Plant Archives Vol. 25, Supplement 1, 2025 pp. 1749-1753 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.239

GENETIC FIDELITY ASSESSMENT OF *OPERCULINA TURPETHUM* (L.) SILVA MANSO: A THREATENED MEDICINAL PLANT OF SOUTH INDIA

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(Date of Receiving : 21-09-2024; Date of Acceptance : 17-11-2024)

ABSTRACT

Operculina turpethum (L.) Silva Manso. a species indigenous to Western Ghats and popularly known as 'trivrit' is a perennial climber belonging to the family Convolvulaceae. Shoot was multiplied using nodal explants cultured on MS medium supplemented with various concentrations and combinations of growth regulators like BAP, Kinetin and NAA. But while micropropagating, the explants subjected to different media composition, various concentrations of growth regulators, light intensity and *in-vitro* stresses might result in the development of somaclonal variations causing mutation, chromosomal aberrations or damage in nucleic acid. The present investigation was carried out at ICAR- Indian Institute of Horticultural Research (ICAR-IIHR), Hesaraghatta, Bengaluru in order to check the homogeneity of micropropagated plants with the mother plant using ten RAPD (Random Amplified Polymorphic DNA) markers. All the banding patterns obtained from the micropropagated plants were monomorphic and similar to the mother plant, indicating no genetic variations among the *in-vitro* raised plants of *O. turpethum.* This study is having significance as it could be commercially utilized for the production of true-to-type plants in *O. turpethum.*
***Keywords*:** *Operculina turpethum*; *In-vitro* propagation; Genetic fidelity; RAPD markers.

Introduction

O. turpethum (L.) Silva Manso. is a threatened medicinal plant used in the Indian system of medicine, belonging to the family Convolvulaceae. It is a perennial climber with purplish stem and milky white exudates (Kohli *et al*., 2010). It is commonly known as "Indian Jalap", "Trivrit" or "Turpeth" in English, "Nisoth" or "Pitohri" in Hindi, "Bangadaballi" or "Bilitigade" in Kannada, "Tegada" in Telugu, "Caralam" or "Civatai" in Tamil and "Thrikoolpakkonna" in Malayalam (Nafees *et al*., 2020). The species is distributed across India, Bangladesh, Nepal, China, Srilanka, Australia, Africa, South America, Pakistan, Taiwan, Myanmar, Indonesia, Malaysia, Thailand, Papua New Guinea, Philippines and is naturalized in West Indies (Sharma and Singh, 2012). In India, it is commonly found in the dry zones of Karnataka and Tamil Nadu (Hoq and Tamanna, 2019). It is a very potent herb having wide pharmacological actions and therapeutic significance. Roots having α and β turpethin as active principles are used in the treatment of fever, cough, asthma, edema, anaemia, ascites, anorexia, constipation, gout, rheumatism, hepatitis, haemorrhoids, intoxication, ulcers, fistulas abdominal tumors, wounds, worm infestation, scorpion sting and snake bite (Thamizhmozhi and Nagavalli, 2017). Some of the ayurvedic formulations like Trivrit Arishta, Trivrit Avaleha, Trivritadi Modak, Trivritadi Kalka and Avipattikar Churna contains *O. turpethum* as a vital ingredient (Sharma and Singh, 2012). But rapid depletion of natural habitat has greatly reduced the distribution of this species and increased the risk of

genetic diversity loss (Alam *et al*., 2010). Apart from this, destructive harvesting of root as a source of raw material for ayurvedic drug preparation and other anthropogenic activities have made the species severely threatened in nature. It is Red-listed (vulnerable) medicinal species by IUCN (International Union for Conservation of Nature). Hence, there is an urgent need for conservation.

In-vitro raised plantlets may show somaclonal variations and genetic fidelity assessment helps in determining the genetic stability of these plantlets. This can be done using Random Amplified Polymorphic DNA (RAPD) marker due to its simplicity and efficiency even without the prior knowledge of sequence information.

Material and Methods

Experimental site

The experiment was carried out at ICAR- Indian Institute of Horticultural Research (ICAR-IIHR), Hesaraghatta, Bengaluru. It is located in the eastern dry zone of Karnataka at $13^0 58^{'}$ North latitude and 78^0 East longitude. It is situated at an altitude of 890 meters above the mean sea level (MSL). It comes under zone-5 of region-3 among the agro-climatic zones of Karnataka and receives South-West and North-East monsoons.

Plant material and cultural conditions

Nodal segments having axillary buds were collected from the mature plants of *O. turpethum* which were grown in the Field Gene Bank of RET (Rare, Endangered and Threatened) medicinal plants, Division of Floriculture and Medicinal crops, ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru. The nodal explants were washed thoroughly under running tap water along with a liquid detergent (Tween 20) for 4-5 minutes. Then they were dipped in 70 per cent (v/v) ethanol for 1-2 minutes and alcohol was decanted by washing the explants thoroughly with sterile distilled water and surface sterilized with $HgCl_2$ (0.2 %) solution for 5-7 minutes under aseptic conditions in a laminar airflow hood. The surface sterilized explants were inoculated on basal MS medium containing different concentrations and combinations of growth regulators. All the cultures were maintained in a Standard Culture Condition (SCC) at a temperature of 26 ± 2^0C under incandescent bulb (25W) with a photoperiod of 16-hour white light and 8-hour dark per day.

DNA extraction and PCR amplification conditions

The genomic DNA of *O. turpethum* was isolated from field grown mother plant and *in-vitro* raised plants using CTAB method described by Doyle and Doyle (1987) with minor modifications. Genetic stability of *in-vitro* raised plants were tested using ten RAPD (Random Amplified Polymorphic DNA) markers. PCR amplification was carried out using 25µl PCR reaction mixture containing 2.5µl of 10mM concentration dNTPs, 3µl of complete buffer, 1µl of Taq polymerase, 13.5µl of nucleus free water, 2.5µl of DNA sample and 2.5µl of primer. PCR amplification was performed in a thermal cycler, which was programmed for initial denaturation at 95°C for 3 min, followed by 35 cycles of 60 seconds denaturation at 95°C, 60 seconds annealing at 54°C and 60 seconds extension at 72°C, with a final extension at 72°C for 5 min. Amplified products were resolved by electrophoresis on 0.8 % agarose gel in TAE buffer stained with ethidium bromide for 2 hours at 75 volts and photographs were taken using the Gel Documenting system.

Statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with three replications. The data was analyzed statistically by following the procedure outlined by Panse and Sukhatme (1967).

Results and Discussion

a) *In-vitro* shoot multiplication

Healthy nodal explants were collected from the Field Gene Bank grown plants and cultured on MS medium supplemented with various concentrations and combinations of growth regulators like BAP, KIN and NAA. Effect of varying plant growth regulators on growth related characters like shoot length, number of shoots and number of leaves was recorded and presented in Table 1.

MS medium supplemented with BAP (1.0 mg/l) + NAA (0.5 mg/l) recorded maximum number of shoots (3.25±0.05) and leaves per explant (4.95±0.04) with minimum (9.15±0.06) days for shoot initiation. Whereas, the highest shoot length (4.65±0.05 cm) was recorded in BAP (1.0 mg/l). Similar results were obtained by Alam *et al.* (2010), where BAP (1.0 mg/l) alone was responsible for rapid shoot bud proliferation with maximum number of shoots in each bud. The findings of Bisht *et al*. (2012) showed similar results in *Hedychium coronarium*, when seedlings explants were inoculated on MS medium containing BAP (1.0 mg/l) and NAA (0.5 mg/l). Similarly, maximum shoot proliferation with highest shoot length was obtained through rapid multiplication of *Salacia chinensis* (Majid *et al.,* 2016).

Table 1: *In-vitro* shoot multiplication in *Operculina turpethum* (L.) Silva Manso. using different growth regulators

Treatment (mg/l)	Number of days taken for shoot initiation (mean ± SE) *	Shoot length (cm) (mean ± SE) *	Number of shoots per explant (mean ± SE) *	Number of leaves per explant (mean ± SE) *
T_1 - BAP (0.5)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T_2 - BAP (1.0)	10.01±0.06	4.65±0.05	3.01±0.01	4.31±0.04
T_3 - KIN (0.5)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T_4 - KIN (1.0)	20.33±0.52	1.41±0.04	1.04±0.04	1.72±0.05
T_5 - BAP (0.5) + KIN (0.5)	16.26±0.17	2.78±0.07	1.50±0.06	2.85±0.03
T_6 - BAP (1.0) + KIN (0.5)	13.78±0.08	3.93±0.04	2.35±0.07	2.79±0.05
T_7 - BAP (1.0) + KIN (1.0)	17.05±0.06	3.29±0.04	1.18±0.02	3.47±0.05
T_8 - BAP (0.5) + KIN (1.0)	17.57±0.05	2.43±0.04	2.06±0.06	1.94±0.04
T_9 - BAP (1.0) + NAA (0.5)	9.15±0.06	4.20±0.07	3.25±0.05	4.95±0.04
T_{10} - BAP (0.5) + NAA (1.0)	11.76±0.05	3.99±0.01	2.50±0.03	2.46±0.04
T_{11} - KIN (0.5) + NAA (1.0)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
T_{12} - KIN (1.0) + NAA (1.0)	11.02±0.12	3.59±0.03	2.92±0.06	3.75±0.06
S.Em±	**0.17**	**0.04**	**0.04**	**0.04**
CD@1%	**0.66**	**0.16**	**0.17**	**0.16**

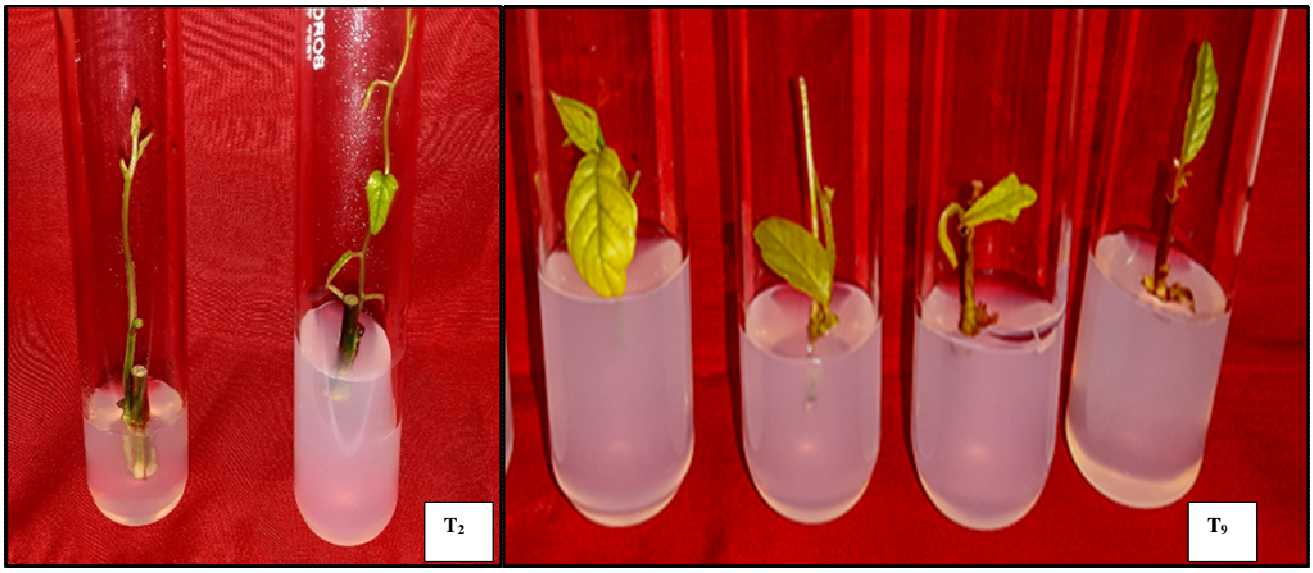

Plate 1 : Best treatments in tissue culture of *Operculina turpethum* (L.) Silva Manso.
{T_2-BAP (1 mg/l), T_9: BAP (1 mg/l) + NAA (0.5 mg/l)}

b) Assessment of genetic stability

RAPD analysis was done to ascertain the genetic stability of *in-vitro* raised plants of *O. turpethum*. The number of clear and distinct bands produced by RAPD analysis were observed and manually scored from the gel profiles using ten randomly selected primers (OPA-O2, OPA-06, OPB-07, OPB-11, OPB-20, OPC-02, OPC-07, OPC-10, OPD-02 and OPD-08). A thick band without any smear on upper part of the gel in the *in-vitro* raised plants and mother plant indicated good quality of genomic DNA. The presence of band was scored as '1' and the absence of band was scored as '0'. All the ten primers subjected to amplification resulted in generation of 95 RAPD bands. The number

of bands resolved per amplification was primer dependent which varied from 8 to 12 with an average of 9.5 bands per primer. Scoring revealed that the highest number of bands (12) were obtained using OPA-06 and OPD-02 primers and the lowest number of bands (8) were obtained using OPA-02 and OPC-07. The size of the amplified products estimated by comparing them to a standard DNA ladder ranging from 100bp to 3000bp revealed that their size varied depending on the primer used for amplification as well as the plant from which DNA was isolated. The range of amplicons obtained using ten primers was from 200bp to 2000bp (Table 2). The highest range of amplification was obtained using primer OPC-10 (325bp – 2000bp) and the lowest range was obtained using OPD-08 (325 – 1050bp). All the banding patterns obtained from the micropropagated plants were monomorphic and similar to the mother plant, indicating no genetic variations among the *in-vitro* raised plants of *O. turpethum.*

There are several reports in which assessment for true to type clones in different species were conducted. In *Chlorophytum borivilianum*, genetic fidelity was assessed by Samantaray and Maiti (2010) using RAPD markers and no genetic variations were noticed in the micropropagated plants. Mallon *et al.* (2010) reported that *in-vitro* raised plants of *Centaurea ultreiae* showed genetic uniformity with the mother plant when assessed using RAPD markers. Kumar *et al.* (2015) came out with the similar results after the assessment of genetic fidelity in the i*n-vitro* raised plants of *Decalepis hamiltonii* and found that the amplified products of the regenerated plants showed similar banding pattern as that of the mother plant. The assessment of genetic stability in micropropagated *Alpinia galanga* plants using RAPD analysis revealed that the bands were monomorphic and similar to the mother plant (Parida *et al.*, 2011; Sahoo *et al.,* 2020). Similarly, Kumar and Anand (2017) observed no somaclonal variations in the *in-vitro* raised plants of *Celastrus paniculatus* in comparison with the mother plant, and these findings were also similar to the fidelity assessment made by Senapathi *et al.* (2013). Evaluation of genetic stability in *Oroxylum indicum* (endangered medicinal tree) confirmed the similar banding pattern of *in-vitro* raised plants and mother plant after RAPD analysis (Rami and Patel, 2017; Panchaksharaiah *et al.,* 2018). Rout and Das (2002) found that the micropropagated plants and mother plant of *Plumbago zeylanica* showed similar banding pattern indicating the monomorphism among the micropropagated plants.

Table 2: List of the primers, total number of bands and size of amplified fragments generated by RAPD primers in the mother plant and micropropagated plants of *Operculina turpethum* (L.) Silva Manso.

Sl. No.	Primers	Total no. of bands produced	Range of amplicons (bp)
1	OPA-02	08	300-1150
2	OPA-06	12	225-1450
3	OPB-07	09	225-1100
4	OPB-11	10	200-1750
5	OPB-20	10	225-1500
6	OPC-02	09	450-1500
7	OPC-07	08	225-1200
8	OPC-10	10	325-2000
9	OPD-02	12	275-1150
10	OPD-08	07	325-1050
Total		**95**	
Mean		**9.5**	

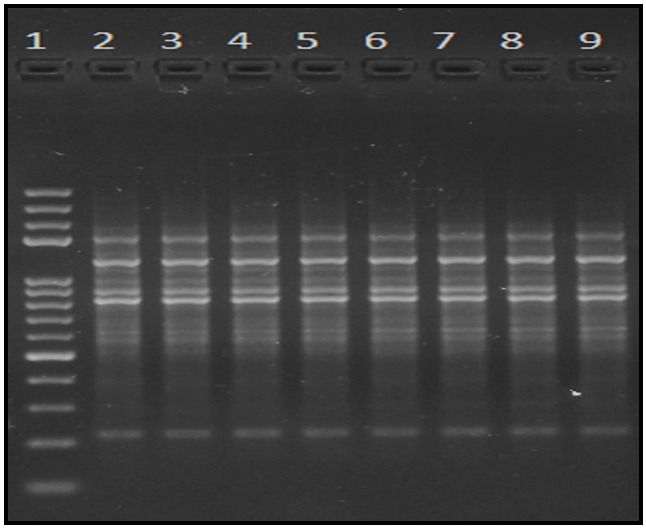

Plate 2: RAPD banding pattern of field grown mother plant and micropropagated plants of *Operculina turpethum* (L.) Silva Manso. using OPC-07 primer (Lane 1: Marker, Lane 2: Field grown mother plant, Lane 3-9: micropropagated plants)

Conclusion

The present study provides the first report on genetic stability of micropropagated *O. turpethum* plantlets obtained from the nodal explants using RAPD markers. There was similarity between the *in-vitro* raised plants and mother plant which confirmed that these plants were true-to-type. Hence, the treatment combination BAP (1.0 mg/l) + NAA (0.5 mg/l) can be considered effective for large-scale

multiplication and conservation with low risk of genetic variations.

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