ABSTRACT

Aim: To develop in vitro propagation protocol of a rare, vulnerable and endangered important medicinal plant Operculina turpethum (L.) Silva Manso (Trivrit) through organogenesis.

Materials and methods: Seeds collected from Institute’s garden were pretreated and inculated on Murashige and Skoog (MS)medium. Cotyledon, axillary bud and nodal segments of in vitro grown plants were used as explants. Explants were cultured on half MS, MS, whites plain medium and MS supplemented with different concentrations and combinations of plant growth regulators viz., BAP, AS, Kn, NAA, IAA, IBA. Cultures were incubated at 22°C ± 2°C and 8 hours photoperiod with light intensity of 3000 lux. Observations were recorded at an interval of 15 to 25 days.

Result: The average maximum number of shoots 19.72 ± 0.240 achieved on MS supplemented with BAP (3 mg/L). 100% root induction was obtained on half MS, MS, Whites medium alone and the combination of MS with 1 to 4 mg/L concentrations of NAA, IBA, IAA. In vitro developed plantlets were transferred in the pots; which were easily acclimatized and established in the soil.

Conclusion: The developed micropropagation protocol is beneficial for the rapid proliferation of shoots and root. The protocol would be helpful for mass multiplication as well as to conserve the rare and endangered plant of Trivrit.

Keywords: Axillary bud, Cotyledon, Endangered, Micropropagation, Node, Rare, Trivrit.

INTRODUCTION

Operculina turpethum (L.) Silva Manso is a perennial twining herb belongs to family Convolvulaceae. It is an important Ayurvedic medicinal plant used in Indian systems of medicines. It is commonly known as Indian Jalap or Turpeth. In Ayurveda, it is popularly known as Trivrit, Nishottar, Tribhandi, Saral, Rechani. About 135 Ayurvedic formulations and preparations are being prepared by using root as the main ingredient. The most popular are Trioritadichurna, Trioritadigutika, Trioritadikvath, Trioritadighrit, Avipattikar churna, Punarnavamandura, Chandraprabhavati, Yogarajaguggulu and Ashwagandharishta.1,2

As per Ayurvedic concept, the root is madhura (sweet), ushna (hot), katu (pungent), tikta (bitter) and having laghu (light), tikshna (sharp) and ruksha (dry) properties.3 It exhibits anti-inflammatory, stimulant, anticancer, hepatoprotective, antioxidant, cardioprotective, thermogenic, anticancer, antiobiotic, carminative, anthelmintic, expectorant, antipyretic, purgative and hydrogogue activities.1,2,4,5 Roots are used in constipation, paralysis, bronchitis, obesity, helminthiasis, leucoderma, ulcers, hemorrhoids, tumors, jaundice and ophthalmia.1,2 It also reported that root bark, stem, and leaves exhibit medicinal properties. Trivrit root is one of the important ingredients in Avipattikar churna which is largely used in the treatment of constipation, skin disorders, and acid peptic disorders.2 It is also an important ingredient of Unani medicine viz. Habbe-e-Ayarij, Habbe-e-Suranjan, Majoon anjeer.5

The root contains turpethin which is responsible for the purgative action. The active compound oleandrin isolated from leaf possesses anti-inflammatory properties.5,6

Trivrit is reported as rare and vulnerable in India7,8 and endangered in Bangladesh8 because of over-exploitation. In vitro propagation technique has the potential to provide very high multiplication rate for the conservation of rare, endangered and vulnerable medicinal plants. Therefore, efforts have been made to develop an efficient in vitro propagation protocol to multiply the rare and

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Original Article

Micropropagation of Operculina turpethum (L.) Silva Manso through Nodal Segment, Apical Bud and Cotyledon

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endangered medicinal plant of Ayurvedic importance. The achievements are presented in this study.

MATERIALS AND METHODS

Plant Material and Source of Explants

Seeds were collected from the plants growing in the garden of Regional Ayurveda Institute for Fundamental Research, Pune.

Identification and Authentication of Plant

The plant was identified by Botanist of the Institute and authenticated with the help of flora of Maharashtra. Herbarium specimens were prepared and deposited in the herbarium section of the Institute with the voucher specimen number 4222.

Explants Source and Preparation of Explants

Mature seeds were collected from the plants growing in the Institutes garden. Seeds were pretreated with Conc. H₂SO₄ for 25 to 30 minutes and thoroughly washed thrice with distilled water to remove acid traces. Treated seeds were surface sterilized with 0.1% Mercuric chloride solution (HiMedia) and inoculated on MS plain medium. Seed germination observed within ten days of inoculation. Cotyledon, apical bud and nodal segments of _in vitro_ grown plantlets were used as explants. These explants were inoculated on MS medium supplemented with BAP, AS, and Kn singly in different concentrations. _In vitro_ grown shoots were subjected on 1/2 MS, MS plain, White’s medium and MS fortified with IAA (1 to 4 mg/L), IBA (1 to 4 mg/L) and NAA (1 to 4 mg/L) for root induction.

Media Preparation and Inoculation

Murashige and Skoog (MS) medium was prepared by adding 3% sucrose and 0.8% agar (w/v) (HiMedia, India) and pH maintained at 5.8 to 5.9. MS medium augmented with different concentrations of phytohormones viz., BAP, Kn, AS, NAA, IBA, and IAA; sterilized at 121°C for 20 minutes.

Treated seeds and _in vitro_ grown node, cotyledon, and an apical bud were inoculated on MS plain alone or in combination with different concentrations of BAP, Kn, AS, NAA, IBA and IAA.

Culture Conditions

All cultures were kept under a light intensity of 3000 lux for 8-hour photoperiod at 22°C ± 2°C. Observations on cultures were recorded at an interval of 15 to 25 days. Experiments were conducted thrice with 16 replicates.

Hardening Trials

Rooted shoots were transferred in the plastic glass containing sterile soil: sand (1:1) proportion. Plantlets were provided with 15 mL half MS medium twice a week up to 60 to 90 days. The plastic glass was covered with transparent plastic bags; punched at few points for gaseous exchange. These plants were shifted in the nursery conditions and kept there for three weeks and then planted in the field. Initially, plants were covered with thatch to avoid direct sunlight.

RESULTS

Nodal segments, apical buds, and cotyledon were used as explants from the _in vitro_ grown plantlets from _in vitro_ germinated seeds (Figs 1A and B).

Nodal explants were implanted on MS medium and MS enriched with different concentrations of phytohormones such as BAP (1 to 4 mg/L), Kn (1 to 4 mg/L) and AS (1 to 4 mg/L).

Nodal segments inoculated on to MS plain developed into shoot formation along with minor callus at the cut end of the nodal segment. Whereas, nodal segments placed in MS fortified with BAP (1 to 4 mg/L) form a little callus at the cut ends along with shoot development. Among these, maximum average 19.72 ± 0.24 number of shoots per explants with an average height of 1.11 ± 0.23 cm was achieved on BAP (3 mg/L). Multiple shoots of an average number of 6.0 per explants were achieved on BAP (2 mg/L) with 1.94 cm height. The minimum number of shoots 1.25 with 1.90 cm height was recorded in BAP (4 mg/L). BAP at all concentration induced the formation of shoot and root. Observations are displayed in Table 1 (Figs 1C to E, 2 and 3).

The response of AS on nodal segments was assessed, where the formation of the shoot was recorded. Nodal explants placed on MS supplemented with AS not responded for root induction. Maximum 7.91 ± 0.16

| Table 1: Effect of BAP on Nodal segments of _Operculina turpethum_ (L.) Silva Manso |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Medium          | Percent (%) of shoot formation | Percent (%) of root formation | Average no. of shoots with ±SE | Average height of shoots (cm) with ±SE |
| MS plain        | 100              | 0.0             | 0.90 ± 0.140    | 1.79 ± 0.180    |
| MS + BAP (1 mg/L) | 100              | 0.0             | 1.72 ± 0.191    | 2.06 ± 0.090    |
| MS + BAP (2 mg/L) | 100              | 0.0             | 6.0 ± 0.260     | 1.94 ± 0.171    |
| MS + BAP (3 mg/L) | 100              | 0.0             | 19.72 ± 0.240   | 1.11 ± 0.230    |
| MS + BAP (4 mg/L) | 100              | 0.0             | 1.25 ± 0.121    | 1.90 ± 0.271    |

± SE Standard error
number of multiple shoots recorded in explants inoculated on MS augmented with 1mg/L AS and minimum 1.25 ± 0.098 number of shoots developed from AS fortified with 4 mg/L. Whereas, maximum height 1.79 ± 0.14 was achieved on MS plain. Detailed observations are shown in Table 2 (Figs 2 and 3).

Nodal segments implanted on MS supplemented with kinetin responded towards 100% shoot formation. Callus and root induction had not been observed. MS salt medium enriched with 1 and 2 mg/L Kn showed formation of average number of 1.2 ± 0.11 and 1.27 ± 0.13 shoots with average of 2.19 ± 0.029 and 1.82 ± 0.034 cm height of shoot, respectively. Whereas, Kn (3 and 4 mg/L) developed average of 1.09 ± 0.210 and 1.00 ± 0.170 number of shoots with average 1.20 ± 0.028 and 1.15 ± 0.021 cm height. Details are given in Table 3 (Figs 2 and 3).

Apical buds inoculated on MS plain alone and MS supplemented with Kn (1 to 4 mg/L) responded towards shoot formation. Average multiple shoots 4.54 ± 0.181 numbers were developed from MS supplemented with Kn (1 mg/L), 2.6 ± 0.111 average of shoots from MS + Kn (2 mg/L) and 0.90 ± 0.079 number of shoot developed.

**Table 2: Effect of AS on Nodal segments of Operculina turpethum (L.) Silva Manso**

| Medium                  | Percent (%) of shoot formation | Percent (%) of root formation | Average no. of shoots with + SE | Average height of shoots (cm) with + SE |
|-------------------------|-------------------------------|-----------------------------|---------------------------------|----------------------------------------|
| MS plain                | 100                           | 0.0                         | 0.90 ± 0.074                    | 1.79 ± 0.140                           |
| MS + AS (1 mg/L)        | 100                           | 0.0                         | 7.91 ± 0.161                    | 1.5 ± 0.180                            |
| MS + AS (2 mg/L)        | 100                           | 0.0                         | 2.45 ± 0.123                    | 1.20 ± 0.221                           |
| MS + AS (3 mg/L)        | 100                           | 0.0                         | 1.45 ± 0.108                    | 1.18 ± 0.291                           |
| MS + AS (4 mg/L)        | 100                           | 0.0                         | 1.25 ± 0.098                    | 0.87 ± 0.033                           |

**Fig. 2:** Effect of BAP, AS, Kn on number of shoots of Operculina turpethum (L.) Silva Manso

**Fig. 3:** Effect of BAP, AS, Kn on height of shoot of Operculina turpethum (L.) Silva Manso
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Table 3: Effect of Kn on Nodal segments of Operculina turpethum (L.) Silva Manso

| Medium          | Percent (%) of shoot formation | Percent (%) of root formation | Average no. of shoots with ±SE | Average height of shoots (cm.) with ±SE |
|-----------------|-------------------------------|------------------------------|--------------------------------|----------------------------------------|
| MS plain        | 100                           | 0.0                          | 0.90 ± 0.150                  | 1.79 ± 0.032                           |
| MS + Kn (1 mg/L)| 100                           | 0.0                          | 1.2 ± 0.114                   | 2.19 ± 0.029                           |
| MS + Kn (2 mg/L)| 100                           | 0.0                          | 1.27 ± 0.131                  | 1.82 ± 0.034                           |
| MS + Kn (3 mg/L)| 100                           | 0.0                          | 1.09 ± 0.210                  | 1.20 ± 0.028                           |
| MS + Kn (4 mg/L)| 100                           | 0.0                          | 1.00 ± 0.170                  | 1.15 ± 0.021                           |

± SE Standard error

from MS + 3 mg/L Kn. Whereas, apical buds inoculated on MS plain and MS augmented with 4 mg/L Kn unable to induce callus, shoot and root formation. Details of observations recorded are exhibited in Table 4.

Effect of BAP, AS and Kn on cotyledon explants was assessed by placing cotyledon on MS plain, and MS fortified with different concentration of BAP (1 to 4 mg/L), AS (1 to 4 mg/L), Kn (1 to 4 mg/L). Among all phytohormones tried, only Kn (1 to 4 mg/L) responded for induction of shoot from the cotyledon explants. Induction of shoot observed in Kn (3 mg/L and 4 mg/L). Maximum 4.54 ± 0.120 number of shoots formed on 3 mg/L Kn. Details of observations are shown in Table 5.

Rooting

In vitro grown shoots were transferred on root induction medium which includes half MS, MS, Whites medium and MS enriched with NAA (1 to 4 mg/L), IBA (1 to 4 mg/L) and IAA (1 to 4 mg/L). Root formation was not a constraint to the in vitro grown shoots. Almost all media and rooting hormones responded positively towards root formation within a week. One hundred percent root developed in all combination tried. Formation of maximum 7 to 8 numbers of robust, long roots observed in IBA (1 to 4 mg/L) within 3 to 5 days. Whereas, induction of 3 to 5 numbers of root observed within 3 to 5 days in IAA and NAA (1 to 4 mg/L). Early and best root induction recorded in IBA (3 mg/L). Details of observations are given in Table 6 (Figs 1F and G).

Hardening

Rooted plantlets were transferred in plastic pots containing sterile soil and sand in 1:1 proportion. Pots were covered with transparent polythene bags and kept in hardening room at ambient temperature. After 60-90 days, these plants were shifted in to nursery conditions and planted in the open field. Seventy five to 80% plants were survived and attended the maturity (Fig. 1H).

DISCUSSION

Because of the lack of systematic in vivo propagation and cultivation practices on medicinal plants; it is now an urgent need to develop in vitro protocols on important, rare and endangered plants. However, many workers had made efforts to develop an in vitro protocols by using
different explants and various permutations and combinations of different phytohormones. Alam et al. reported maximum 11.65 numbers of shoots on MS enriched with BAP (1 mg/L) from nodal explant. Average 2.36, 7.16, 5.60 and 8.33 number of shoots were recorded on MS + Kn (1 mg/L), MS + BAP (1 mg/L) + Kn (0.5 mg/L), MS + BAP (1 mg/L) + NAA (0.2 mg/L) and MS + BAP (1 mg/L) + GA3 (0.1 mg/L), respectively.9 Whereas in our experiment, average maximum 19.72, 7.91 and 4.54 number of shoots achieved on MS supplemented with BAP (3 mg/L), MS fortified with AS (1 mg/L) from nodal explant and MS augmented with Kn (1 mg/L) from apical bud, respectively.

Similarly, In Merremia quinquefolia (L.) Hall., another genus of Convolvulaceae, maximum average 6.11 shoots per node was obtained on MS medium supplemented with 4.09 mg.dm⁻³ BAP.13 Benzyl amino purine was also found to be more effective for shoot induction in Cucumis melo L.,14 Satureja punctata (Benth.) Briq.15 when in vitro germinated seedlings were used as the mother plant.

Sebastianraj et al. (2013)16 reported maximum 16.10, 12.70 and 3.33 number of shoots developed from cotyledon explants on MS + Kn (1.5 mg/L) + IAA (0.1 mg/L), MS + Kn (1.5 mg/L) and MS + BAP (1 mg/L).13 In the present study, MS + Kn (1-4 mg/L) produced an average of 4.54 number of shoots.

In vitro grown shoots cultured on half MS, MS, Whites medium and MS enriched with NAA (1 to 4 mg/L), IBA (1 to 4 mg/L), IAA (1 to 4 mg/L) for root induction. One hundred percent root formation achieved on all combinations whereas Sebastianraj,16 achieved 90% root formation on MS medium fortified with 0.5 mg/L IBA. While Alam et al. reported best root establishment on 1.0 mg/L IAA.

From the result, it is clearly indicated that the MS medium enriched with 3 mg/L BAP was found suitable, as it produces, the maximum number of shoots (19.72 ± 0.24) along with roots from nodal explants. Thus, the developed in vitro propagation protocol is simple, efficient and reproducible for raising plantlets on large scale.

CONCLUSION

Because of an unsystematic collection from wild sources, the plant becomes rare and endangered. The developed in vitro protocol would be highly beneficial for conservation, fast multiplication of Operculina turpethum (L.) Silva Manso. It would be helpful to conserve and multiply the plants on large scale.

REFERENCES

1. Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants Used in Ayurveda. Vol.5. New Delhi: Central Council for Research in Ayurveda and Siddha; 2002. pp 171-186.
2. Kohli KR, Nipaniukar SU, Kadhbhanne KP. A Comprehensive Review on Trivrit [Operculina turpethum]. International Journal of Parma and Bio Sciences 2010,14(4):443-452.
3. Sharma Veena, Singh Manu. Operculina turpethum As a Panoramic Herbal Medicine: A Review. International Journal of Pharmaceutical Sciences and Research. 2012;3(1):21-25.
4. Satyapal Singh, Sangeeta Sudharsa, I. Kodituwakku, Rajendra Prasad, Tripathi JS, Rai NP. A Review on Therapeutic Potential of Trivrita (Operculina turpethum) Linn. Int. J. Ayu. Pharm. Chem. 2016,5(1):339-348.
5. Tasleem Ahmad, Mohd Kashif Husain, Mohd Tariq, Javed Inam Siddiqui, Mohd Khalid, Mohammed Wasim Ahmed, Munawwar Hussain Kazmi. A Review on Operculina turpethum : A Potent Herb of Unani system of Medicine. Journal of Pharmacognosy and Phytochemistry 2017,6(1):23-26.
6. Gupta Shweta, Ved akash. Operculina turpethum (Linn.) Silva Manso as a Medicinal Plant Species: A Review on Bioactive Components and Pharmacological Properties. Pharmacognosy Review 2017;11(22):158-166.
7. Envis FRLHT. Medicinal Plant species of conservation concern identified for Maharashtra (MH): 2010, (http://envis.frlht.org).
8. Envis FRLHT. Medicinal Plant species of conservation concern identified for Andhra Pradesh (AP): 2010, (http://envis.frlht.org).
9. Alam Jahangir M, Iftekhar Alam, Shamima Akhtiar Sharmin, M Mizanur Rahman, M. Anisuzzaman, Mohammad Firoz Alam. Micropropagation and antimicrobial activity of Operculina turpethum (syn. Ipomoea turpethum), an endangered medicinal plant. Plant Omics Journal 2010,3(2):40-46.
10. Singh NP, Lakshminarasimhan S, Karikeyan, PV Prasanna. Flora of Maharashtra State – Dicotyledones vol. II. Calcutta: Botanical Survey of India; 2001. pp. 814.
11. Rao RR, Sharma BD. A Manual for Herbarium collections. Calcutta : Botanical Survey of India. 1990. pp. 5-20.
12. Murashige T, Skoog F. A Revised medium for Rapid growth and Bio assays with Tobacco Tissue Culture. Physiol. Plant 1962;15:473-497.
13. Kher MM, Nataraj M, Parmar HD, Buchad H. Micropropagation of Merremia quinquefolia (L.) Hallier f. from nodal explants. Journal of Horticultural Research 2015,23(1): 13-16.
14. Parvin S, Kausar M, Enamul Haque M, Khalekuzzaman Siddar B, Asadul Islam M. In vitro propagation of Musklemelon (Cucumis melo L.) from nodal segments, shoot tips and cotyledonary nodes. Journal of life and earth and agricultural sciences, 2013;41:71-77.
15. Teshome Indrias, Shiferaw T, Teshome Soromessa, Tileyeye Feyissa. Development of an efficient in vitro propagation protocol for Saturja ounctata – A rare aromatic and medicinal plant. Taiwania 2016,61(1):41-48.
16. Sebastianraj J, John Britto S, Senthil Kumar SR. Micropropagation of Operculina turpethum (L.) Silva Manso. Using Cotyledonary Node Explants. Academic Journal of Plant Sciences 2013,6(2):77-81.
हिंदी सारांश

नोडल सेग्मेंट, एपिकलबड एवं कोटिलेजन के माध्यम से ओपरकुलिना टरमियम (एल.) सिल्वामानसो का माइक्रोप्रोपेगेशन

सारांश: जीवोपति के माध्यम से दुरुलम, गेय्ड एवं संकटपान महसुसार चिकित्साय उपचार के माध्यम से ओपरकुलिना टरमियम (एल.) सिल्वामानसो का इस मिट्टी प्रोपेगेशन प्रोटोकॉल का विकास करना।

सामग्री एवं विधि: संस्थान के उद्धार से एकरंज बील ने एमएस ग्रेनियम पर पूर्व उपचार एवं टिकाकृत किया गया। एक्सप्रेंट के रूप में कोटिलेजन, एपिकलबड एवं नोडल सेग्मेंट का प्रयोग इन बिट्टों उत्पादन पादयों में किया गया। डाक्टर एमएस, एमएस, कामेट लेने मीडियम एवं एमएस स्लोमेंट को पादय विकास के निर्माण गठन भी भी, एमएस, कैंसर, एमए, आईएस के विभिन्न संयोजनों एवं संयोजनों पर एक्सप्रेंट का संबंधित किया गया। 22 मिट्टी सेंटीग्रेड 2 मिट्टी सेंटीग्रेड एवं 3000 लंग्स. पर 8 घंटे तक प्रकाश तीमा के साथ संरचनात्मक प्रक्रिया का संचालन किया गया।

परिणाम: एमएस रॉमेंट को भी (5 मिलीग्राम/लीटर) के रूप पर 19.72±0.240 के उद्धार संग्रह में टिकाइया प्राप्त हुई। एमएस, एमएस, क्योट लेने मीडियम पर 100% जड़ अनुममा पाना गया। जबकि 1–4 मिलीग्राम/लीटर के एमएस, आईएस, आईएस संरचना के रूप पर एमएस पाया गया। इन बिट्टों द्वारा विकसित किये गए पादयों के बर्नरों ने समनात्मक संरचना किया गया जिसे सीलर पैकिंग मिट्टी में रेशारर कर दिया गया।

निष्कर्ष: जड़ों एवं उद्खालों के लर्नर प्रसार के लिए माइक्रोप्रोपेगेशन प्रोटोकॉल लाभकारी है। यह प्रोटोकॉल पादयों के विकास के लिए लाभकारी होगा एवं सहायक होगा।

शापुकृति: भित्रय, माइक्रोप्रोपेगेशन, कोटिलेजन, एपिकलबड, नोडल, दुरुलम, संकटपान।