Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 11, pp.9488-9493, November, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

MACROPROPAGATION OF *OPERCULINA TURPETHUM* (TIHUDI), A RED-LISTED MEDICINAL PLANT OF ODISHA

Manisha Mohapatra and *Uday Chand Basak

Seed Bank and Seed Biology Division, Regional Plant Resource Centre, R & D Institute of Forest and Environment Department, Government of Odisha, Bhubaneswar-15, India

ARTICLE INFO

Article History:
Received 05th August, 2014
Received in revised form
16th September, 2014
Accepted 24th October, 2014
Published online 18th November, 2014

Key words:
Auxins, nodal cuttings,
Operculina turpethum,
vegetative propagation.

ABSTRACT

Operculina turpethum is a Red-Listed (vulnerable category) medicinal plant distributed sporadically in Odisha. Propagation of 'tihudi' through seeds suffer from the problems like poor seed viability, low percentage of germination. As an alternative, it can be mass propagated through vine-nodal cuttings. The cuttings were treated with two types of auxin combinations along with control. Maximum rooting, growth and development were influenced by the treatment T_1- NAA (3000 ppm) + IBA (2000 ppm). The nodal cuttings with single node plus single leaf (C_1) was found to be the best for vegetative propagation where the major outcomes like rooting percentage (79.91±1.08), root number (7.00±1.56), root length (10.61±1.63 cm), new flushes (3.00±0.94), survivability percentage (81.48±0.01) were obtained. Through utilization of this technique, mass production of quality planting materials of this valuable but vulnerable medicinal plant can be achieved.

INTRODUCTION

Operculina turpethum (L) Silva Manso (Tihudi) is an important but Red-Listed medicinal plant of the family Convolvulaceae (Ved *et al.,* 2008). It is mostly used in the traditional Indian system of medicines (Kamboj, 2000). The plant is found throughout tropical dry and moist deciduous regions in central India and sometimes cultivated in tropical Africa, Madagascar, and South Asia to Australia (Kohli *et al.,* 2010). In Odisha, it is found in Jajpur, Bargarh, Balangir, Khurda and Sambalpur district in a sporadic manner (Saxena and Brahmam, 1995). It is a perennial creeper with stout quadrangular stem. Root and root barks are cathartic and laxative. Chemical constituents in *O. turpethum* are resins 9-13% which is a mixture of α and β-turpethin, glycosides, coumarin, scopoletin lupeol (Austin, 1982; Kohli *et al.,* 2010). Alcoholic extract showed the presence of glycosides, saponins, Flavonoids, steroids and carbohydrates (Sharma *et al.,* 2012). The root has anthelmintic, purgative, antipyretic and alexiteric property. It is used in ascites, leucoderma, itching, ulcer, constipation, piles, snakebites, fever, muscle pain, anaemia and abdominal trouble etc (Kirtikar and Basu, 1994). Alcoholic extract of root exhibited antibacterial activity (Kumari *et al.,* 2010). Unfortunately, rapid fragmentation of natural habitats is greatly narrowing the distribution of this plant and increasing the risk of losing genetic diversity (Alam *et al.,* 2010).

****Corresponding author: Uday Chand Basak,***
Seed Bank and Seed Biology Division, Regional Plant Resource Centre, R & D Institute of Forest and Environment Department, Government of Odisha, Bhubaneswar-15, India.

Moreover, conventional seed propagation of *O. turpethum* is facing problems due to poor seed viability, low percentage of germination and survivability. Therefore, vegetative macropropagation is thought to be suitable for production of quality planting materials. Mass macropropagation can be enhanced by using different type of explant materials with the aid of exogenous application of rooting hormones. Thus, present paper is all about the standardization of protocol for vegetative macropropagation of *O. turpethum,* an important RET medicinal plant of Odisha.

MATERIALS AND METHODS

Plant materials

The propagating materials (leafy stem cuttings) were collected from wild sources (Khurda Dist.) and the voucher specimen was examined and matched with the herbarium specimens present in the institutional herbarium. The voucher specimen was also cross verified through the reference book 'The Flora of Orissa' (Saxena and Brahmam, 1995).

Type of Explants

Three types of explants of nodal cuttings were used as detailed below (Fig. 1):

Type-1: nodal cuttings with single node plus single leaf (C_1)
Type-2: nodal cutting with single node plus two leaves (C_2)
Type-3: nodal cuttings with single node plus small Shoot-bud (C_3)

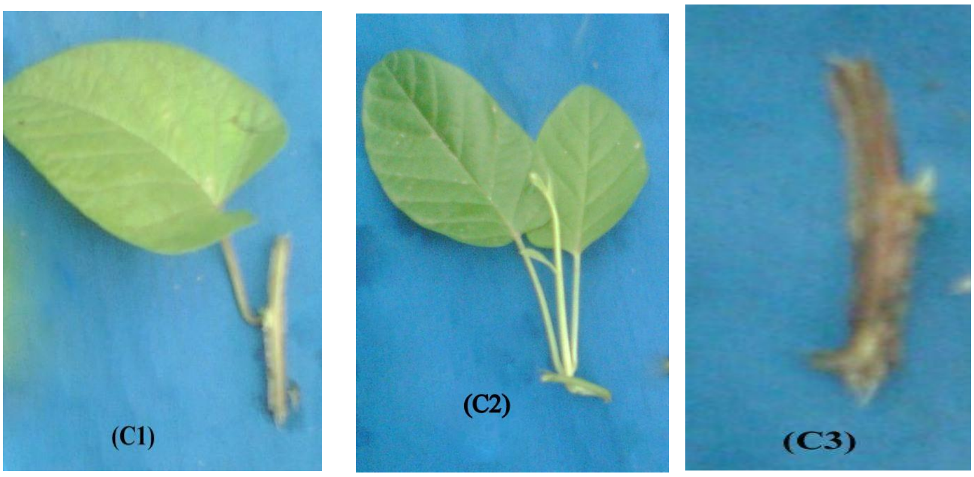

Fig. 1. Type of stem cuttings of *O. turpethum* used for propagation experiment

C_1: nodal cuttings with single node plus single leaf
C_2: nodal cuttings with single node plus two leaves
C_3: nodal cuttings with single node plus small Shoot-bud

Treatment

All the above mentioned cuttings were treated with various rooting hormones including 'control' (without hormone) as described below:

T_0 - Control
T_1 - NAA (3000 ppm) + IBA (2000 ppm)
T_2 - NAA (2000 ppm) + IBA (3000 ppm).

The rooting hormones were applied following quick dip method (Basak *et al.,* 2000). The treated cuttings were placed in polypots (6×5") filled with the rooting media sand: soil: soil rite mixture (1:1:1) kept under shade-net house for rooting.

Data recording

Rooting parameters like % of rooting, root numbers, root length and % of survivability were recorded after 2 weeks of treatment. The other related growth and development parameters like shoot length, % of shooting, number of new leaves (flushes) were recorded after 4 weeks of treatment.

Statistical Analysis

All the data were subjected to statistical analysis by using two way RM ANOVA followed by Geisser-Greenhouse's epsilon test using GraphPad Prism (Version 6.0). All the percentile values were converted into angular transformation for analysis (Table 1).

RESULTS

Root number per cutting

When treated with control, C_1 cuttings showed maximum average root number per cuttings (3.00±0.66) and C_3 cuttings showed minimum average root number per cuttings (2.00±0.94). In case of explants, those were treated with T_1 treatment; C_1 cuttings showed maximum average root number per cuttings (7.00±1.56) and C_3 cuttings showed minimum average root number per cuttings (5.00±1.05). In case of explants, those were treated with T_2 hormone treatment; C_1 cuttings showed maximum average root number per cuttings (7.00±0.82) and C_3 cuttings showed minimum average root number per cuttings (4.00±0.94).

Table 1. Analysis of variance (ANOVA) for different parameters of propagated nodal cuttings of *O. turpethum*

Parameter	Source of Variation	Df	F	P value
Root Number **	Treatment	2	28.000	P = 0.0339
	Type of Stem Cuttings	2	9.000	P = 0.0331
Root Length **	Treatment	2	8.410	P = 0.0874
	Type of Stem Cuttings	2	9.028	P = 0.0329
% of Rooting **	Treatment	2	0.4808	P = 0.5603
	Type of Stem Cuttings	2	5.524	P = 0.0707
% of Shooting ***	Treatment	2	169.4	P = 0.0034
	Type of Stem Cuttings	2	26.55	P = 0.0049
Shoot Length **	Treatment	2	25.91	P = 0.0342
	Type of Stem Cuttings	2	38.11	P = 0.0025
No of new flushes ns	Treatment	2	4.809	P = 0.1125
	Type of Stem Cuttings	2	0.205	P = 0.8227
% of Survivality **	Treatment	2	4.216	P = 0.1359
	Type of Stem Cuttings	2	33.58	P = 0.0032

NB-*** Highly Significant at 0.05% level of probability, ** Significant at 0.05% level of probability, ns Not Significant

Root length per cutting

The maximum root length per cutting (5.80±0.87 cm) in case of control (T_0) treatment was recorded in C_1 cuttings and minimum root length per cutting (4.30±0.85 cm) was recorded in C_3 cuttings. When treated with T_1 treatment, C_1 cuttings showed maximum average root length per cuttings (10.61±1.63 cm) and C_3 cuttings showed minimum average root length per cutting (5.70±1.16 cm). In case of explants, those were treated with T_2 treatment, C_1 cuttings showed maximum average root length per cuttings (7.90±0.77 cm) and C_3 cuttings showed minimum average root length per cuttings (5.10±0.43 cm).

Percentage of Rooting

When treated with control, C_1 cuttings showed maximum rooting percentage (66.92±1.08%) and C_3 cuttings showed minimum rooting percentage (54.14±0.98%). In case of explants, those were treated with T_1 treatment, C_1 cuttings showed maximum rooting percentage (79.91±1.08%) and C_3 cuttings showed minimum rooting percentage (51.20±1.17%). In case of explants, those were treated with T_2 hormone treatment, C_1 cuttings showed maximum rooting percentage (66.14±0.91%) and C_3 cuttings showed minimum rooting percentage (59.11±1.52%).

Percentage of Survivability

When treated with control, C_1 cuttings showed maximum survivability percentage (65.12±1.28%) and C_3 cuttings showed minimum survivability percentage (38.76±1.22%). In case of explants, those were treated with T_1 treatment, C_1 cuttings showed maximum survivability percentage (81.48±1.53%) and C_3 cuttings showed minimum survivability percentage (50.21±1.28%). In case of explants, those were treated with T_2 hormone treatment, C_1 cuttings showed maximum survivability percentage (74.07±1.37%) and C_3 cuttings showed minimum survivability percentage (40.23±1.54%).

Shoot length

The maximum shoot length per cutting (7.55±0.39 cm), in case of control (T_0) treatment, was recorded in C_1 cuttings and minimum shoot length per cutting (4.10±0.45 cm) was recorded in case of C_3 cuttings. When treated with T_1 treatment, C_1 cuttings showed maximum average shoot length per cuttings (10.91±1.06 cm) and C_3 cuttings showed minimum average shoot length per cuttings (6.50±0.71 cm). In case of explants, those were treated with T_2 treatment, C_1 cuttings showed maximum average shoot length per cuttings (7.17±0.71 cm) and C_3 cuttings showed minimum average shoot length per cuttings (4.63±0.65 cm).

Percentage of shooting

When treated with control, C_1 cuttings showed maximum shooting percentage (47.21±1.05%) and C_3 cuttings showed minimum shooting percentage (39.51±1.57%). In case of explants, those were treated with T_1 treatment, C_1 cuttings showed maximum shooting percentage (69.22±1.12%) and C_3 cuttings showed minimum shooting percentage (59.36±1.49%). In case of explants, those were treated with T_2 hormone treatment, C_1 cuttings showed maximum shooting percentage (55.80±1.03%) and C_3 cuttings showed minimum shooting percentage (47.13±1.44%).

Number of new flushes

When treated with control, C_1 cuttings showed maximum number of new flushes (2.00±0.94) and C_3 cuttings showed minimum number of new flushes (1.30±0.82). In case of explants, those were treated with T_1 treatment; C_1 cuttings showed maximum number of new flushes (4.30±0.95) and C_3 cuttings showed minimum number of new flushes (3.00±0.82). In case of explants, those were treated with T_2 hormone treatment; C_1 cuttings showed maximum number of new flushes (4.00±1.15) and C_3 cuttings showed minimum number of new flushes (2.50±1.08).

Table 2. Rooting efficiency and related growth parameters of propagated nodal cuttings of *O. turpethum* under T_0 (Control) treatment

Parameters	C_1	C_2	C_3
No. of Roots/cutting	3.00 ± 0.66	3.00 ± 1.05	2.00 ± 0.94
Root Length/ cutting (cm)	5.80 ± 0.87	5.10 ± 0.71	4.30 ± 0.85
Shoot Length (cm)	7.55 ± 0.39	4.90 ± 0.75	4.10 ± 0.45
No. of New Leaves	2.00 ± 0.82	1.50 ± 0.53	1.30 ± 0.94
% of Rooting	66.92 ± 1.08	60.26 ± 1.14	54.14 ± 0.98
% of Shooting	47.21 ± 1.05	43.56 ± 1.93	39.51 ± 1.57
% of Survivability	65.12 ± 1.28	58.67 ± 1.67	38.76 ± 1.22

Abbreviation*s*: C_1- nodal cuttings with single node plus single leaf; C_2-nodal cuttings with single node plus two leaves; C_3-nodal cuttings with single node plus small Shoot-bud

Table 3. Rooting efficiency and related growth parameters of propagated nodal cuttings of *O. turpethum* under T_1= NAA (3000 ppm) + IBA (2000 ppm) treatment

Parameters	C_1	C_2	C_3
No. of Roots/ cutting	7.00 ± 1.56	6.00 ± 1.25	5.00 ± 1.05
Root Length/cutting (cm)	10.61 ± 1.63	7.80 ± 1.35	5.70 ± 1.16
Shoot Length(cm)	10.91 ± 1.06	7.20 ± 0.78	6.50 ± 0.71
No. of New Leaves	4.30 ± 0.95	3.00 ± 0.94	3.00 ± 0.82
% of Rooting	79.91 ± 1.08	63.40 ± 1.89	51.20 ± 1.17
% of Shooting	69.22 ± 1.12	67.48 ± 1.73	59.36 ± 1.49
% of Survivability	81.48 ± 1.53	62.37 ± 1.80	50.21 ± 1.28

Abbreviation*s*: C_1- nodal cuttings with single node plus single leaf; C_2-nodal cuttings with single node plus two leaves; C_3-nodal cuttings with single node plus small Shoot-bud

Table 4. Rooting efficiency and related growth parameters of propagated nodal cuttings of *O. turpethum* under T_2=NAA (2000 ppm) + IBA (3000 ppm) treatment

Parameters	C_1	C_2	C_3
No. of Roots /cutting	7.00 ± 0.82	5.00 ± 0.82	4.00 ± 0.94
Root Length/ cutting (cm)	7.90 ± 0.77	5.70 ± 0.72	5.10 ± 0.43
Shoot Length(cm)	7.17 ± 0.71	5.11 ± 0.64	4.63 ± 0.65
No. of New Leaves	4.00 ± 1.15	3.00 ± 0.82	2.50 ± 1.08
% of Rooting	66.14 ± 0.91	64.34 ± 1.27	59.11 ± 1.52
% of Shooting	55.80 ± 1.03	50.12 ± 1.11	47.13 ± 1.44
% of Survivability	74.07 ± 1.37	66.47 ± 1.37	40.23 ± 1.54

Abbreviation*s*: C_1- nodal cuttings with single node plus single leaf; C_2-nodal cuttings with single node plus two leaves; C_3-nodal cuttings with single node plus small Shoot-bud

The C_1 cuttings under control treatment showed highest development in all the rooting parameters like root number per cutting, root length per cutting, rooting percentage and survivability percentage. Similar results were observed in post developmental parameters like shooting percentage, shoot length and number of new flushes. When hormone type was considered, with T_1 treatment, C_1 cuttings showed maximum development in all the rooting as well as other post developmental parameters. Considering the type of treatments used, control showed the least developmental results and in case of explant types, C_1 found to be the best type of cutting followed by C_2 and C_3 irrespective of treatments (Table 2, 3 and 4, Fig. 2, 3 and 4).

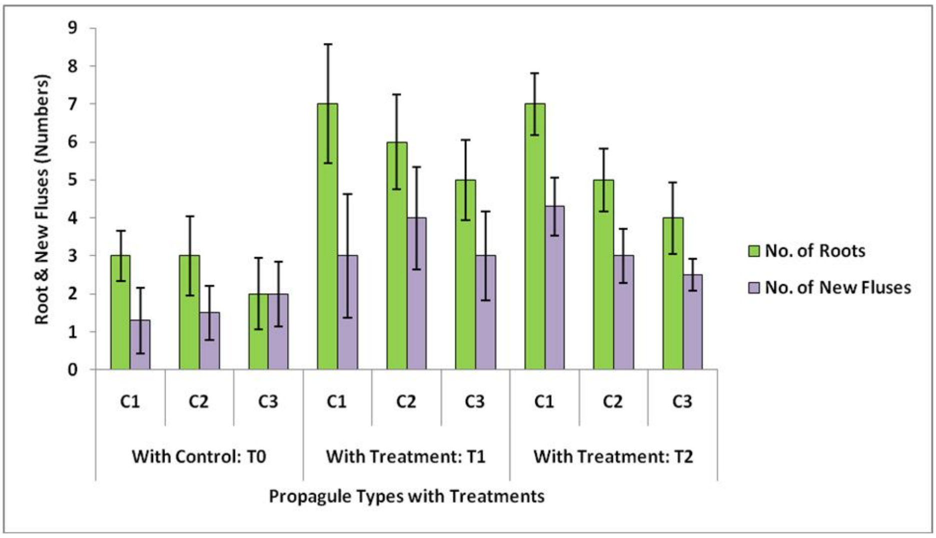

Fig. 2. Root number and new flushes of propagated stem cuttings of *O. turpethum* under different hormonal treatments

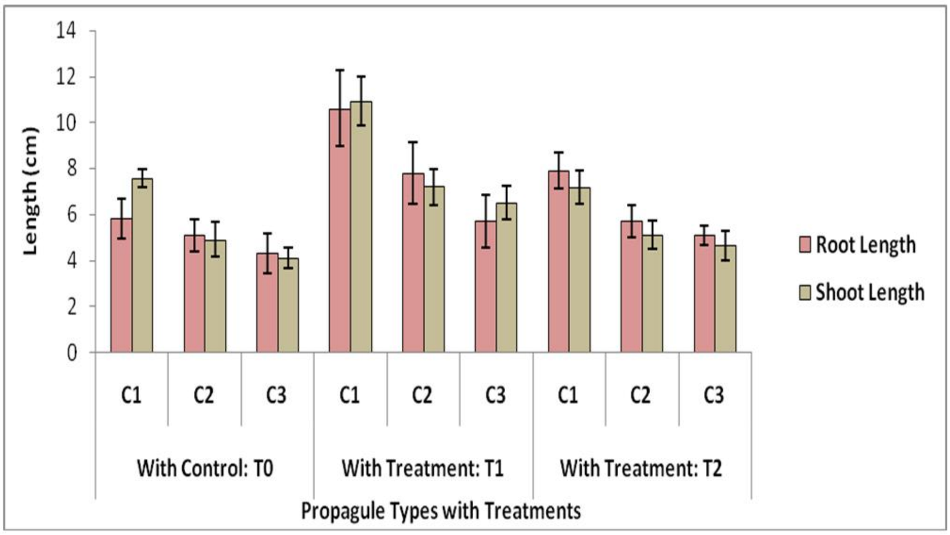

Fig. 3. Root and shoot length of propagated stem cuttings of *O. turpethum* under different hormonal treatments

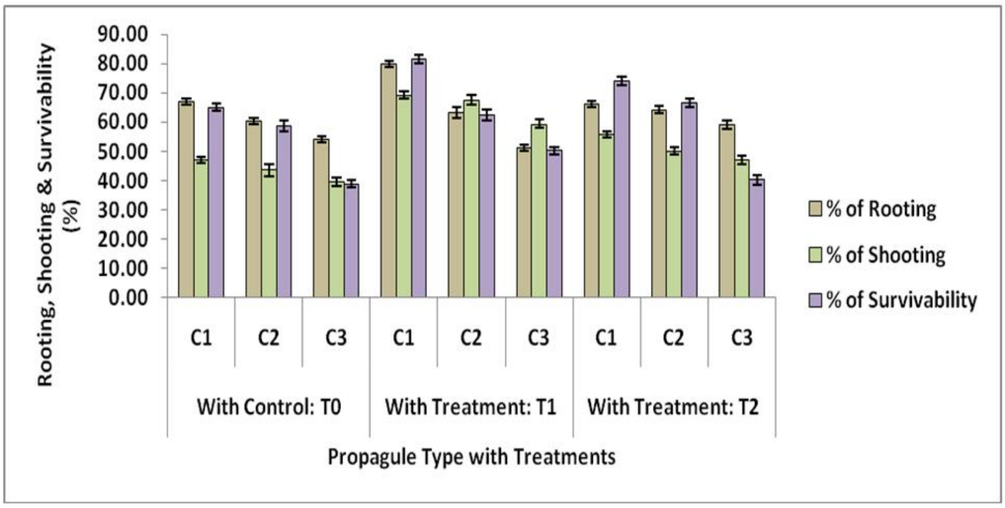

Fig. 4. Rooting, shooting and survivability % of propagated stem cuttings of *O. turpethum* under different hormonal treatments

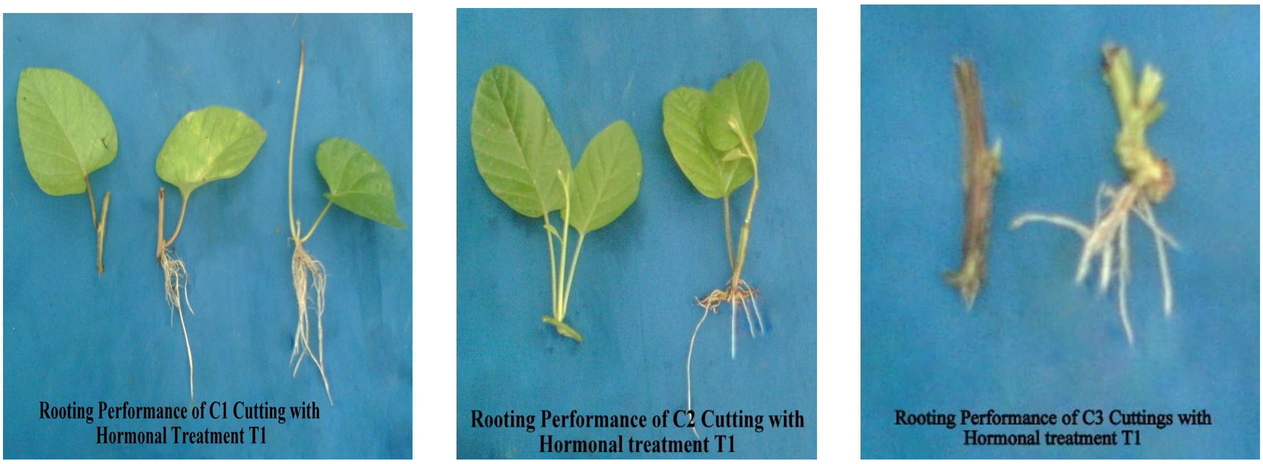

Fig. 5. Rooting Performance of three different types of stem cuttings of *O. turpethum* under T_1 hormonal treatment

Fig. 6. Established *O. turpethum* Saplings propagated from three types of stem cuttings under T_1 treatment

(1) Sapling propagated from nodal cuttings with single node plus single leaf (C_1)
(2) Sapling propagated from nodal cuttings with single node plus two leaves (C_2)
(3) Sapling propagated from nodal cuttings with single node plus small shoot-bud (C_3)

DISCUSSION

It was observed that production and development of adventitious roots in *O. turpethum* cuttings are higher when treated with hormones. Application of auxins at different concentrations strongly influenced the quality of the shoot and the root system at the end of the rooting period. The root length tends to reduce with higher concentration of IBA and NAA. The root elongation phase is very sensitive to auxin concentration and it is inhibited by application of high concentration of auxin (Kollmeier *et al*., 2000). According to the results, the nodal cuttings, that are treated with T_1 showed better growth in comparison to control and T_2 treatment. Nature of explants does play a vital role in development rate of plant. In this case nodal cuttings with single node plus single leaf (C_1**)** showed better growth rate in comparison with other cuttings. Similar type of result was viewed in several other studies. In an experiment different hormonal concentrations were utilized for vegetative propagation of two aromatic medicinal plants *Angelica glauca* Edgew. and *A. archangelica* Linn. by Vashistha *et al.* (2009). They had observed sprouting and rooting percentage using different hormonal composition of IBA, NAA and GA_3. From their study, IBA 100 ppm showed promising results for propagation procedure (Vashistha *et al.,* 2009). However effect of auxins and light on rooting efficiency of stem cuttings of *Populus nigra, Salix tetrasperma, Ipomea fistulosa* and *Hibiscus notodus* was observed by Nanda *et al.,* 1969. Exogenous application of auxins enhanced rooting in all the above cuttings (Nanda *et al.,* 1969). In this experiment, the impact of auxins on the growth and development of different types of nodal cuttings of *O. turpethum* was observed and supported by several other studies given above. According to statistical analysis, rooting percentage and survivability percentage showed significant variation when compared on basis of types of treatments and types of explants used. Furthermore post development parameters like % of shooting and shoot length have also shown significant variations at 0.05% level of probability. When type of stem cuttings were subjected for evaluation of growth rate without and with combination of hormones, significant difference in growth pattern was observed.

Moreover, types of stem cuttings played a very vital role in growth and development of the plantlet. The C_1 cuttings showed excellent growth in T_1 treatment with highest percentage of rooting and survivality as compared to control (Significant at 0.005% level). This may be due to the fact that the C_1 cuttings were having minimum leaf area, sufficient for photosynthesis, which was absent in C_3 cuttings. Furthermore, maximum no of leaves present in C_2 cuttings might lead to excess loss of water from the cuttings through transpiration. This fact is being supported by Ofori *et al.,* (1996). Effect of different auxin concentrations, leaf areas and propagation media on the rooting ability of leafy stem cuttings of *Milicia excelsa* in Ghana was compared and according to them addition of IBA increased root number by approximately 80% and percentage rooting and root number were positively related to leaf area (Ofori *et al.,* 1996).

Conclusion

Vegetative macropropagation of *O. turpethum* was achieved with the application of auxins taking different types of nodal stem cuttings as explants. The hormonal treatment IBA (2000 ppm) + NAA (3000 ppm) can be used for high rate of production of quality planting materials. Furthermore the reduced leaf area in stem cuttings showed optimum growth and development with hormonal treatment in comparison to other explants. The nodal cuttings with single node and single leaf was found to be the most suitable explants material for macropropagation of the red listed medicinal plant *O. turpethum.*

Acknowledgement

The authors are grateful to National Medicinal Plants Board (NMPB), Govt. of India for the financial support through Project Grant No. GO/OR-1/2009

REFERENCES

Alam JM, Alam I, Sharmin AS, Rahman MM, Anisuzzaman M and Alam FM. 2010. Micro-propagation and antimicrobial activity of *Operculina turpethum* (syn. *Ipomoea turpethum*), an endangered medicinal plant. *Plant Omic. J.,* 3 (2): 40-46.

Austin DF. 1982. *Operculina turpethum* (convolvulaceae) as a medicinal plant in Asia. *Eco. Bot.,* 36 (3): 265-269.

Basak UC, Das AB and Das P. 2000. Rooting response in stem cuttings from five species of mangrove trees: Effect of auxins and enzyme activities. *Mar. Biol.*, 36: 185-189.

Kamboj VP. 2000. Herbal medicine. *Curr. sci.,* 78: 35-39.

Kirtikar KR and Basu DB. 1994. Indian Medicinal Plant. Sing MP (Ed.). *Dehradun.*, 3: 1730.

Kohli KR, Nipanikar SU and Kadbhane KP. 2010. A comprehensive review on Trivrit [*Operculina turpethum* syn. *Ipomoea turpethum*]. *Int. J. of Pharma. and Bio Sci.,* 1: 443-452

Kollmeier M, Felle HH and Horst WJ. 2000. Genotypical differences in aluminum resistance of maize are expressed in the distal part of the transition zone. Is reduced basipetal auxin flow involved in inhibition of root elongation by aluminum, *Plant Physiology,* 122: 945-956.

Kumari S, Meena AK, Shukla VJ, Ota S and Rao MM. 2010. Quality Assessment of *Ipomoea species* plants by HPTLC. *J. of Analytical Chem.,* 1 (1): 1-8.

Nanda KK, Purohit AN and Kochhar VK. 1969. Effect of Auxins and Light on Rooting Stem Cuttings of Populus nigra Salix tetrasperma, Ipomea fistulosa and Hibiscus notodus in Relation to Polarity. *Physiologia Plantarum.,* 22 (6): 1113-1120.

Ofori DA, Newton AC, Leakey RRB and Grace J. 1996. Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentration, leaf area and rooting medium. *Forest Ecology and Management,* 84 (1-3): 39-48.

Saxena HO and Brahmam M. 1995. The Flora of Orissa. Regional Research Laboratory and Orissa Forest Development Corporation Ltd. I-IV.

Sharma V and Singh M. 2012. *Operculina turpethum* as a panoramic herbal medicine: a review. *Int. J. of Pharmaceutical Sci. and Research*, 3 (1): 21-25.

Vashistha RK, Chaturvedi AK, Nautiyal BP and Nautiyal MC. 2009. Vegetative propagation of *Angelica glauca* Edgew. and *Angelica archangelica* Linn.: two high value medicinal and aromatic herbs of the Himalaya. *Nature and Sci.,* 7 (8): 76-82.

Ved DK, Kinhal GA, Ravi KK, Sankar VR, Sumathi R, Mahapatra AK and Panda PC. 2008. Conservation assessment and management prioritization for medicinal plants of Orissa. Regional Plant Resource Centre, *Bhubaneswar and Foundation for Revitalization of Local Health Traditions, Bangalore*, 77-78.

****