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Short Communication

In vitro callus induction and plant regeneration from immature zygotic embryos of black pepper (*Piper nigrum* L.)

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Abstract

Immature zygotic embryos of *Piper nigrum* L. were cultured in different concentrations and combination of auxins and cytokinins. Callus formation was highest (92.2%) when the explants were cultured on Murashige and Skoog (MS) medium supplemented with 2.0 mg/l IAA and 0.5 mg/l kinetin. Calli were transferred to same basal medium containing 1.5 mg/l BAP and 0.5 mg/l NAA, where they developed maximum number of adventitious buds within three weeks of culture. Regenerated shoots were elongated on MS medium containing 1.5 mg/l BAP and 0.5 mg/l NAA and subsequently rooted in half-strength MS medium with 1.5 mg/l IBA.

Keywords: Black pepper, Callus induction, Shoot regeneration, Root induction

Introduction

Black pepper (*Piper nigrum* L.) belongs to the family of Piperaceae and is one of the most valuable spices crop and has leading position in spices trade. Black pepper locally known as "golmarich" in Bangladesh. The spice is produced from the fruits of this vine. Black pepper is grown successfully in Bangladesh (Haque and Hossain, 1985) and it is produced in very small quantity which is not recorded in the official statistics (Ahmad, 1985). Till now stem cutting is the only method of propagation and vegetative propagation from selected clones is highly desirable. Rapid clonal propagation of desired genotypes is one of many applications of plant tissue culture. Although over 1000 plant species have been reported to be enable to culture *in vitro* (Brown and Thorpe, 1986). Improvement of this spice is necessary in order to make it to be quite good with respect to size and pungencies through plant biotechnological method. Very little information is available on *in vitro* culture in black pepper (Rahman *et al.* 2000; Rahman 2006). Tissue culture technique could offer a valuable alternative and reliable procedure for large scale propagation of black pepper. The present study, describes a technique for production of multiple shoots through callus culture from immature zygotic embryos of black pepper.

Materials and Methods

The experiment was carried out in the Biotechnology Laboratory of the Institute of Biological Sciences, University of Rajshahi, Rajshahi. After surface sterilization seeds were air-dried and excised embryos were placed in 100×15 mm petridishes containing an agar-gelled medium for callus induction. The dishes were sealed with parafilm and stored in dark at 26±1^{0}C. Cultures were scored for number of embryos induced callus and days to callus initiation. The growth media consisted of MS basal formula (Murashige and Skoog, 1962) with 0.5 - 4.0 mg/l IAA. With every concentration of IAA, 0.5 mg/l kinetin was also used. The proliferated calli were subcultured in MS medium containing different concentrations (0.5 - 4.0 mg/l) of BAP with 0.5 mg/l NAA for adventitious shoot bud induction. The elongated shoots (>3.0 cm long) were placed for rooting in half-strength MS medium with IBA and IAA in different concentrations.

The data on both callus induction and shoot regeneration frequency (%) were recorded after four weeks of inoculation and the results are presented as mean ± standard error with analysis of variance. The experiments were carried out in a Randomized Complete Block (RCB) design.

Results and Discussion

Irrespective of growth regulator concentrations used, when cultured on media containing IAA and kinetin the explants swelled within 4-6 days and callus formation started from the middle portion of the embryo within 8-16 days of initiation. It was noticed that media supplemented with IAA 1.5-2.5 mg/l and kinetin 0.5 mg/l yielded high amount of callus (Table 1) and they were creamish in colour (Fig. A). Maximum frequency (92.2%) of explants produced callus in media containing 2.0 mg/l IAA and 0.5 mg/l kinetin. The callus appeared as loosely-packed friable regions of vacuolated cells, which produced more callus after subculture in shoot induction medium (BAP with NAA). It was also observed that low (0.5-1.0 mg/l) and high concentration (3.0-4.0 mg/l) of IAA with kinetin failed to induce callus sufficiently. The results were summarized in Table 1. Bhat *et al.* (1992) described optimum conditions for initiation and growth of calli from *Piper longum* seedling nodal segment explants and they suggested that IAA was suitable for the purpose. Rahman *et al.* (2000) used IAA for inducing callus from seedling apical shoot and nodal segment of *Piper nigrum* and produced greenish, hard textured embryogenic calli. Rahman (2006) also observed similar findings from cotyledon explants.

Table 1. Effect of different concentrations of IAA with 0.5 mg/l kinetin in MS medium on callus induction from immature zygotic embryos of *Piper nigrum*

Growth regulators (mg/l)		Days to callus initiation	% of callus formation
IAA	Kinetin		
0.5	0.5	14-16	42.4
1.0		12-14	48.3
1.5		10-12	82.6
2.0		08-10	92.2
2.5		10-12	90.3
3.0		10-12	54.4
3.5		14-15	40.3
4.0		14-16	38.6

When the irregularly shaped calli were transferred to medium containing deferent concentration (0.5-4.0 mg/l) of BAP with NAA 0.5 mg/l and developed adventitious shoot buds. Under appropriate condition of auxin and cytokinin, the six-weeks old calli showed the formation of a number of adventitious shoot buds in compact masses of many of them developed into shoots (Fig. B). The highest frequency (90.4%) of calli produced shoots, number of shoots per callus (62.8) and shoot length (1.8cm) were recorded in media with BAP 1.5 mg/l and NAA 0.5 mg/l (Table 2). Similar results were observed in apical shoot, nodal segment (Rahman *et al.*, 2000) and in cotyledon explants (Rahman, 2006) derived calli of *piper nigrum.*

Table 2. Effect of different concentrations of BAP with 0.5 mg/l NAA in MS medium on shoot regeneration from immature zygotic embryo derived calli

Growth regulators (mg/l)		% of inoculum with shoot buds	Number of shoot per inoculum $\overline{X} \pm SE$	Shoot length (cm) $\overline{X} \pm SE$
BAP	NAA			
0.5	0.5	58.4	31.7 ± 0.81	0.8 ± 0.04
1.0		88.8	35.5 ± 0.23	1.2 ± 0.06
1.5		90.4	62.8 ± 0.06	1.8 ± 0.06
2.0		82.2	58.4 ± 0.11	1.1 ± 0.09
2.5		74.3	48.8 ± 0.10	1.0 ± 0.05
3.0		72.8	45.4 ± 0.04	0.8 ± 0.10
3.5		46.2	32.6 ± 0.09	0.6 ± 0.11
4.0		32.4	13.2 ± 0.07	0.5 ± 0.08

The regenerated shoots were excised from stock cultures and subcultures individually into medium containing 1.5 mg/l BAP and 0.5 mg/l NAA for shoot elongation. The elongated shoots (> 3 cm long) were rooted in half-strength MS medium containing various concentrations of IBA and IAA. IBA was found to be more effective in respect of root induction, and 82.5% shoots produced roots within in media having 1.5 mg/l IBA and mean number of roots per shoot was 6.8 (Table 3). Rooted plantlets were transferred to pots and more than 88% plants survived (Fig. C). The present investigation provides a method that ensures a multiple shoot induction from immature zygotic embryos explants of black pepper via intermediate callus phase.

Table 3. Effect of auxins of root formation from *in vitro* grown shoot of *Piper nigrum*

Auxin	concentration (mg/l)	Rooting (%)	Number of roots per shoot $\overline{X} \pm SE$
IAA	0.2	-	-
	0.5	10.2	1.3 ± 0.18
	1.0	18.5	2.2 ± 0.23
	1.5	27.5	2.4 ± 0.21
	2.0	20.2	1.8 ± 0.14
IBA	0.2	16.4	3.2 ± 0.16
	0.5	28.8	4.4 ± 0.29
	1.0	54.6	5.2 ± 0.23
	1.5	82.5	6.8 ± 0.18
	2.0	51.2	4.9 ± 0.26

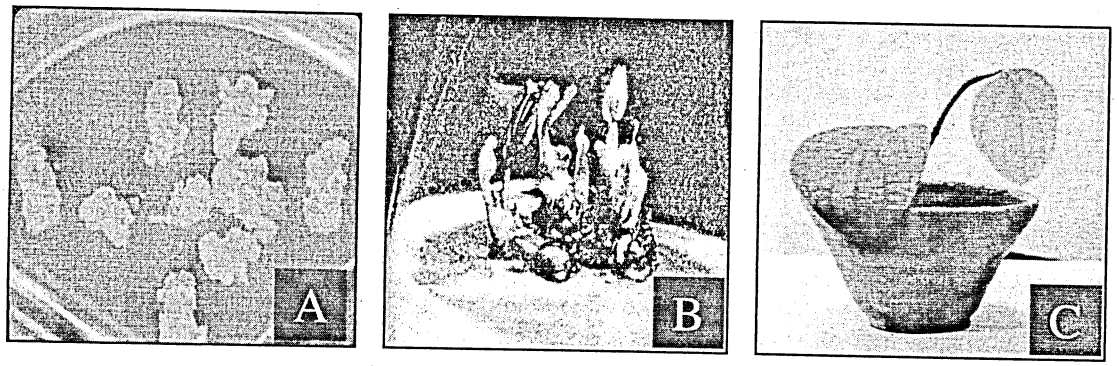

Fig. 1. Callus induction and plant regeneration in black pepper. **A.** Induction of callus on immature zygotic embryo of black pepper in MS medium supplemented with 2.0 mg/l IAA and 0.5 mg/l kinetin, after 4 weeks of culture. **B.** Multiple shoot formations from callus in MS medium supplemented with 1.5 mg/l BAP and 0.5 mg/l NAA, after 4 weeks of culture. **C.** Potted plantlet three weeks after transfer.

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