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Direct Regeneration from Leaves and Nodes Explants of Physalis Minima Linn

[1]E. Sheeba, [2]S. Parvathy and [2]S. Palanivel

[1]Department of Microbiology, J.J College of Arts and Science, Pudukottai, Tamilnadu, India
[2]Department of Botany, Government Arts College, Karur, Tamilnadu, India

Abstract: An efficient micropropagation protocol was developed for the medicinal plant *Physalis minima* (Solanaceae) by the *in vitro* culture of leaf and nodes explants.Murashige and Skoog's medium supplemented with BAP alone or in combination with IAA and IBA induced shoots. Full strength MS solid medium with 2.0mg/l IBA exhibited the best *in vitro* rooting.The highest shoot regeneration frequency was 86%. Sixty five percent of the rooted shoots survived when transferred to green house and subsequently to the field.

Key words: *Physalis minima* · *In vitro* plant regeneration · MS medium · Tissue Culture

INTRODUCTION

Physalis minima Linn. is one of the important medicinal plant species belonging to the family Solanaceae. The plants of *Physalis minima Linn.* are bitter, appetizing, tonic, diuretic, laxative and useful in inflammations, enlargement of the spleen and abdominal troubles. The fruit is considered to be a tonic, diuretic and purgative. The mundas (a tribe) of Chhotaa Nagpur mix the juice of the leaves with water and mustard oil and use it as a remedy against ear ache. Increasing human and live-stock populations have already affected the status of wild plants; particularly those used in herbal medicine [1]. Thus, application of biotechnology may lead to alternative methods for propagation and genetic modification of *Physalis minima.* Plant *in vitro* regeneration is a biotechnological tool that offers a potential solution to the problem of medicinal plants decimation [2]. We also highlight the need for the utilization of this technology for the mass propagation of this medicinal plant.

MATERIALS AND METHODS

Seeds of *Physalis minima L.* were collected from wild plants, sun dried and surface sterilized with savlon (2%), tween 20 (6-8 drops) and Mercuric chloride (0.05%). Finally, washed thrice with sterilized distilled water inside the laminar air flow chamber. Surface sterilized seeds were inoculated on plain agar medium containing 3% sucrose for germination. The aseptically germinated seedlings were used for further studies.

The various explants such leaves and nodes were inoculated on M.S medium (1962) fortified with auxins(IAA and IBA) and different concentrations of cytokinin (BAP) [3-7]. The pH of the medium was adjusted to 5.6-5.8, solidified with 1% agar and autoclaved at15 lbs pressure for 15'. The auxins concentration was constant (0.25mg/l) and cytokinin concentrations were ranged from 1mg/l - 5mg/l .The cultures were incubated at 25±1°C with 16h photoperiod. Differentiated shoots from the above cultures were transferred to MS medium supplemented with IBA (0.5-2.5 mg/l) for root induction [8, 9]. Plantlets were removed from culture tubes and their roots were washed in running tap water, transferred to pots containing sterile vermiculite and moss in 1:1 ratio and covered with plastic bags to maintain humidity [10, 11]. The plants were transferred to field condition after 45 days.

RESULTS

Leaves Explants: Leaves explants were incubated on MS medium fortified with different concentrations of BAP (1-5mg/l) IAA (0.25mg/l). After four weeks multiple shoots emerged directly from the explants. The response was good at 5mg/l BAP + 0.25mg/l IAA combination (Table 1) where 9.0 ± 4.15 shoots developed (Fig.I) and at 1mg/l BAP + 0.25mg/l IAA, the response was less, only 2.2 ± 0.87 shoots developed.

Corresponding Author: E. Sheeba, Department of Microbiology, J.J College of Arts and Science, Pudukottai, Tamilnadu, India, 622404, Tel: +918095148199, E-mail: sheeba.elavalli@gmail.com.

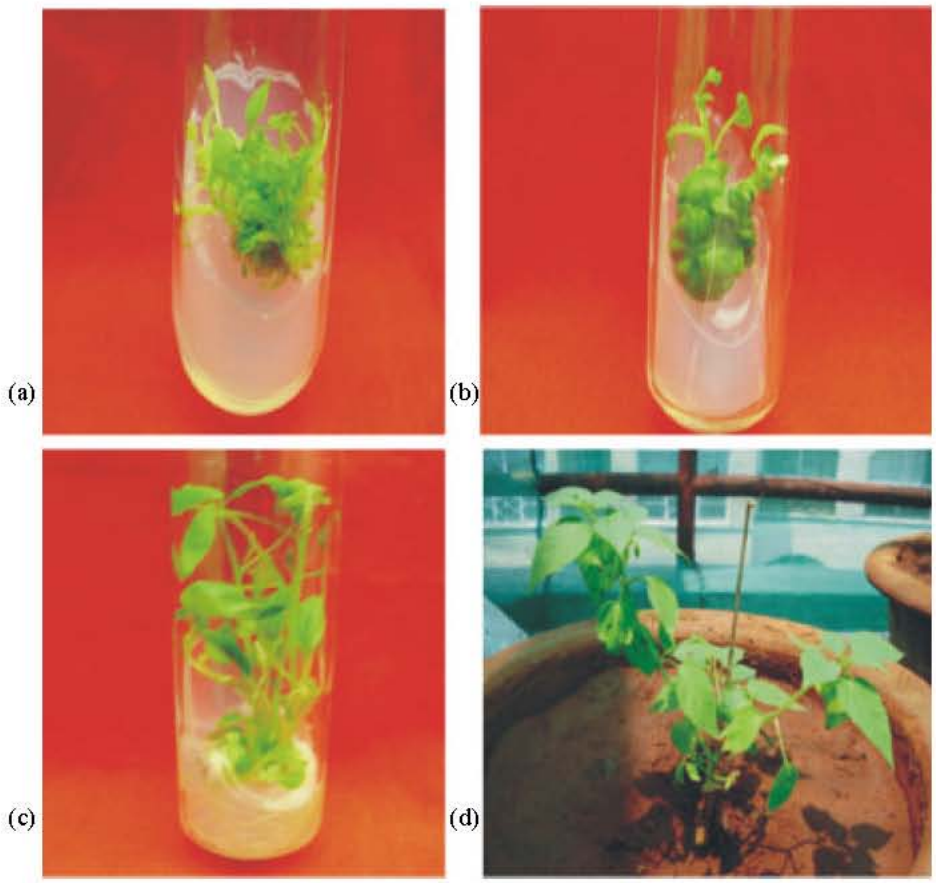

Fig. 1: Plant regeneration of *Physalis minima Linn.*(a) Regeneration of multiple shoots from nodal explants; (b) Regeneration of multiple shoots from leaf explants; (c) Rooting of regenerated shoots from nodal explants; (d) Hardened plant from nodal explants.

Table 1: Effect of plant growth regulators (BAP + IAA) on shoot regeneration from leaf segments of *Physalis minima* in M S medium after four weeks of culture

Growth regulators(mg/l) BAP+IAA	% regeneration	No. of shoots (Mean ± S.D)
1.0 + 0.25	43	2.2 ± 0.87
2.0 + 0.25	67	2.9 ± 0.75
3.0 + 0.25	74	5.7 ± 1.68
4.0 + 0.25	51	2.3 ± 1.10
5.0 + 0.25	92	9.0 ± 4.15

Nodes Explants: Nodal explants were incubated on MS medium fortified with different concentrations of BAP (1-5mg/l) alone and in combination with IAA (0.25mg/l) or IBA (0.25mg/l). After three weeks multiple shoots emerged directly from the explants. The response was good at 2mg/l BAP (Table 2) where 19.0 ± 8.59 shoots developed (Fig.1) and at 4mg/l BAP + 0.25mg/l IBA the response was less, only 1.6±1.11 shoots developed.

Table 2: Effect of plant growth regulators on shoot regeneration from nodal segments of *Physalis minima* in M S medium after three weeks of culture

Growth regulators(mg/l)				No. of Shoots/node
BAP	IAA	IBA	% regeneration	(Mean ± S.D)
1.0			53	2.7 ± 1.27
2.0			84	19.0 ± 8.59
3.0			79	12.1 ± 7.60
4.0			62	11.0 ± 2.57
5.0			68	11.4 ± 1.91
1.0	0.25		62	3.4 ± 1.91
2.0	0.25		78	6.3 ± 2.28
3.0	0.25		71	6.9 ± 2.51
4.0	0.25		79	12.5 ± 1.20
5.0	0.25		83	15.6 ±2.46
1.0		0.25	46	1.9 ± 0.83
2.0		0.25	63	5.2 ± 0.98
3.0		0.25	57	3.2 ± 0.98
4.0		0.25	41	1.6 ± 1.11
5.0		0.25	52	2.8 ± 1.54

Table 3: Effect of IBA on root induction from *in vitro* raised shoots of *Physalis minima* after 4 weeks of culture.

Growth regulator IBA (mg/l)	No.of roots/shoot (Mean ± S.D)	Root length(cm) (Mean ± S.D)
1.0	4.9 ± 1.81	4.7 ± 2.49
2.0	6.7 ± 2.61	6.1 ± 2.21
3.0	1.8 ± 0.87	4.4 ± 2.58
4.0	5.1 ± 1.70	4.4 ± 2.06
5.0	3.8 ± 2.60	3.5 ± 2.25

After four weeks on the shoot- growth medium, the shoots were transferred to the root induction medium containing IBA (2.0mg/l). Ninety two percent of shoots produced well-developed roots (Table 3 and Fig - I) within two weeks on MS medium supplemented with 3% sucrose and IBA (2.0mg/l). Eighty five percent of rooted shoots were further transferred to soil under humid conditions and sixty five percent of plantlets survived. Regenerated plants were uniform with normal leaf, flower shape and colour. No morphological variation was observed.

DISCUSSIONS

In *Solanum trilobatum* (another important medicinal plant belonging to the same family) a combination of 5mg/l BAP + 0.5mg/l IAA was reported to be the most suitable concentration for multiple shoot induction [12]. In *Psoralea corylifolia*, MS medium supplemented with 5µM BA and 0.5µM NAA recorded the highest shoot regeneration [13]. A simple and reproducible method of *in vitro* multiplication and conservation has been optimized in *Physalis minima*. Nodal segments were the effective explants for the multiple shoot regeneration.The maximum number of roots in regenerated shoots of *Physalis minima* on half strength MS medium with 0.3mg/l NAA within 15 days [14]. Tissue culture techniques have been increasingly applied for micropropation and conservation of medicinal plants especially those with vegetative mode of propagation or those for which either the roots or the whole plant is used in drug preparation.The high production cost of nursery plants and the time required for restored plants to complete their life cycle are commonly considered the barriers to successful propagation. In case of *Physalis minima,* growth occurred mainly in rainy season. Hence, *in vitro* propagation is more important for further studies like secondary metabolite production and *Agrobacterium* mediated transformation.

REFERENCES

1. Alagumanian, S., V. Saravana Perumal, R. Balachandar, K. Rameshkannan, and M.V. Rao, 2004. Plant regeneration from leaf and stem explants of *Solanum trilobatum L.* Current Sci., 86(11): 1478-1480.
2. Afolayan, A.J. and P.O. Adebola, 2004. *In vitro* propagation: A biotechnological tool capable of solving the problem of medicinal plants decimation in South Africa. African J. Plant Biotechnol., 3 (12): 683-687.
3. Anwar Shahzad, Hashma Hasan. and S.A. Siddiqui, 1999. Callus induction and regeneration in *Solanum nigrum L.in vitro*. Phytomorphol., 49(2): 215-220.
4. Neelam Sharma. and S. Vimala Devi, 2006. In *vitro* multiplication and conservation of *Peristrophe bicalyculata Nees*. -A lesser known medicinal plant with potential value. Pharmacol., 56(3&4): 121-125.
5. Sadhana Agrawal, N. Chandra. and S.L. Kothari, 2006. Plant regeneration in tissue cultures of pepper(*Capsicum annuum L.cv. mathania*). Plant cell Tissue and Organ Culture, 16(1): 47-55.
6. Sri Rama Murthy, K., D. Raghu Ramulu, J. Chandrasekhara Rao, S. Emmanuel and T. Pullaiah, 2006. *In vitro* flowering of *Spathoglottis plicata BL.* Phytomorphol., 56(3&4): 117-120.
7. Syiem, D., M.A. Laskar, J.P. Lyngdoh and J.J. Buam, 2005. Plantlet regeneration via adventitious shoot bud proliferation from leaf explants in Potentilla fulgens Wall.ex *Hook.*-A plant possessing hypoglycemic activity. Indian J. Biotechnol., 4: 257-260.
8. Chikara, J., Aneesha Singh. and J.B. Pandya, 2004. Plant regeneration from callus cultures in *Suaeda nudiflora (Wild). Moq*. Indian J. Biotechnol., 3: 457-459.
9. Siddique, N.A., M.A. Bari, Sharmin Shahnewaz; M.H. Rahman, M.R. Hasan, M.S.I. Khan and M.S. Islam, 2004. Plant regeneration of *Withania somnifera (L.) Dunal* (Ashwagandha) from nodal segments derivedcallus an endangered medicinal plant in Bangladesh. J. Biological Sci., 4(2): 219-223.
10. Rao,Y.V., A. Ravi Shankar, T.V.R. Lakshmi and K.G. Raja Rao, 2004. Plant regeneration in *Physalis pubescens L.* and its induced mutant. Plant Tissue Culture, 14(1): 9-15.
11. Vadawale, A.V., D.M. Barve and A.M. Dave, 2006. *In vitro* flowering and rapid propagation of *Vitex negundo L.* - A medicinal plant. Indian J. Biotechnol., 5: 112-116.

12. Arockiasamy, D.I., B. Muthukumar, E. Natarajan and S. John Britto, 2002. Plant regeneration from node and inter node explants of *Solanum trilobatum L.* Plant Tissue Culture, 12(2): 93-97.
13. Mohammad Anis. and Mohd Faisal, 2005. In vitro regeneration and mass multiplication of Psoralea corylifolia- An endangered medicinal plant. Indian J. Biotechnol., 4: 261-264.
14. Farhana Afroz, A.K.M. Sayeed Hassan, Laila Shamroze Bari, Rebeka sultana; Nadira Begum; Miskat Ara Akter Jahan and Rahima Khatun, 2009. *In vitro* Shoot Proliferation and Plant Regeneration of *Physalis minima* L.-a Perennial Medicinal Herb.Bangladesh J. Scientific and Industrial Res., 44(4): 453-456.