Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 483-485
Received: 07-03-2019
Accepted: 09-04-2019

Vishal Prajapati
Department of Plantation Spices Medicinal and Aromatic Crops, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

MM Patel
Department of Plantation Spices Medicinal and Aromatic Crops, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

Suman Kumar Jha
Department of Forest Biology and Tree Improvement, College of Forestry, ACHF, Navsari Agricultural University, Navsari, Gujarat, India

Kalpesh Makwana
Department of Plantation Spices Medicinal and Aromatic Crops, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

Correspondence
Suman Kumar Jha
Department of Forest Biology and Tree Improvement, College of Forestry, ACHF, Navsari Agricultural University, Navsari, Gujarat, India

De novo organogenesis from leaf explants in *Piper longum* L.

Vishal Prajapati, MM Patel, Suman Kumar Jha and Kalpesh Makwana

Abstract
De novo regeneration pathway from leaf callus was established in *Piper longum* L. Within three weeks of culture, leaf explants showed the callogenic response in media supplemented with various concentrations of TDZ. However no callus observed growth regulator free media. The callogenic response found highest (60%) in media supplemented with 0.5 mg l^{-1} TDZ. However, the per cent callus showing shoot bud (organogenic callus) was maximum (46.0%) in MS media supplemented with 0.25 mg l^{-1} TDZ which also showed maximum number (eight) of shoot bud per callus clump. These organogenic callus clumps when placed in MS + 1.5 mg l^{-1} BAP gave average 3-4 elongated shoots in each culture. The *in vitro* raised plants were successfully rooted in MS + 1.0 mg l^{-1} IBA and rooted shoots were subsequently hardened in Soil: Sand: FYM (1:1:1) with 70% success.

Keywords: *Piper longum*, callus, TDZ, regeneration

Introduction
Long pepper (*Piper longum* L), belonging to family Piperaceae, is one of the valuable medicinal plant. This unisexual perennial is indigenous to the hotter parts of India and found in wild in the Western Ghats (Soniya and Das 2002) [1]. The whole plant part is useful in treating various diseases related to respiratory tracts like asthma and bronchitis. Piperine is the main medicinal active component which has been proved to have several health benefits, especially against chronic diseases, such as reduction of insulin-resistance, anti-inflammatory effects, and improvement of hepatic steatosis (Derosa *et al.* 2016) [2].
The heedless extraction of the plant due to its medicinal importance has declined its population in wild (Nair 2000) [3]. Moreover, poor seed viability and seed set along with low percentage of rooting is major drawback for large-scale propagation of this species. Hence development of alternate regeneration pathway is required urgently in this species (Soniya and Das 2002) [1]. Some reports on micropropagation of *P. longum* is available in literatures (Soniya and Das 2002; Bhat *et al.* 1995; Madhusudhanan and Rahiman 2000; Parida and Dhal 2011; Rani and Dantu 2012) [1, 4-7] however very scanty report on the callus through regeneration pathway (Bhat *et al.* 1992) [8] is available in this species. The present work demonstrates efficient *de novo* regeneration protocol from leaf callus in *P. longum*.

Material and methods
Leaves were isolated from aseptically raised nodal culture and were cultured on full strength MS media supplemented without any cytokinin or supplemented with various concentrations of TDZ (0.25, 0.5, 1.0 and 1.5 mg l^{-1}). Data on number of explants responding for callus induction were recorded after four week of culture. Experiment was repeated five with 10 explants in each treatment. The callus obtained in various media were sub-cultured in same media composition viz., without any cytokinin or supplemented with various concentrations of TDZ (0.25, 0.5, 1.0 and 1.5 mg l^{-1}) for shoot bud induction. Per cent of callus producing shoot bud and average number of shoot bud per callus were recorded after four week of culture. Experiment was repeated five with 10 explants in each treatment. Microshoots (1.5–2.0 cm in length) were excised and transferred to half-strength MS medium supplemented with 1.0 mg l^{-1} IBA (earlier found effective for rooting of microshoots in same laboratory). The rooted plants were hardened as described above in the section. The rooted plants were removed from the culture bottles, washed free of agar with sterile distilled water and transferred to plastic pots with sterile Soil:Sand:FYM (1:1:1) media. The plantlets were maintained at 70% relative humidity by initially covering with transparent polyrthene. The plants were kept in 28^{0}C under a 12-h photoperiod for acclimatization. The plants were fertilized with 1/8th MS macro nutrients twice during the course of acclimatization at an interval of 4–5 wk.

The data recorded were analysed for ANOVA (Analysis of Variance) for Completely Randomized Design (CRD). The mean were compared using crtical difference at 5% significance level. All contaminated cultures were removed from the initiation experiments, thus limiting the scope of thorough statistical analysis. Wherever necessary, the data transformation (square root or angular) applied before analysis to normalize the data.

Results and discussion

There was no response of callogenesis even after four weeks in growth regulator free media. Even the lower concentration of TDZ induced callus on leaf explants in P. longum. The callogenic response found highest (60%) in media supplemented with 0.5 mg l^{-1} TDZ followed by 1.0 mg l^{-1} TDZ (54%). However, the per cent callus showing shoot bud (organogenic callus) was maximum (46.0%) in MS media supplemented with 0.25 mg l^{-1} TDZ which also showed maximum number of shoot buds per callus clump i.e., 8 (Table 1, Fig 1).

Table 1: Effect of different concentrations of TDZ on callus formation response (%), callus showing shoot bud (%) and average number of shoot buds per callus in leaf derived callus of Piper longum L. (Data recorded after four weeks of culture)

Treatments (mg/l)	Callogenesis (%)* (##)	Organogenic callus (%)* (##)	Average number of shoot buds per callus* (#)
Control	0.00 (0.52)	0.00 (0.52)	0.00 (0.71)
0.25 TDZ	44.00 (41.52)	46.00 (42.68)	8.00 (2.91)
0.5 TDZ	60.00 (50.80)	39.90 (39.11)	6.20 (2.58)
1.0 TDZ	54.00 (47.29)	25.32 (30.02)	7.60 (2.84)
1.5 TDZ	48.00 (43.83)	21.00 (27.24)	5.00 (2.34)
SEm±	1.33	1.44	0.06
CD at 5%	3.91	4.25	0.18
CV%	8.06	11.54	6.11

*Figures in prentheses are transformed values # square root transformation ## arcsine transformation.

In present study, TDZ found effective for callus induction from leaf explants. Recently, there is an increased use of TDZ *in vitro* propagation of plants including medicinal and horticultural crops (Deepa *et al.* 2018) [9]. TDZ successfully used for indirect regeneration in various plants (Chen *et al.* 2016; Jose and Thomas 2015; Raghu *et al.* 2011; Sherif *et al.* 2016; Tsai *et al.* 2015; Wei *et al.* 2015) [10-15].

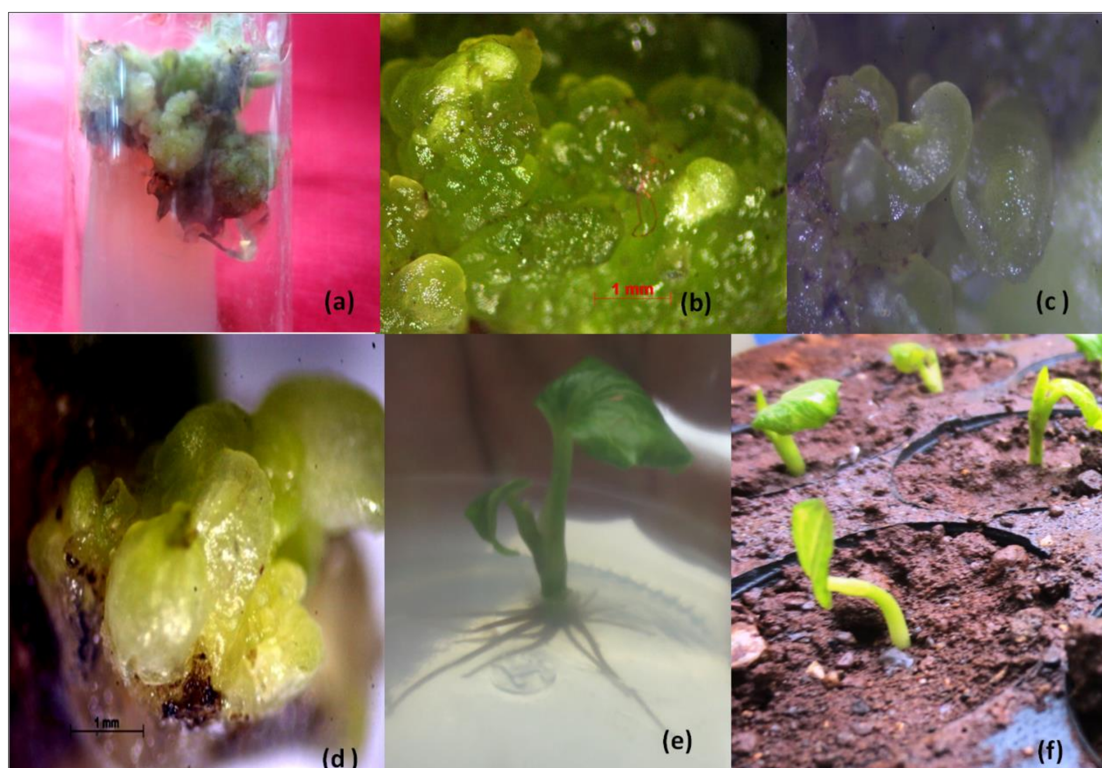

Fig 1: *De novo* organogenesis in *P. longum* a) callus formation from leaf explants b) organogenic callus c) shoot bud formation d) shoot buds elongation e) rooting e) hardening.

These organogenic callus clumps when placed in MS + 1.5 mg l^{-1} BAP gave average 3-4 elongated shoots in each culture (elongation media established in same laboratory for micropropagation in *P. longum*). These shoots were further rooted in MS + 1.0 mg l^{-1} IBA and subsequently hardened. Effectiveness of IBA in *in vitro* rooting is already reported in medicinal plants (Jani *et al.* 2015; Jani *et al.* 2015; Nagar *et al.* 2015) [16-18]. Rooted explants successfully hardened in Soil: Sand: Vermiculite (1:1:1) with 70% success.

Conclusion

Successfully indirect regeneration protocol has been established from leaf explants. The *de novo* regeneration pathway in *P. longum* can be useful for metabolic engineering

and *in vitro* manipulation of piperine through cell culture along with mass propagation for conservation and multiplication of improved planting material.

Acknowledgements
Authors acknowledge the support and facility extended by Dean, ACHF, Navsari and Principal, College of Forestry. We also acknowledge all the member of Tissue culture Laboratory, College of Forestry, NAU, Navsari, for their support during investigation.

References

1. Soniya EV, Das MR. *In vitro* micropropagation of *Piper longum* – an important medicinal plant. Plant Cell, Tissue and Organ Culture. 2002; 70(3):325-327.
2. Derosa G, Maffioli P, Sahebkar A. Piperine and Its Role in Chronic Diseases. In: Anti-inflammatory Nutraceuticals and Chronic Diseases. Edited by Gupta SC, Prasad S, Aggarwal BB. Cham: Springer International Publishing, 2016, 173-184.
3. Nair K. Manual of non-wood forest produce plants of Kerala, Krala forest research institute, Kerala, In. 2000, 268-270.
4. Bhat SR, Chandel KPS, Malik SK. Plant regeneration from various expiants of cultivated Piper species. *Plant* Cell Reports. 1995; 14(6):398-402.
5. Madhusudhanan K, Rahiman BA. The effect of activated charcoal supplemented media to browning of *in vitro* cultures of Piper species. Biologia Plantarum. 2000, 43(2):297-299.
6. Parida R, Dhal Y. A study on the micro-propagation and antioxidant activity of *Piper longum* (an important medicinal plant). Journal of Medicinal Plants Research. 2011; 5(32):6991-6994.
7. Rani D, Dantu PK. Direct shoot regeneration from nodal, internodal and petiolar segments of *Piper longum* L. and *in vitro* conservation of indexed plantlets. *Plant Cell,* Tissue and Organ Culture (PCTOC). 2012; 109(1):9-17.
8. Bhat SR, Kackar A, Chandel KPS. Plant regeneration from callus cultures of *Piper longum* L. by organogenesis. Plant Cell Reports. 1992; 11(10):525-528.
9. Deepa AV, Anju M, Dennis Thomas T. The Applications of TDZ in Medicinal Plant Tissue Culture. In: Thidiazuron: From Urea Derivative to Plant Growth Regulator. Edited by Ahmad N, Faisal M. Singapore: Springer Singapore, 2018, 297-316.
10. Chen Y, Zhang Y, Cheng Q, Niu M, Liang H, Yan H *et al*. Plant regeneration via direct and callus-mediated organogenesis from leaf explants of *Chirita swinglei* (Merr.) W. T. Wang. *In vitro* Cellular & Developmental Biology – Plant. 2016, 52(5):521-529.
11. Jose S, Thomas TD. High-frequency callus organogenesis, large-scale cultivation and assessment of clonal fidelity of regenerated plants of *Curcuma caesia* Roxb., an important source of camphor. Agroforestry Systems. 2015; 89(5):779-788.
12. Raghu AV, Unnikrishnan K, Geetha SP, Martin G, Balachandran I. Plant regeneration and production of embelin from organogenic and embryogenic callus cultures of *Embelia ribes* Burm. f. a vulnerable medicinal plant. *In vitro* Cellular & Developmental Biology – Plant. 2011; 47(4):506-515.
13. Sherif NA, Kumar TS, Rao MV. *In vitro* regeneration by callus culture of *Anoectochilus elatus* Lindley, an endangered terrestrial jewel orchid. *In vitro* Cellular & Developmental Biology – Plant. 2016; 52(1):72-80.
14. Tsai K-L, Chen EG, Chen J-T. Thidiazuron-induced efficient propagation of *Salvia miltiorrhiza* through *in vitro* organogenesis and medicinal constituents of regenerated plants. Acta Physiologiae Plantarum. 2015; 38(1):29.
15. Wei Q, Cao J, Qian W, Xu M, Li Z, Ding Y. Establishment of an efficient micropropagation and callus regeneration system from the axillary buds of *Bambusa ventricosa*. Plant Cell, Tissue and Organ Culture (PCTOC). 2015; 122(1):1-8.
16. Jani JN, Jha SK, Nagar DS. Root explant produces multiple shoot from pericycle in *Psoralea corylifolia* – a leprosy destroyer medicinal plant. Industrial Crops and Products. 2015, 67:324-329.
17. Jani JN, Jha SK, Nagar DS, Chauhan RS, Hegde H. Phloroglucinol plays role in shoot bud induction and *in vitro* Tuberization in *Tinospora cordifolia*-a medicinal plant with multi-therapeutic application. Advanced Techniques in Biology & Medicine, 2015, 1-5.
18. Nagar DS, Jha SK, Jani J. Direct adventitious shoot bud formation on hypocotyls explants in *Millettia pinnata* (L.) Panigrahi- a biodiesel producing medicinal tree species. Physiology and Molecular Biology of Plants. 2015; 21(2):287-292.