Journal of Medicinal Plants Research Vol. 5(32), pp. 6991-6994, 30 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.1067

Full Length Research Paper

A study on the micro-propagation and antioxidant activity of *Piper longum* (an important medicinal plant)

Reena Parida* and Yogamaya Dhal

Utkal University, Bhubaneswar-751004, Odisha, India.

Accepted 18 November, 2011

An efficient micro-propagation technique has been developed for cloning *Piper longum* plantlets by nodal segment multiplication. The result showed that the optimal medium for shoot multiplication was Murashige and Skoog (MS) medium supplemented with 1 mg/L benzyladenine and 1 mg/L indoleacetic acid. The best medium for rooting was in MS medium with 1 mg/L benzyladenine and 0.2 mg/L indoleacetic acid. The present study reveals the antioxidant activity and *in vitro* multiplication of *P. longum* plants.

Key words: *Piper longum*, growth regulators, *in vitro* propagation, antioxidant.

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 and grows wild in the evergreen forests of Western Ghats. It is well known as 'pippali', used for its medicinal value and also as a spice ingredient. The leaf of *P. longum* possesses antidiabetic, antiplatelet, antiulcer, antifertility, cardiotonic, antitumour, antimutagenic, hypotensive, respiratory depressant and anthelmintic activities. 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 (Sivarajan and Balachandran, 1994). The harmful free radicals play an important role in most major health problems such as cancer, cardiovascular diseases, rheumatoid arthritis, cataract, alzheimer's disease and other degenerative diseases associated with aging. Whereas the antioxidants are beneficial components that neutralize the free radicals before they attack cells and prevents damage to cell proteins, lipids and carbohydrates. So a wide range of antioxidants both natural and synthetic can be used in treatment of human diseases. The antioxidant supplements containing foods might be used to help human body cells to reduce oxidative starch (Halliwell and Gutteridge, 1999). As the plants are excessively extracted from its natural resource, the species has now become very rare in some forests as Kerala (Nair, 2000).

Conventional propagation is beset with problems of poor seed viability, low percentage of germination and scanty, delayed rooting of vegetative cuttings. Therefore, there is a need for alternative propagation methods (Sarasan et al., 1993). Tissue culture techniques might be applied to generate large number of true to type clonal propagules, germplasm conservation and plant improvement of *Piper* species. There are very few reports on micro-propagation of *P. longum* but there is no report on its antioxidant activities. So the present study reports on *in vitro* multiplication and antioxidant activities of leaf sample of *P. longum* plantlets.

*Corresponding author. E-mail: ms.reenas@gmail.com.

Abbreviations: BA, Benzyladenine; **KIN,** Kinetin; **IAA,** Indoleacetic acid; **IBA,** Indolebutyric acid; **NAA,** Napthaleneacetic acid.

MATERIALS AND METHODS

Plant materials collection

P. longum plants were collected from Mayurbhanj district of Odisha and were maintained in the medicinal plant garden. The young nodes of mature plants of *P. longum* were used as explants.

Surface sterilization, inoculation of explants and acclimatization

Nodal segments of *P. longum* were trimmed to 1 to 1.5 cm length

Table 1. *In vitro* shoot multiplication in *Piper longum* using different plant growth regulators.

S/N	MS Media + Growth regulators (mg/L)	Percentage of shoot initiation (Mean ± SE)	No. of shoots/explants (Mean ± SE)	No. of roots/explants (Mean ± SE)
1	BA(0.5)	35.2 ± 0.3[a]	2.0 ± 0.3[a]	5.5 ± 0.2[e]
2	BA(1)	50.2 ± 0.7[b]	3.8 ± 0.5[b]	4.5 ± 0.8[b]
3	BA(3)	85.0 ± 0.2[f]	4.6 ± 0.4[c]	5.8 ± 0.5[c]
4	BA(5)	40.8 ± 0.5[a]	2.5 ± 0.2[a]	4.0 ± 0.3[b]
5	BA(1)+IAA(0.2)	48.6 ± 0.7[b]	5.2 ± 0.7[d]	6.3 ± 0.2[e]
6	BA(1)+IAA(0.5)	60.8 ± 0.1[c]	5.5 ± 0.3[d]	5.5 ± 0.8[c]
7	BA(1)+IAA(1)	65.0 ± 0.2[c]	7.5± 0.5[e]	4.6 ± 0.6[b]
8	BA(3)+IAA(0.5)	80.2 ± 0.6[e]	5.8 ± 0.2[d]	3.0 ± 0.2[a]
9	BA(3)+IAA(1)	78.8 ± 0.5[e]	4.3 ± 0.9[c]	3.6 ± 0.3[a]
10	BA(1)+NAA(0.5)	35.4 ± 0.8[a]	3.5 ± 0.2[b]	3.6 ± 0.3[a]
11	BA(1)+NAA(1)	38.8 ± 0.9[a]	3.3 ± 0.4[b]	4.3 ± 0.7[b]

* a-f: Mean having the same letter in a column were not significantly different at $p < 0.05$ level.

and washed with 2% bavistin for 10 min, and then the explants were washed under running tap water followed by a neutral liquid detergent (Extran, Merck) for 3 to 5 min. Again these treated explants were washed repeatedly with distilled water and finally surface sterilized with 0.1% mercuric chloride for 3 to 7 min in a laminar flow cabinet. The surface sterilized explants were washed 3 to 4 times with autoclaved water aseptically to remove any trace of mercuric chloride prior to inoculation. Outer scale leaves were removed aseptically and explants were inoculated in MS medium (Murashige and Skoog, 1962). Cultures were maintained at 25 ± 2°C under 16/8 h photoperiod provided by cool white fluorescent tubes (60 µ mol m^{-2} s^{-1}). *P. longum* explants were cultured on MS medium fortified with various concentrations of BA (0.5 to 5 mg/L), IAA (0.2 to 1 mg/L) and NAA (0.5 to 1 mg/L). The explants were sub-cultured to a fresh media after every 2 weeks and the data was recorded for multiple shoot induction and rooting respectively. *In vitro* grown plants having well developed shoots and roots were washed gently under running tap water to remove agar from roots and planted in earthen pots containing soil, cow dung and sand mixture in 1:1:1 ratio. The plantlets were kept in greenhouse for acclimatization for 30 days and then transferred to normal field condition for growth until maturity. The percentage of survival was noted.

DPPH antioxidant assay

Fresh *in vitro* leaves of *P. longum* were rinsed several times with running tap water to make it dust and debris free. Then these were dried in the shady condition for about one week until they became moisture free. Dried leaves were grinded in a mortar and pestle to get fine powder form. Using a Soxhlet apparatus, the dried powdered leaf samples (each 50 g) were extracted successively with double distilled water, followed by ethanol (400 ml) for 10 to 12 hours. Then the collected solutions were filtered through Whatman No.1 filter paper. The extracts were evaporated to dryness under reduced pressure at 90°C by using rotary vacuum evaporator to obtain the respective extracts thereby stored at 4°C for further analysis.

The evaluation of radical scavenging activity (antioxidant activity) was conducted by the method of Blois (1958) with some modifications. The following concentrations of leaf extracts were prepared 40, 60, 100, 130 and 160 µg/ml, respectively. A stock solution of the sample (100 mg/ml) was diluted up to five concentrations. Each concentration was tested in triplicate samples. The portion of sample solution (0.5 ml) was mixed with 3.0 ml of 0.1 mM 1, 1-diphenyl-2-2picrylhydrazyl (DPPH, in 95% distilled ethanol) and allowed to stand at room temperature for 30 min under light protection. The absorbance was measured at 518 nm spectrophotometrically. The scavenging activity of the samples at corresponded intensity of quenching DPPH lower the absorbance of the reaction mixture and indicates higher free radical scavenging activity. The difference in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as (%) scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the equation given below. In the DPPH test, antioxidants were typically characterized by their IC50 value (Inhibition Concentration of Sample required to scavenge 50% of DPPH radicals). The equation is:

Scavenging effect (%) = (1-As/Ac) ×100 (As is the absorbance of the sample at t =0 min; Ac is the absorbance of the control at t=30 min).

RESULTS

In vitro shoot multiplication and field transfer of regenerants

Multiple shoots were induced from nodal segments of conventionally grown plants of *P. longum* in MS medium supplemented with BA (0.5 to 5 mg/l), IAA (0.2 to 1 mg/l) and NAA (0.5 to 1 mg/l). After 3 days, on the 4th day, growth in the explants was observed. Highest percentage of growth response, that is 85.0 ± 0.2 was seen in MS media containing BA (3 mg/L) (Table 1 and Figure 1a,b). The shooting and rooting are facilitated in different concentrations of same cytokinins as shown in Table 1. Maximum numbers of shoots were observed in BA (1 mg/l) and IAA (1 mg/L); 7.5 ± 0.5 and maximum number of roots; 6.3 ± 0.2 were observed in BA (1 mg/L) and IAA (0.2 mg/L). Shoots were cut into segments and rooted on

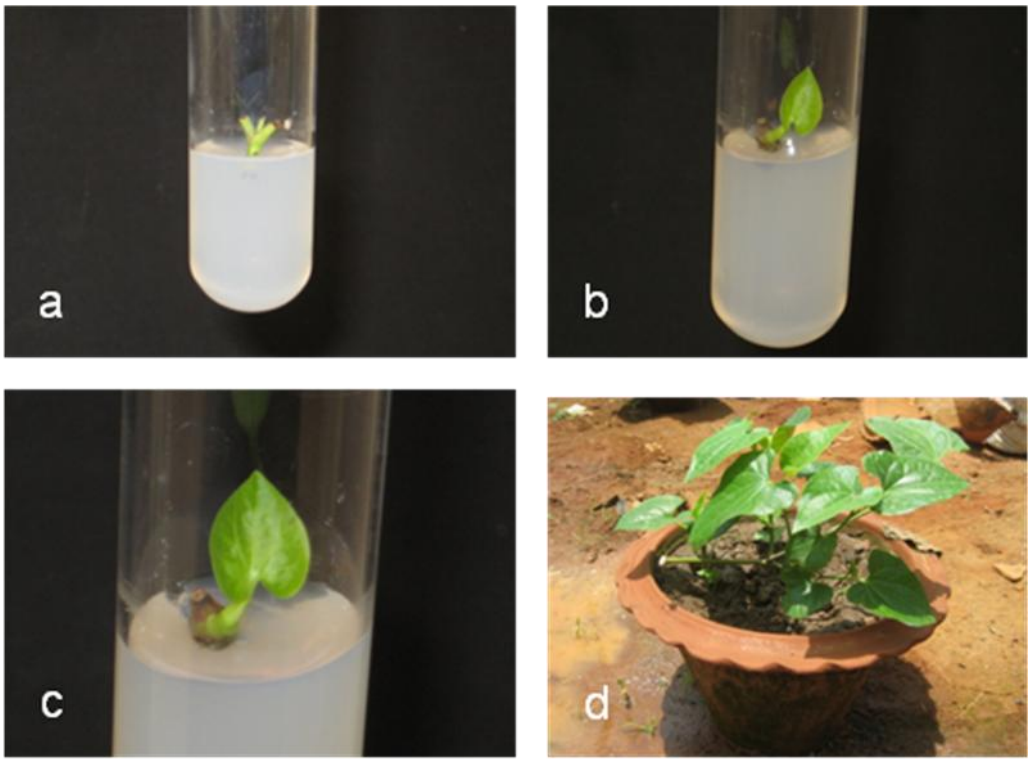

Figure 1. a. Piper longum explant; b. shoot formation; c. rooting of shoots; d. *in vitro* grown plantlets kept in the greenhouse condition.

Table 2. IC_{50} Value of *Piper longum* taking water and ethanol as solvent.

Solvents used	Parts used	Crude extract (g)	IC_{50} Value (µg/ml)
Water	Leaf Sample	1.45	>50
Ethanol	Leaf Sample	1.90	115

rooting medium. No additional step was required for rooting of the shoot only a little decrease in IAA concentration. Shoot tips excised from these shoots were transferred to fresh medium containing the same hormonal combinations which showed similar trend in multiplication in the subsequent subcultures. After 1 month, full growth of the plantlets was observed when the plantlets attained considerable growth in number and size from culture tubes and these were then transferred to greenhouse condition for acclimatization. About 70% of plants survived and grew to maturity in field condition (Figure 1d).

Antioxidant activity

The yield of extracts using water and ethanol in case of *P. longum* was 1.45 and 1.90 g, respectively. The variation in yield may be due to the polarity of the solvents used in the extraction process (Table 2). Table 3 shows the results of the free radical (DPPH) scavenging activity in (%) inhibition in *P. longum.* The result revealed that the ethanol fraction of leaves exhibited highest radicals scavenging activity, that is, 63.84 ± 0.05. The *P. longum* leaf extracts obtained from ethanol shows the value as 63.84 ± 0.05 which is the highest scavenging activity with 160 µg/ml of the crude extract followed by its water extract as 33.84 ± 0.05 with the same concentration. In overall comparison the ethanolic extract of leaves in *P. longum* shows the highest scavenging activity followed by the water.

DISCUSSION

In vitro shoot multiplication and antioxidant activity

In our study, MS media containing BA and IAA were more effective for shoot multiplication. Similarly in earlier studies of Bhat et al. (1995), BA and IAA were effective

Table 3. Specific antioxidant activity of different concentration of *in vitro* leaf extracts of *Piper longum* with the solvents water and ethanol.

Antioxidant activity (%)		
Concentration of extracts (µg/ml)	**Water**	**Ethanol**
40	25.38±0.02	53.07±0.04
60	27.69±0.08	56.92±0.06
100	30.00±0.10	60.00±0.09
130	32.30±0.07	61.53±0.07
160	33.84±0.05	63.84±0.05

for the multiplication of *in vitro* raised plants of *P. longum, Piper betle* and *Piper nigrum*. But in the report of Soniya and Das (2002) *P. longum* produced maximum number of shoots in MS medium supplemented with BA 2 mg/l and KIN 1 mg/l. Few reports on multiplication of *P. longum* are reported (Sarasan and Nair, 1991; Sarasan et al., 1993; Bhat et al., 1995; Philip et al., 2000). Very limited work has been done on tissue culture of Piper species because the *in vitro* establishment of *P. longum* is severely affected by bacterial contamination (Bhat et al., 1995). Similarly in our study there were more chances of bacterial contamination but these were reduced by dipping the explants in 2% bavistin for 10 min.

During antioxidant activity, ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity as also reported by Karadeniz et al. (2005). Reports on *P. longum* antioxidant activities have been done by Veeru et al. (2009) and in other species of *Piper* by Manigauha et al. (2009). The plant exhibited strong anticancer, hepato-protective, antiviral and several other activities. These properties may be due to its antioxidant activity. Among the extracts listed the ethanolic crude extracts of *P. longum* was found to be a better antioxidant in comparison to water. As the plant extract of *P. longum* is safe and bears good antioxidant activity so it can be used as food supplements as well as abundantly used for medicinal purposes having higher therapeutic value for various diseases. So in this study an effort was made for *in vitro* multiplication of *P. longum* as an alternative method to conventional harvesting along with its antioxidant properties which is an important medicinal plant.

REFERENCES

Bhat SR, Chandel KPS, Malik SK (1995). Plant regeneration from various explants of cultivated *Piper* species. Plant Cell Rep., 14: 398-402.

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181: 1199-1200.

Halliwell B, Gutteridge JMC (1999). Free radicals in biology and medicine, 3rd ed. Oxford University Press Oxford.

Manigauha A, Ali H, Maheshwari MU (2009). Antioxidant activity of ethanolic extract of *Piper betel* leaves. J. Pharm. Res., 2(3): 491-494.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

Nair KKN (2000). Manual of Nonwood Forest Produce Plants of Kerala Kerala Forest Research Institute, Kerala, India, pp. 268-270.

Philip S, Banerjee NS, Das MR (2000). Genetic variation and micropropagation in three varieties of *Piper longum* L. Current Science, 78: 169-173.

Sarasan V, Nair GM (1991). Tissue culture of medicinal plants:morphogenesis, direct regeneration and somatic embryogenesis. In: Prakash J & Pierik RLM (eds) Horticulture - New Technologies & Applications Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 237-240.

Sarasan V, Thomas E, Lawrence B, Nair GM (1993). Plant regeneration in *Piper longum* L. (Piperaceae) through direct and indirect shoot development. J. Spices Arom. Crops, 2: 34-40.

Sivarajan VV, Balachandran I (1994). Ayurvedic Drugs and their Plant Sources. Oxford and IBH Pubishing Co. Pvt.Ltd, pp. 374-376.

Soniya EV, Das MR (2002). *In vitro* micropropagation of *Piper longum* - an important medicinal plant. Plant Cell, Tissue and Organ Culture, 70: 325-327.

Veeru P, Mishra PK, Mishra M (2009). Screening of medicinal plant extracts for antioxidant activity. J. Med. Plants Res., 3(8): 608-612.