



Vandana Publications

IJRASB

Volume-5, Issue-1, January 2018

International Journal for Research in Applied Sciences and Biotechnology

Page Number: 10-13

In Vitro Shoot Regeneration and Rooting of Piper Longum L.: A valuable Medicinal Plant

DLCK Fonseka¹, WWUI Wickramaarachchi²^{1,2}Faculty of Agriculture, Department of Crop Science, University of Ruhuna, SRI LANKA¹Corresponding Author: kumarifonseka23@gmail.com

ABSTRACT

Piper longum L. (Long pepper), of family Piperaceae, is an important medicinal plant in Sri Lanka. Cultivation of Piper longum L. till recently was not very common and still it is extensively collected from the wild owing to the increasing demand from the pharmaceutical industry, threatening the very existence of the plant. Poor seed viability and low germination, scanty and delayed rooting of vegetative cuttings restrict its propagation through sexual and vegetative propagation methods indicating a need of alternative approaches such as in vitro techniques for large scale propagation of this medicinally important plant. Therefore, this study was conducted to develop an effective in vitro propagation protocol for Long pepper using nodal segments as ex plants, obtained from a shade house grown mature plant. Murashige and Skoog (MS) medium was used throughout the experiment. For shoot proliferation, MS medium supplemented with combination of 1-Naphthalene acetic acid (NAA), Kinetin (KIN) and different levels of N6-benzylaminopurine (BAP) were used. In vitro rooting was achieved to 50% strength MS basal medium containing 1mg/L. The highest frequency of multiple shoot regeneration, that is 87.5% and the maximum number of, 36-40 shoots/ex plants in one sub culture (within 8 weeks after inoculation) were observed in MS media containing BAP (3 mg/L), KIN (0.5mg/L) and NAA (0.2mg/L). The maximum number of roots (6-8 per plantlet) were obtained in 50% MS basal medium + glucose (15 g/L) + Ascorbic acid (100 mg/ L) and gelled with 0.8% (w/v) agar supplemented with NAA (1 mg/L). In vitro rooted shoots were successfully acclimatized in the shade house conditions. Therefore, it is possible to deduce that the current protocol is promising for in vitro mass propagation of Piper longum L. to solve the reproduction and cultivation problem of the plant.

Keywords— Growth Regulators, In Vitro, Large Scale, Medicinal, Piper Longum L., Propagation

I. INTRODUCTION

Piper longum L. of family Piperaceae, commonly known as long pepper is a unisexual perennial climber which is indigenous to the hotter parts of India [1]. It is well known as 'Thippili' in Sri Lanka, used for its medicinal value and also as a spice ingredient. The principal pharmacological constituents are piperine and piplartin [2]. The compound of medicinal interest in Piper longum L. is present in the female spike (inflorescence) and leaf possesses antidiabetic, antiplatelet, antiulcer, antifertility, cardiotoxic, antitumour, antimutagenic, hypotensive, respiratory depressant and anthelmintic activities [3]. Not only that, almost all its parts including the roots, stems and fruits are medicinally used in the treatment of diseases of respiratory tract like bronchitis and asthma [4].

Due to its high pharmaceutical value and wide spread use in traditional medicinal systems, plants are over exploited in natural habitats. Even, in India where the plant is indigenous, the species has now become very rare in some forests as Kerala [5].

Owing to poor seed germination and scanty and delayed rooting of vegetative cuttings, mass propagation of Piper longum L. to meet the demand seems impossible [6].

Tissue culture techniques might be applied to generate large number of true to type clonal propagules, germplasm conservation and plant improvement of Piper species. So the present study reports on + shoot regeneration and rooting of Piper longum, a valuable medicinal plant.

II. TYPES OF FLOW CONTROL DEVICES

Plant Materials Collection and Ex-Plant Surface Sterilization

The nodal segment explants (2-4 cm) were obtained from freshly emerged sprouts of mature mother plants of *Piper longum*, maintained at the Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka under shade house conditions. Explants were washed thoroughly in running tap water and washed with detergent Teepol for 5 min and surface sterilized with 10% Clorox for 10 minutes followed by 70% ethanol for few seconds. The disinfected explants were washed (3-4 washes) with sterile double distilled water to remove traces of sterilant.

Hormonal Combination and Plant Proliferation

MS medium with 30 g/l sucrose and gelled with 0.8% (w/v) agar and the pH was adjusted to 5.8 and sterilized in an autoclave under 15 psi and 121°C. The medium was supplemented with combinations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L), NAA (0.2mg/L) and KIN (0.5mg/L). All the cultures were incubated at $25 \pm 2^\circ\text{C}$ with 16/8 photoperiod under white fluorescent tubes. Numbers of shoots/explant produced were recorded in each combination and shoot proliferation rate was calculated.

In Vitro Rooting of Micro-Propagated Shoots

Well developed shoots after 8 weeks of shoot proliferation were rooted on 50% MS basal medium with glucose (15g/L) + Ascorbic acid (100mg/L) and gelled with 0.8% (w/v) agar supplemented with NAA 1 mg/L. The root number was taken by visually counting the number of the roots.

Acclimatization

For acclimatization in vitro grown plants with well-developed shoots and roots were washed gently with Luke warm water and under running tap water to remove agar from roots. These plantlets were planted in polybags containing sand, compost and coir dust in 1:1:1 ratio. These were kept in shade house under high relative humidity (80–90 %). for acclimatization for 30 days and the percentage of survival was noted.

Experimental Design and Statistical Analysis

All experiments were arranged as Completely Randomized Design with 20 replicates and design was repeated two times, the obtained data were subjected to analysis of variance and the means were compared with Duncan's Multiple Range Test (DMRT).

III. RESULTS & DISCUSSION

All the nodal explants of *Piper longum* L. responded readily on MS medium supplemented with NAA and BAP in combination with various concentrations for bud break. However, highest frequency of multiple shoot regeneration, that is 87.5% was seen in MS media containing BAP (3.0 mg/L), KIN (0.5mg/L) and NAA (0.2mg/L) (Table 1). The effectiveness of BAP in shoot proliferation has been well documented for a number of plant species [7]. Also, a similar result has been obtained for *Piper longum* L. by Parida and Dhal [8] for 3mg/L BAP and 0.5mg/L IAA. To obtain plantlets with uniform growth characteristics of the

mother plant, the direct regeneration is essential. Nodal explants are suitable for a large-scale production and multiple shoots can easily be obtained in vitro from this explant type [9]. The lateral buds were developed into shoots after 30 days of incubation.

According to our observations (Table 1), best response in terms of multiple shoot regeneration was observed on MS medium supplemented with 0.2 mg/L NAA + 2 mg/L BAP + 0.5 mg/L KIN resulting 36-40 shoots/ex explants in one sub culture (within 8 weeks after inoculation).

TABLE I
EFFECT OF DIFFERENT LEVELS OF BAP, NAA AND KIN ON MULTIPLE SHOOT INDUCTION FROM NODAL EXPLANTS OF *C. PANICULATUS*

BAP (mg/L)	NAA (mg/L)	KIN (mg/L)	No. of shoots/explant	Frequency of multiple root regeneration
0.5	0.2	0.5	4.34 ± 0.01^d	65.0%
1.0	0.2	0.5	10.41 ± 0.05^c	50.2%
1.5	0.2	0.5	13.21 ± 0.13^c	65.4%
2.0	0.2	0.5	36.25 ± 0.49^a	87.5%
2.5	0.2	0.5	26.25 ± 0.04^b	78.8%
3.0	0.2	0.5	21.67 ± 0.01^b	63.0%

In the report of Soniya and Das [10] *Piper longum* has produced maximum number of shoots in MS medium supplemented with 2 mg/L BAP and 1 mg/L KIN. This implies that inclusion of these cytokinins have synergic effect in promoting shoot induction. However, maintaining BAP at 2 mg/L and a further increase of Kinetin concentration will result in reduction of shoot induction frequency [11].

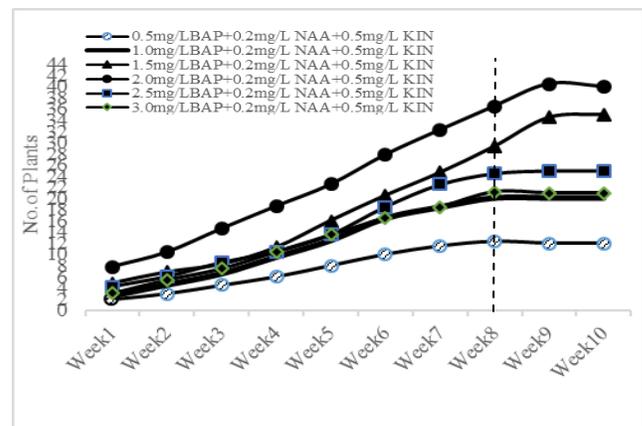


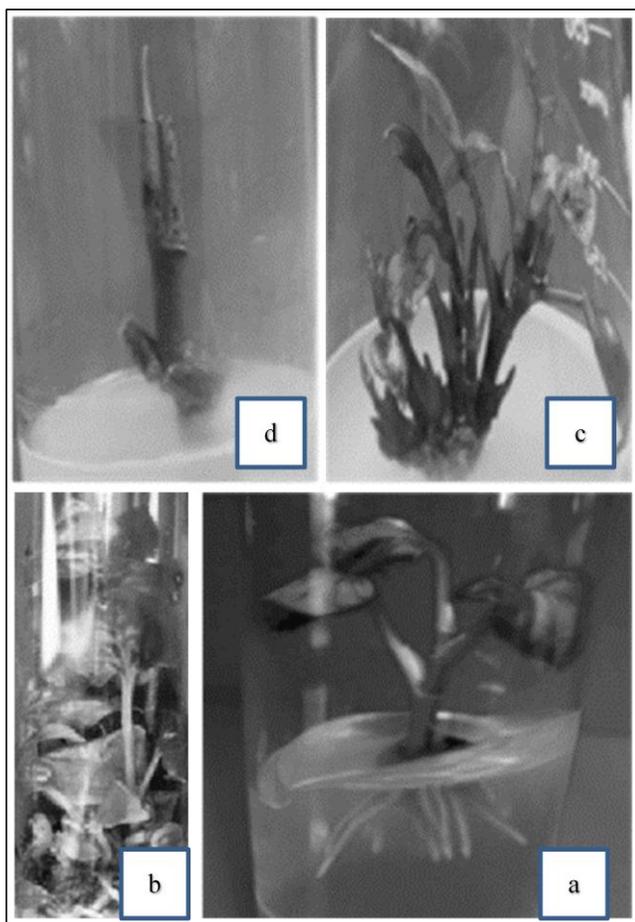
Figure 1: Plantlet proliferation rate of *Piper longum* L.

In commercial cultivation, production of a large number of plantlets within a minimum time duration is essential [12]. In this study, it was observed that by 8th week after culture initiation, nodal explants give rise to maximum number of shoots with high vigor and the trend was continued (Figure 1). Repeated subculture is usually applied

for increasing the shoot bud multiplication rate. Use of repeated subculture technique to increase the number of shoot buds in *Pterocarpus santalinus* has increased in shoot bud multiplication rate up to the six subculture stage [13].

Effective rooting was undertaken on 50% MS basal medium + glucose (15 g/L) + Ascorbic acid (100 mg/L) and gelled with 0.8% (w/v) agar supplemented with NAA 1 mg/L with highest number of roots (6-8), 12-15 days after inoculation. Phenolic exudates cause browning and necrosis of plantlets and this was overcome by toting up the root induction medium with 100mg/L ascorbic acid. Similar result was also obtained from 1 mg/L NAA [14]. The root development was recorded on MS0 without adding auxin 40-45 days after inoculation. The plantlets with well-developed shoots and roots were transferred to poly bags for acclimatization.

All the acclimatized plantlets showed 100% survival percentage after 30 days.



Different steps of in vitro regeneration of *Piper nigrum*: (a) Shoot formation on MS medium with 3.0 mg/L BAP, 0.5mg/L KIN and 0.2 mg/L NAA. (b) Multiple shoot formation on same medium mentioned in Fig. a. (c) Multiple

shoot elongation on the same medium as in Fig. a, (d) In vitro root formation on 50% MS medium with 1 mg/L NAA.

IV. CONCLUSION

This study provides an efficient in vitro propagation method that could be commercially feasible for *Piper longum* L. using a simple and cost-effective protocol for producing true to type plants in a relatively short period and with high multiplication rate. Hence, this protocol is found to be optimal for in vitro multiplication of the plant *Piper longum* L. to solve the commercialization and conservation bottle necks of this economically valuable medicinal plant.

REFERENCES

- [1] R. Parida, Y. Dhall, A study on the micro-propagation and antioxidant activity of *Piper longum* L. (an important medicinal plant). *Journal of Medicinal Plants Research*, 5: 6991-6994.2011.
- [2] V.V. Sivarajan, I. Balachandran, Ayurvedic drugs and their plant resources. Oxford and IBH Publishing Co Pvt. Ltd., USA, 1994.
- [3] A.M. Majumdar, J.N. Dhuley, V.K. Deshmukh, P.H. Raman and S.R. Naik, Anti-inflammatory activity of piperine, *Japanese Journal of Medical Science and Biology*, 43: 95-100, 1990.
- [4] B.S.Jung, M.K.Shin, Encyclopedia of illustrated Korean natural drugs, Araliaceae. In: Kwon SB (Ed) *Encyclopedia of World Medicinal Plants*. Seoul, 439-443, 1998.
- [5] K.K.N.Nair, *Manual of Nonwood Forest Produce Plants of Kerala*, Kerala Forest Research Institute, Kerala, India, 268-270, 2000.
- [6] V. Sarasan, V. Thomas, B. Lawrence, G.M. Nair, Plant regeneration in *Piper longum* L. (Piperaceae) through direct and indirect shoot development, *Journal of Spices and Aromatic Crops* 2: 34-40, 1993.
- [7] M. Phulwaria, K. Ram, K. Harish, A.K. Gupta and N.S. Shekhawat, Micropropagation of mature *Terminalia catappa* (Indian almond), a medicinally important forest tree, *Journal of Forest Research*, 17:202-207, 2012.
- [8] R. Parida and Y. Dhal, A study on the micro-propagation and antioxidant activity of *Piper longum* (an important medicinal plant), *Journal of Medicinal Plants Research* Vol. 5(32): 6991-6994, 2011.
- [9] M. Karim, R. Jannat, M. Rahman and M. Haque, Micropropagation of *Stevia* Plant from Nodal Segments. *Progress. Agriculture*, 19: 20-28, 2013.
- [10] E.V. Soniya, M.R. Das MR, In vitro micropropagation of *Piper longum* -an important medicinal plant. *Plant Cell, Tissue and Organ Culture*, 70: 325-327, 2002.
- [11] K. Sathelly, S. Podha, Establishment of Efficient Regeneration System from Leaf Discs in Long Pepper an Important Medicinal Plant (*Piper longum* L.), *Med Aromat Plants* 5:3, 2016.

[12] G. Cristiano, B. Murillo-Amador and B. De Lucia, Propagation Techniques and Agronomic Requirements for the Cultivation of Barbados Aloe (*Aloe vera* (L.) Burm. F.)—A Review. *Frontiers in Plant Science*, 7, 2016.

[13] E. Prakash, P.S. Sha Valli Khan, T.J.V. Sreenivasa Rao, E.S. Meru, Micropropagation of red sanders (*Pterocarpus santalinus* L.) using mature nodal explants. *J.For. Res.* 11, 329–335, 2006.

[14] B. Rock-Okuyucu, M. Bayraktar, I. Akgun and A. Gurel, Plant Growth Regulator Effects on In vitro Propagation and Stevioside Production in *Stevia rebaudiana* Bertoni. *Hort Science*, 51(12): 1573-1580,2016.