf segments from *in vitro* grown shoots were further cultured on MS medium containing different concentrations and combinations of PGRs. Among the different combinations, MS + 2.0 mg/l BAP + 1.0 mg/l IAA was found to be the best for induction of maximum number of shoot buds (4.13 ± 0.11) per nodal explant with the highest percentage (95%) of shoot bud formation within 15-18 days of culture. Nodal segments proved to be better than leaf explants for callus induction. Maximum 86% of nodal segments produced brownish compact calli on MS medium fortified with 1.0 mg/l 2, 4-D + 0.5 mg/l Kn. It was noticed that the brownish friable calli did not undergo any kind of differentiation. The highest increase in length (cm) of shoot bud (3.60 ± 0.11cm) was noted on MS medium augmented with 1.0 mg/l BAP + 0.5 mg/l NAA. The elongated multiple shoot buds were individually transferred to rooting media. Maximum numbers of roots (5.87 ± 0.17) per micro shoot was recorded in half-strength MS medium supplemented with 1.0 mg/l IBA. The stout rooted plantlets were hardened in pots at 75% survival rate. Both micro propagated plants were stable and showed uniform morphological and growth characteristic following their establishment.

## Introduction

*Physalis minima* is commonly known as wild gooseberry, is an important medicinal plant belongs to the family Solanaceae (Sheeba et al. 2010). It is frequently found in organic matter-rich waste as well as agrarian land, distributed around the tropical and subtropical

*Author for correspondence: <kashem.bot@cu.ac.bd>.

regions of Asia (Chothani and Vaghasiya 2012). This species is used as tonic, diuretic, laxative, anti-inflammatory, Splenomegaly, and bladder ulcers (Mungole et al. 2011). Its fruit is edible (Cribb and JW 1976). Crushed leaves and fruits are applied over snakebite (Karthikeyani and Janardhanan 2003). Extracts of the plant have anticancer activity (Duke and Ayensu 1985) and extracts of leaves have antimicrobial activity (Nayeemulla et al. 2006). Mixture of mustard oil and water along with leaf extracts of *P. minima* has been used as a remedy for earache (Chopra et al. 1986). Pietro et al. (2000) reported that extract of *Physalis* containing physalins display anti-mycobacterial activity against *Mycobacterium tuberculosis*, *M. avium*, *M. kansii*, *M. malmoense* and *M. intracellulare*.

Despite the plant has enormous therapeutic potential, over-exploitation and ongoing environmental pollution are putting it in risk of extinction. Consequently, it is essential to develop conservation strategies for enhancing its survival. Micropropagation offers a significant opportunity for the preservation and large-scale multiplication of beneficial species (Usman et al. 2008, Afroz et al. 2009). However, there are very few reports in the current literature have been found about *P. minima* micropropagation experiments. Thus, the main objective of the present investigation was to develop an effective reproducible *in vitro* protocol for mass scale propagation and sustainable supply of this plant in future drug discovery.

## Materials and Methods

The plant materials used in this experiment were collected from the botanical garden, University of Chittagong, Chattogram. The nodal segments were washed thoroughly under running tap water and later soaked them in 1% Savlon (ACI Ltd., Bangladesh) for about 10-15 min, washed 3-4 times again by running tap water and three to four times by distilled water. The nodal segments were surface sterilized by dipping them with 0.1% (w/v) mercuric chloride for 2 min, followed by 3-4 rinses with sterile double distilled water in a laminar air flow cabinet. They were then surface sterilized with 70% (v/v) ethanol for 1 min. and washed them 4-5 times with sterile double distilled water. The surface sterilized explants were sized to 1.0-1.5 cm in length containing a single node with an axillary bud. The explants were inoculated in agar gelled MS basal media supplemented with auxin and cytokinins at different concentrations for shoot induction and proliferation. Shoots induced from the *in vitro* cultures were further used as explants for adventitious shoot proliferation. For *in vitro* rooting, individual shoots (3-5 cm) were excised from the proliferated shoot cultures and implanted on half strength MS media with different concentrations and combinations of IBA, IAA and NAA. All the media were supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. The pH of each medium was adjusted to 5.80 before autoclaving at 121°C for 20 min and the medium was dispensed into 25 × 150 mm culture tubes or 250 ml conical flasks. The cultures were incubated for 14 h photoperiod at 25 ± 2°C under fluorescent light (Intensity: 2000-3000 lux).

For acclimatization of the *in vitro* developed plantlets in outside environment, the culture vessels were kept outside the culture room with gradual increasing the time up to three days. Finally, the plantlets were taken out of the culture vessels and washed with running tap water for removal of agar attached to the roots. Then the plantlets were transplanted to small plastic pots containing garden soil and compost in the ratio of 1:1 and kept in room temperature for 3-5 days. The soil was treated with 0.1% agrosan (fungicide) solution. After gradual acclimatization, plantlets were finally transferred to large pots and kept in outside natural environment. Each treatment was repeated thrice. The graphs and mean values of parameters were compared by analysis of variance using SPSS (Ver. 26) software.

## Results and Discussion

Various concentrations of BAP and Kn (1.0-2.0 mg/l) singly and in combination with NAA and IAA were used in MS medium to observe their effects on the shoot proliferation in nodal explants of *P. minima*. The nodal segments undergo direct organogenesis producing multiple shoot buds within 18-30 days of culture. The highest

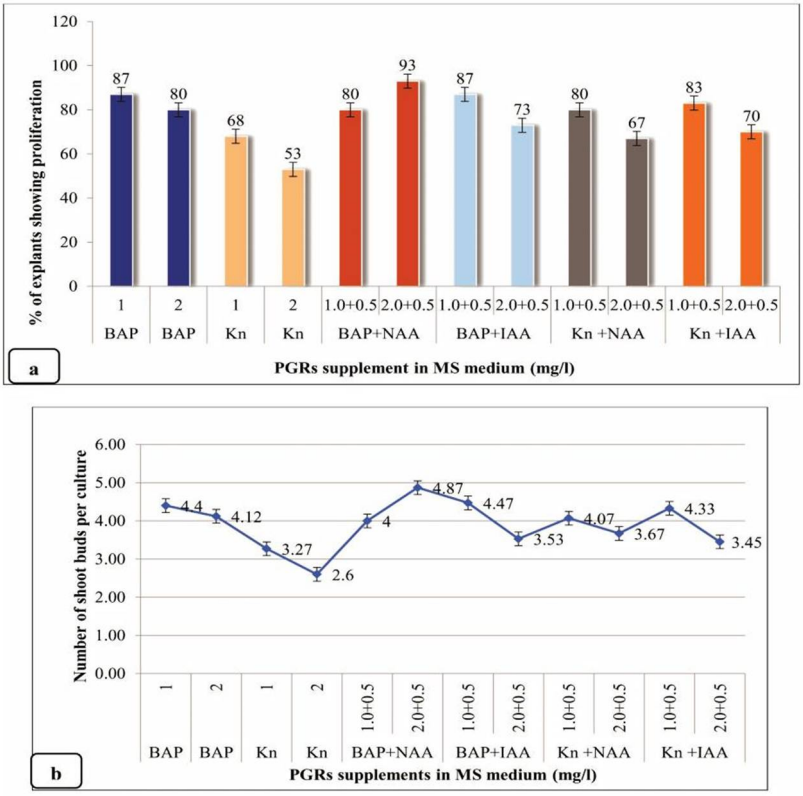


Fig. 1(a-b). Effect of MS medium with different concentration and combination of PGRs on *in vitro* shoot formation from nodal explants of *Physalis minima*: (a) percentage of proliferation, and (b) number of shoot buds per culture after 18-22 days.

number of nodal segments (93%) produced multiple shoot buds when cultured on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA (Fig. 1a) and this medium also produced maximum number of shoot buds (4.87 ± 0.12) per nodal segment (Fig. 1b). It is proved that higher amount of cytokinin in combination with lower amount of auxin promoted direct organogenesis which was better in combination of BAP and NAA rather than that of any other combination of PGRs. The importance of plant growth regulators on shoot propagation was highlighted in various studies. Particularly, the synergistic effect of BAP with NAA has been shown in many other medicinal plants like, *Centella asiatica* (Hossain et al. 2000), *Stemona tuberosa* (Biswas et al. 2011), *Aloe vera* (Das and Rout 2018), *Withania somnifera* (Goswami et al. 2022), *Leucas biflora* (Paul and Biswas 2024), *Spilanthes acmella* (Sana and Rani 2025), *Actinidia deliciosa* (Uddin et al. 2025).

For callus induction, leaf and nodal segments were cultured on MS medium supplemented with different concentrations of auxin and cytokinin. The nodal explants showed better response than leaf segments and produced brownish compact callus on different PGRs supplemented medium. The maximum callus tissue (86%) produced from nodal explants when cultured on MS medium containing 1.0 mg/l 2, 4-D + 0.5 mg/l Kn (Fig. 2). The nature of callus in all the media compositions was brownish compact. It was found that MS medium in combination with 2,4- D and Kn was better than the other tested combination in terms of callus induction. Similar proliferation of callus tissue from nodal segments was also noted in other medicinal plants including *Mirabilis jalapa* (Ling et al. 2009), *Camellia sinensis* (Seran et al. 2007), *Piper carinconnectivum* (dos Santos et al. 2015). In order to promote differentiation, the calli produced from *P. minima* were further grown on a broad spectrum of PGRs supplemented MS medium. It was highly noticed that the callus did not undergo any kind of differentiation and finally died.

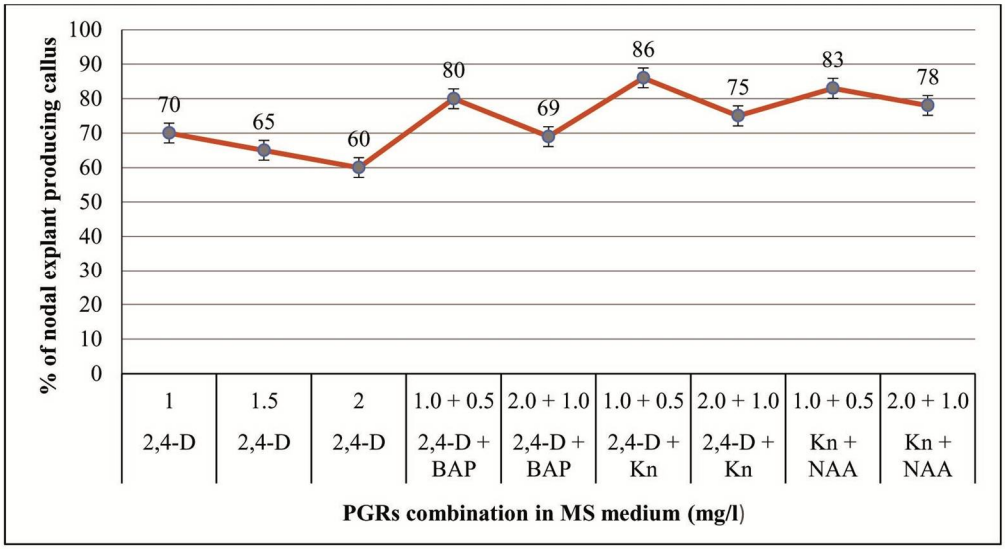


Fig. 2. Callus induction from nodal explants of *Physalis minima*.

**Table 1. Effects of different concentrations and combinations of auxins and cytokinins for the elongation of micro-shoots in *P. minima*.**

| MS-PGRs combination (mg/l) | | | | | | Average* initial length (cm) of individual shoot bud (mean ± SE) | Average* length(cm) of individual shoot buds after 30d of culture (mean ± SE) | Average* increase in length(cm) of individual shoot buds after 30d of culture (mean ± SE) |
|---|---|---|---|---|---|---|---|---|
| BAP | Kn | BAP+NAA | BAP+IAA | Kn+NAA | Kn+IAA | | | |
| 1.0 | - | - | - | - | - | 1.33 ± 0.03$^{bc}$ | 3.93 ± 0.10$^{bh}$ | 2.60 ± 0.07$^{abf}$ |
| 2.0 | - | - | - | - | - | 1.27 ± 0.13$^{bd}$ | 3.74 ± 0.09$^{bdf}$ | 2.47 ± 0.16$^{cdg}$ |
| - | 1.0 | - | - | - | - | 1.07 ± 0.17$^{b}$ | 2.74 ± 0.14$^{cg}$ | 1.67 ± 0.21$^{fg}$ |
| - | 2.0 | - | - | - | - | 1.13 ± 0.10$^{ac}$ | 2.86 ± 0.12$^{bcg}$ | 1.73 ± 0.02$^{h}$ |
| - | - | 1.0 + 0.5 | - | - | - | 1.67 ± 0.11$^{c}$ | 5.27 ± 0.05$^{abd}$ | 3.60 ± 0.11$^{fh}$ |
| - | - | 2.0 + 0.5 | - | - | - | 1.40 ± 0.15$^{df}$ | 4.87 ± 0.07$^{acf}$ | 3.47 ± 0.05$^{bdh}$ |
| - | - | - | 1.0 + 0.5 | - | - | 1.23 ± 0.12$^{cg}$ | 3.36 ± 0.17$^{bef}$ | 2.13 ± 0.14$^{bcd}$ |
| - | - | - | 2.0 + 0.5 | - | - | 1.20 ± 0.08$^{bd}$ | 3.13 ± 0.19$^{bch}$ | 1.93 ± 0.10$^{ce}$ |
| - | - | - | - | 1.0 + 0.5 | - | 1.60 ± 0.10$^{be}$ | 4.80 ± 0.13$^{dh}$ | 3.20 ± 0.15 $^{bch}$ |
| - | - | - | - | 2.0 + 0.5 | - | 1.33 ± 0.04$^{acd}$ | 3.66 ± 0.08$^{acf}$ | 2.33 ± 0.09$^{cdg}$ |
| - | - | - | - | - | 1.0 + 0.5 | 1.80 ± 0.07$^{abe}$ | 5.30 ± 0.15$^{ceg}$ | 3.50 ± 0.17$^{bcg}$ |
| - | - | - | - | - | 2.0 + 0.5 | 1.25 ± 0.17$^{d}$ | 3.58 ± 0.10$^{bgh}$ | 2.33 ± 0.14$^{cd}$ |

d = days. *values are the means of five replicates with 15 explants. All test values with different superscripts in the same column are significantly different at $p \leq 0.05$.

For promoting rapid elongation, the *in vitro* grown shoot buds of *P. minima* that developed directly from nodal segments were further cultured on elongation media. MS medium supplemented with different concentrations and combinations of PGRs were used for this purpose. The highest increase of shoot buds (3.60 ± 0.11cm) was recorded in MS medium containing 1.0 mg/l BAP + 0.5 mg/l NAA (Table 1, Fig. 4D). The combination of auxin and cytokinin stimulates cell division and affect the path of differentiation (Thomas and Chaturvedi 2008). The effect of BAP in combination with NAA has been noticed in many other medicinal plant species such as *Plumbago indica* (Bhadra et al. 2004), *Vitex negundo* (Hiregoudar et al. 2006), *Gynura procumbens* (Keng et al. 2009), and *Lilium lancifolium* (Sun et al. 2013).

Finally, in order to get complete plantlets the elongated shoot buds were individually grown on rooting media. The rooting media were prepared by half-strength MS medium with or without low concentrations of auxin (IBA, IAA and NAA). Among the different combinations and concentrations of PGRs, half-strength MS medium fortified with 1.0 mg/l IBA gave better response in induction and development of root. The highest percentage was 93% in term of maximum number of roots (5.87 ± 0.17) per shoot (Fig. 3a) and the highest increase in root length (3.07 ± 0.12) (Fig. 3b) after 30 days of culture (Fig. 4E). In this study, the application of IBA demonstrated strong rooting efficiency similar to some previously reported cases. The effeciency of IBA for *in vitro* root induction in *Withania coagulans* has been reported by Valizadeh and Valizadeh (2011), *Physalis*

*peruviana* (Otroshy et al. 2013), *Bacopa monnieri* (Jain et al. 2014), *Satureja punctate* (Teshome et al. 2016). The present results are different from these reports in terms of early root induction, large number of roots, vigorous growth. Generally, IBA has widely been used as rooting substance to propagate various plants. IBA acts as a slow-releasing hormone, enabling its effects to remain longer in the culture medium and resulting in the constant promotion of roots (Epstein and Ludwig-Müller 1993). Sivanesan and Jeong (2009) demonstrated that half-strength MS medium supplemented with 2.0 mg/l IBA promoted better rooting in *Sphagneticola trilobata* which supports the present observation.

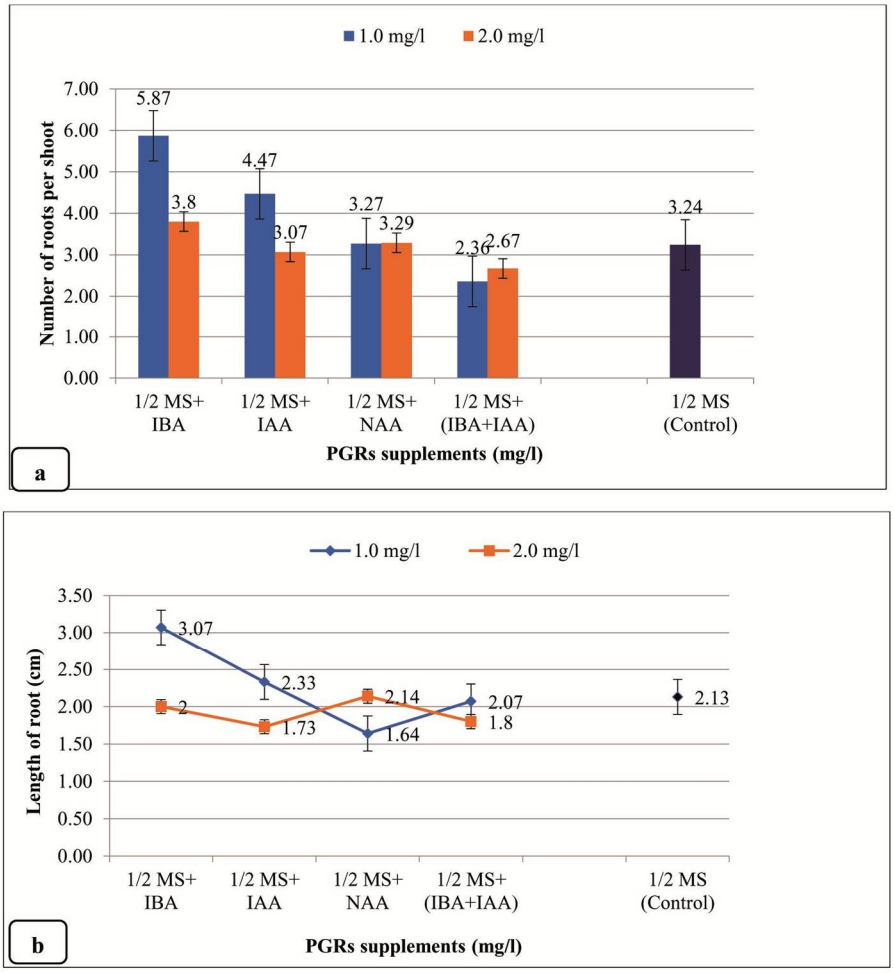


Fig. 3(a-b). Effect of ½MS with different auxins on root development of *P. minima*: (a) numbers of roots and (b) length of roots.

After establishment of well profuse rooted plantlets the *in vitro* raised complete seedlings with healthy root system were transferred to outside environment and planted in earthen pot through successive phase of acclimatization described in Materials and

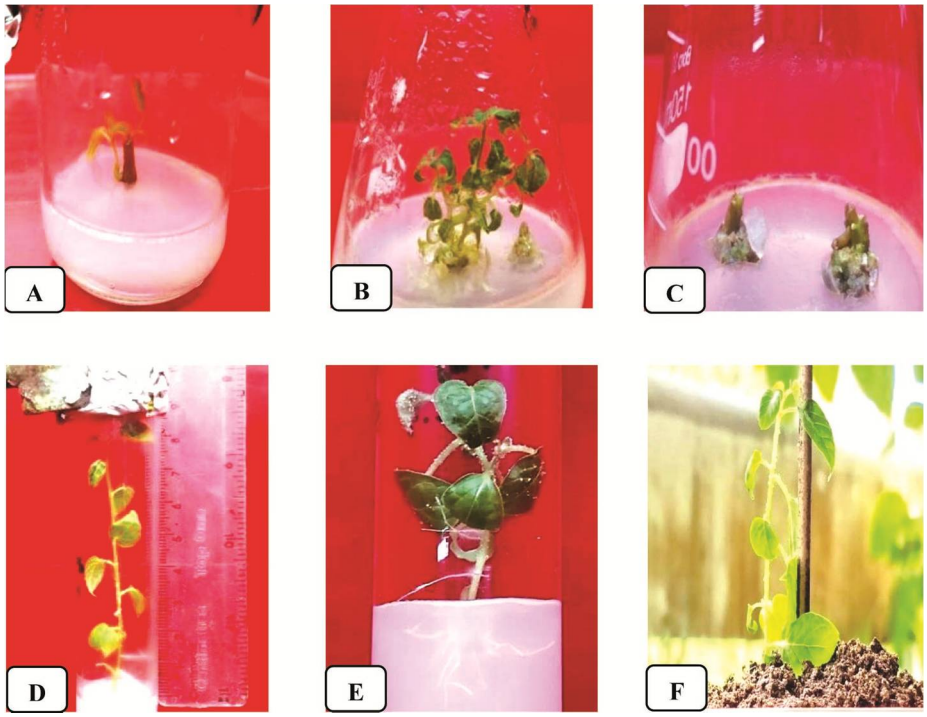


Fig. 4(A-F). *In vitro* propagation of *Physalis minima* through direct organogenesis: (A) initiation of shoot buds in nodal segments, (B) maximum proliferation of shoot buds on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA, (C) initiation of brownish friable callus in nodal segments, (D) maximum elongation of shoot buds on MS + 2.0 mg/l BAP and 1.0 mg/l NAA, (E) highest number of roots per micro shoot in half-strength MS + 1.0 mg/l IBA, and (F) establishment of *in vitro* raised plantlets in plastic pots.

Methods. Hardening of *in vitro* grown plantlets for adjustment to natural environment is a difficult aspect of plant tissue culture. These plantlets of *P. minima* were developed through *in vitro* culture technique were at last transferred to earthen pots in outside environment (Fig. 4F). Ultimately 75% of the plantlets survived and established showing normal growth.

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## References

**Afroz A, Hassan SMKA, Bari SL, Sultana R, Begum N, Jahan AAM** and **Khatun R** (2009) *In vitro* shoot proliferation and plant regeneration of *Physalis minima* L. a perennial medicinal herb. Bang. J. Sci. Indus. Res. **44**(4): 453-456.

**Bhadra SK, Kabir MR** and **Rahman S** (2004) *In vitro* clonal propagation of *Plumbago indica*. Plant Tiss. Cult. Biotech. **14**(1): 25-32.

**Biswas A, Bari MA, Roy M** and **Bhadra SK** (2011) *In vitro* propagation of *Stemona tuberosa* Lour., a rare medicinal plant through high frequency shoot multiplication using nodal explants. Plant Tiss. Cult. Biotech. **21**(2): 151-159.

**Chopra RN, Nayar SL** and **Chopra IC** (1986) Glossary of Indian medicinal plants (including the Supplement). Council of Scientific and Industrial Research (CSIR), New Delhi, India.

**Chothani DL** and **Vaghasiya H** (2012) A phyto-pharmacological overview on *Physalis minima* L. Indian J. Nat. Prod. Res. **3**(4): 477-482.

**Cribb AB** and **JW** (1976) Wild food in Australia. Fontana ISBN 0-00-634436-4.

**Das J** and **Rout GR** (2018) Plant regeneration through somatic embryogenesis in leaf-derived callus of *Aloe vera.* Biol. Plantarum. **45**(2): 299-302.

**Dos Santos MRA, De Souza CA, Guimarães MD, Smozinski CV, Magalhães GDO, Nogueira WO** and **Paz ES** (2015) Growth pattern of friable callus from *Piper carniconnectivum* leaf explants. Aust. J. Basic Appl. Sci. **9**(27): 226-231.

**Duke JA** and **Ayensu ES** (1985) Medicinal plants of China. Fedd. Rep. **2**(98): 398-398.

**Epstein E** and **Ludwig-Müller J** (1993) Indole-3-butyric acid in plants: occurrence, synthesis, metabolism and transport. Physiol . Plant. **88**: 382-389.

**Goswami B, Khan S, Banu TA, Akter S, Islam M** and **Habib A (**2022) *In vitro* mass propagation of *Withania somnifera* (L.) Dunal: an important medicinal plant of Bangladesh. Bangladesh J. Bot. **51**(2): 191-197.

**Hiregoudar LV, Murthy RN, Bhat JG, Nayeem A, Hema BP, Hahn EJ** and **Paek KY** (2006) Rapid clonal propagation of *Vitex negundo*. Biologia Plantarum. **50**(2): 291-294.

**Hossain SN, Rahman S, Joydhar A, Islam S** and **Hossain M** (2000) *In vitro* propagation of Thankhuni (*Centella asiatica* L.). Plant Tiss. Cult. Biotech. **10**(1): 17-23.

**Jain A, Pandey K, Benjamin D, Meena AK** and **Singh RK** (2014) *In vitro* approach of medicinal herb: *Bacopa monnieri*. Int. J. Innov. Res. Sci. Eng. Tech. **3**(5): 12088-12093.

**Karthikeyani TP** and **Janardhanan K** (2003) Indigenous medicine for snake, scorpion, and insect bites/stings in Siruvani hills, Western Ghats, South India. Asian J. Microbiol. Biotechnol. Environ. Sci. **5**(4): 467-470.

**Keng CL, Yee LS** and **Pin PL** (2009) Micropropagation of *Gynura procumbens* (Lour.) Merr. an important medicinal plant. J. Med Plants. **3**(3): 105-111.

**Ling APK, Tang KY, Gansau JA** and **Hussein S** (2009) Induction and maintenance of callus from leaf explants of *Mirabilis jalapa* L. Med. Aromat. Plant Sci. Biotech. **3**(1): 42-47.

**Mungole AJ, Doifode VD, Kamble RB, Chaturvedi A** and **Zanwar P** (2011) *In vitro* callus induction and shoot regeneration in *Physalis minima* L. Ann. Biol. Res. **2**(2): 79-85.

**Nayeemulla S, Sudarshana MS, Umesha S** and **Hariprasad P** (2006) Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. Afr. J. Biotech. **5**(10): 946-950.

**Otroshy M, Mokhtari A, Mohammad S, Khodaee M** and **Bazrafshan AH** (2013) Direct regeneration from leaves and nodes explants of *Physalis peruviana* L. Int. J. Farm. Allied Sci. **2**(9): 214-218.

**Paul A** and **Biswas A** (2024) *In vitro* propagation of *Leucas biflora* (Vahl) Sm. - a vulnerable medicinal herb. Plant Tiss. Cult. Biotech. **34**(1): 49-54.

**Pietro RC, Kashima S, Sato DN, Januario AH** and **Franca SC** (2000) *In vitro* anti-mycobacterial activities of *Physalis angulata* L. Phytomedicine. **7**: 355-358.

**Sana H** and **Rani AS** (2025) Establishment of direct shoot regeneration in *Spilanthes acmella* Murr. using leaf explants: an *in vitro* approach for mass propagation. Plant Tiss. Cult. Biotech. **35**(1): 177-184.

**Seran TH, Hirimburegama K** and **Gunasekare MTK** (2007) Production of embryogenic callus from leaf explants of *Camellia sinensis* (L.). J. Natn. Sci. Foundation, Sri Lanka. **35**(3): 191-196.

**Sheeba E**, **Parvathy S** and **Palanivel S** (2010) Direct regeneration from leaves and node explants of *Physalis minima* L. Eur. J. Appl. Sci. **2**: 58-60.

**Sivanesan I** and **Jeong BR** (2009) *In vitro* propagation of *Sphagneticola trilobata* (L.) Pruski. Propag. Ornamen. Plants **9**(1): 10-15.

**Sun L, Zhou Z** and **Cheng K** (2013) Micropropagation from *in vitro* cultured bulb scales of *Lilium lancifolium*. Life Sci. J. **10**(2): 2689-2692.

**Teshome I, Teshome S, Soromessa T** and **Feyisssa T** (2016) Development of an efficient *in vitro* propagation protocol for *Satureja punctate*- a rare aromatic and medicinal plant, Taiwania. **61**(1): 41-48.

**Thomas TD** and **Chaturvedi R** (2008) Endosperm culture: a novel method for triploid plant production', Plant Cell Tiss. Org. Cult. **93**(1): 1-14.

**Uddin M, Goswami B, Uddin N, Hossain S, Akter S, Islam M, Habib A, Khan S** and **Banu TA** (2025) *In vitro* regeneration of exotic kiwi fruit (*Actinidia deliciosa*) in Bangladesh. Plant Tiss. Cult. Biotech. **35**(1): 33-40.

**Usman M, Fatima B, Gillani KA, Khan MS** and **Khan MM** (2008) Exploitation of potential target tissues to develop polyploids in citrus. Pak. J. Bot. **40**(4): 1755-1766.

**Valizadeh J** and **Valizadeh M** (2011) Development of efficient micropropagation protocol for *Withania coagulans* (Stocks) Dunal. Afr. J. Biotech. **10**(39): 7611-7616.

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