GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

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MICROPROPAGATION OF BLACK PEPPER, *CV.* PANNIYUR-1: STANDARDIZATION OF STERILIZATION PROTOCOL AND MEDIA COMPOSITION

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ABSTRACT
The present investigation was aimed to standardize micro propagation technique in Black pepper Cv. Panniyar-1. The explants namely Berries, Shoot tip and Nodal segment were used for this experiment. Treatment involving Dettol (1.0%), ethanol (50%) and $HgCl_2$ (0.1%) proved to be the best treatments in shoot tip and nodal segment as these treatments recorded minimum contaminated cultures along with better per cent of culture establishment. In berries explants, treatment of dettol (1.0%) for 5 min, ethanol (50%) for 30 sec and $HgCl_2$ (0.5%) for 2 min recorded minimum contamination of cultures. MS medium along with 4.5 mg/l BAP, 5.0 mg/l BAP and 4.0 mg/l BAP with 1.0 mg/l IAA observed to be the best combinations for establishment. On this combination shoot tip apex showed (53.82%) establishment whereas nodal segment showed 52.30% established cultures. The berries explants showed zero per cent germination on various establishment media. The best multiple shoot initiation was observed on the media combination of the media SIM_{10} (MS +4.5 mg/l BAP + 1.0 mg/l IAA) showed maximum (69.33%) shoot induction followed by media SIM_9 (67.11%), media SIM_{11} (65.56%) and media SIM_8 (60.67%). The media SIM_3 showed significantly lowest (13.56 %) shoot induction.

KEYWORDS: Black pepper; Panniyur-1; Micropropagation; Berries; Shoot apex; Nodal segment.

INTRODUCTION

Piper nigrum (family Piperaceae) is a valuable medicinal plant. It is one of the most commonly used spices and considered as ''The King of spices'' among various spices. Black pepper is grown in many tropical regions like Brazil, Indonesia and India. The spiciness of black pepper is due to the chemical piperine. Piperine increases bioavailability of many drugs and nutrients by inhibiting various metabolising enzymes[15,17]. *Piper nigrum* L and its active constituent ''Piperine''exhibits diverse pharmaco-logical activities like antihypertensive, anti-platelet, antioxidant, antitumor, anti-asthmatics, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, anti-depressants, immunomodulatory, anticonvulsant, anti-thyroids, antibacterial, antifungal, hepato-protective, insecticidal and larvicidal activities etc[3,7,9]. Continued improvement and future expansion for high yielding cultivars depends on rapid clonal propagation method [11, 13]. Black paper is commonly infected by fungal, bacterial and viruses which are difficult to control and are nearly always transferred by vegetative propagation[16]. Shoot tip cultures provide a means for the mass clonal propagation of elite vines. In vitro cultures are now being used as tools for the study of various basic problems in plant sciences [14]. The conventional propagation method, however, has disadvantages, including genetic variability, short viability period, low rate of germination, and high risk of infection by various diseases and pests[12]. In addition, since the plant also lacks natural vegetative propagation, tissue culture methods provide a novel way for the asexual multiplication of hot pepper plants. It is now possible to propagate all plants of economic importance in large numbers by tissue culture[10]. Tissue culture techniques have taken significant part in clonal propagation, conservation of germplasm and plant improvement in black-pepper.

METHODS AND MATERIAL

1. Collection of explant

The experimental material of the present investigation was collected from the S. P. college of Horticulture, Kharwate -Dahiwali potted and maintained in the green house. These plants were used as a source of different explants such as barriers, shoot apex and nodal segment[2,5] throughout the investigation.

2. Surface Sterilization

Explants – barriers as it is and shoot apex, nodal segment approximately 0 .5 to 1 cm in length were excised, washed thoroughly with running tap water for 5 minutes and then with distilled water. Explants were treated with 1.0% dettol for 5 minute and then rinsed thoroughly with sterile distilled water. The explants were subjected to 50% ethanol for 30 seconds, washed with distilled water and then placed in 0.1% mercuric chloride for 2 minute and again washed with distilled water in laminar airflow cabinet **[1]**. Explants blotted on filter paper before placing it on Murashige and Skoog (MS) media.

3. Culture Media

Standard procedure was followed for the preparation of MS media (Table 1)**[4,6]**. The pH of the media was adjusted

to 5.8 and heat resistant growth regulators (IAA and BAP) were added to the media prior to sterilization done at 15 lbs for 15 min at 121°C. All media were solidified with 8 g/l agar. After autoclaving further work done under Laminar Air Flow. After inoculation of the relative explants, cultures were maintained in glass vessels in a growth room culture at 25 ±2°C with a daily photoperiod of 16 h (25 µmol·m–2·s–1 from cool-white Westinghouse fluorescent lamps [8].

Experimental results

1. Frequency of aseptic culture

There was significant difference observed for frequency of aseptic culture developed among medium, explants and interaction (Table 2).

1.1. Effect of explants

Among the three explants, significantly highest response for frequency of aseptic culture was observed in nodal segment (50.97%) irrespective of media followed by shoot apex (45.70%) and berries (15.94%). Mean frequency of aseptic culture ranged from 15.94% to 50.97% in all the three explants.

1.2. Effect of treatments

The significant differences were observed between sterilization treatments. Treatment T_{10} (Dettol (1%)(5 min) + Ethanol (50%)(30 sec) + $HgCl_2$ (0.1%)(2 min)) showed highest (69.78 %) frequency of aseptic shoot initiation followed by treatment T_{11} (65.56%), treatment T_9 (61.33 %) and treatment T_8 (54.44 %). Treatment T_3 (Dettol (1%) (15 min)) Showed significantly lowest (17.33%) frequency of aseptic culture (Plate 1).

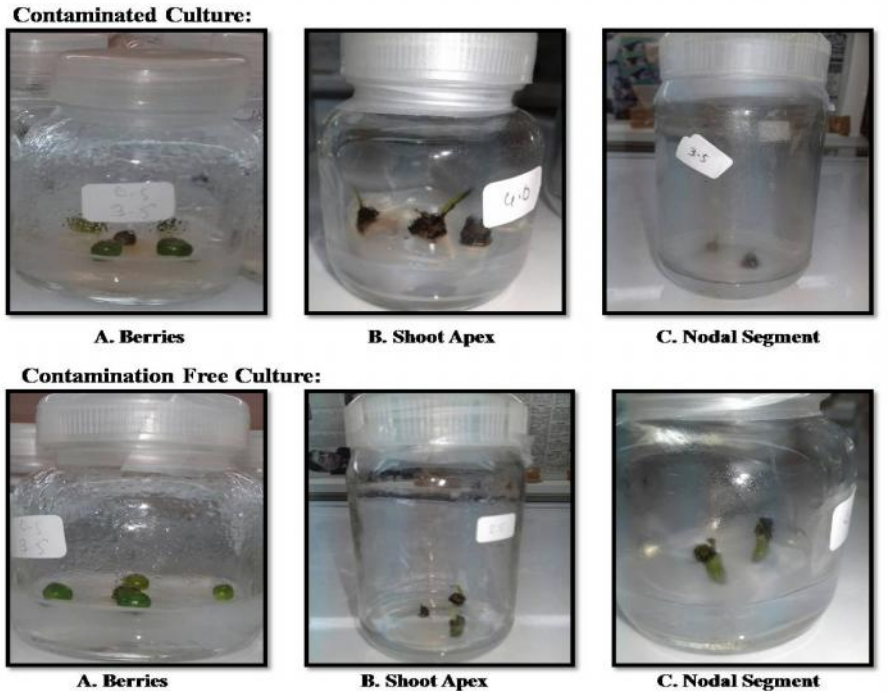

Plate I: Aseptic Culture

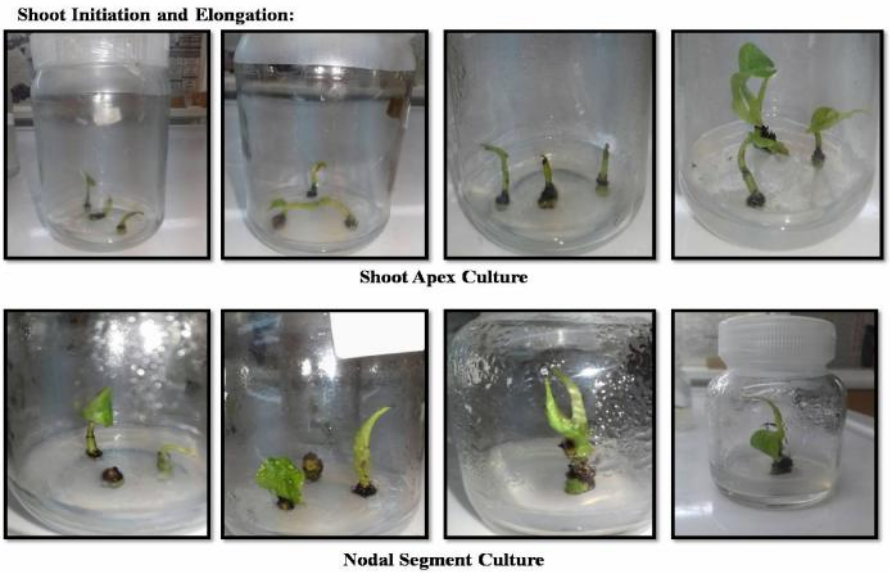

Plate II: Shoot Induction and Elongation

TABLE 1: Composition of MS media

Major salts (macronutrients)		
SR. No.	**Components**	**Quantity**
1	Ammonium nitrate	1,650mg/l
2	Calcium chloride	440mg/l
3	Magnesium chloride	370mg/l
4	Potassium phosphate	170mg/l
5	Potassium nitrate	1,900mg/l
Minor salts (micronutrients)		
6	Boric acid	6.2 mg/l
7	Cobalt chloride	0.025 mg/l
8	Cupric sulphate	0.025 mg/l
9	Manganese sulphate	22.3 mg/l
10	Potassium iodide	0.83 mg/l
11	Sodium molybdate	0.25 mg/l
12	Zinc sulphate	8.6 mg/l
Iron source		
13	Ferrous sulphate	27.8 mg/l
14	EDTA	100 mg/l
Vitamins and organics		
15	Meso-inositol	100 mg/l
16	Pyridoxine-HCL	0.5 mg/l
17	Nicotinic acid	0.5 mg/l
18	Thiamine HCL	0.1 mg/l
19	IAA	5.0 mg/l
20	Glycine	2.0 mg/l
21	**Sucrose**	**30 g/l**
22	**Agar**	**8 g/l**
23	**pH**	**5.8**

TABLE 2. Frequency of aseptic culture

Treatment No.	Treatment details	Explants			Mean (%)
		Berries (%)	Shoot Apex (%)	Nodal segment (%)	
T_1	Control (DDW washing)(5 Min.)	0.00 (00)	0.00 (00)	0.00 (00)	0.00 (00)
T_2	Tween-20	0.00 (00)	0.00 (00)	0.00 (00)	0.00 (00)
T_3	Dettol (1%)(15 min)	0.00 (00)	25.33 (30.22)	26.67 (31.09)	17.33 (24.60)
T_4	Ethanol (50%) (30 sec.)	11.33 (19.67)	26.67 (31.09)	28.67 (32.37)	22.22 (28.12)
T_5	$HgCl_2$ (0.1%)(5min)	13.33 (21.41)	31.33 (34.03)	31.33 (34.03)	25.33 (30.22)
T_6	Dettol (1%) (2 min)+ Ethanol (50%)(30 sec) + $HgCl_2$ (0.1%)(1 min)	19.33 (26.08)	53.33 (46.91)	70.67 (57.20)	47.78 (43.72)
T_7	Dettol (1%) (2 min) + Ethanol (50%)(30 sec) + $HgCl_2$ (0.1%)(2 min)	18.67 (25.59)	55.33 (48.06)	73.33 (58.90)	49.11 (44.49)
T_8	Dettol (1%)(2 min) + Ethanol (50%)(30 sec) + $HgCl_2$ (0.1%)(3 min)	24.67 (29.77)	62.67 (52.33)	76.00 (60.66)	54.44 (47.54)
T_9	Dettol (1%)(5 min) + Ethanol (50%)(30 sec) + $HgCl_2$ (0.1%)(1 min)	27.33 (31.52)	75.33 (60.22)	81.33 (64.40)	61.33 (51.55)
T_{10}	Dettol (1%)(5 min) + Ethanol (50%)(30 sec) + $HgCl_2$ (0.1%)(2 min)	31.33 (34.03)	90.00 (71.56)	88.00 (69.73)	69.78 (546.65)
T_{11}	Dettol (1%)(5 min) + Ethanol (50%)(30 sec) + $HgCl_2$ (0.1%)(3 min)	29.33 (32.79)	82.67 (65.39)	84.67 (66.94)	65.56 (54.06)
	Mean (%)	15.94 (23.53)	45.70 (42.53)	50.97 (45.55)	37.54 (37.78)
		SE (m)±	CD at 1%		
	Treatments	1.858037	6.969591		
	Explants	0.970328	3.63975		
	Interaction	3.218214	12.07168		

TABLE 3. Per cent shooting/ shoot induction

Sr. No.	Combination details	Explants			Mean (%)
		Berries (%)	Shoot apex (%)	Nodal segment (%)	
SIM_1	MS medium (control)	0.00 (00)	0.00 (00)	0.00 (00)	0.00 (00)
SIM_2	MS+0.5 mg/l BAP + 1.0 mg/l IAA	0.00 (00)	0.00 (00)	0.00 (00)	0.00 (00)
SIM_3	MS+1.0mg/l BAP + 1.0 mg/l IAA	0.00 (00)	19.33 (26.08)	21.33 (27.50)	13.56 (21.60)
SIM_4	MS +1.5 mg/l BAP + 1.0 mg/l IAA	0.00 (00)	34.00 (35.66)	28.67 (32.37)	20.89 (27.19)
SIM_5	MS +2.0 mg/l BAP +1.0 mg/l IAA	0.00 (00)	45.33 (42.32)	39.33 (38.84)	28.22 (32.08)
SIM_6	MS+2.5mg/l BAP +1.0 mg/l IAA	0.00 (00)	53.33 (46.91)	58.67 (49.99)	37.33 (37.66)
SIM_7	MS +3.0 mg/l BAP +1.0 mg/l IAA	8.67 (17.12)	75.33 (60.22)	70.67 (57.20)	51.56 (45.89)
SIM_8	MS +3.5 mg/l BAP +1.0 mg/l IAA	11.33 (19.67)	87.33 (69.15)	83.33 (65.90)	60.67 (51.15)
SIM_9	MS +4.0 mg/l BAP +1.0mg/l IAA	17.33 (24.60)	93.33 (75.03)	90.67 (72.21)	67.11 (55.00)
SIM_{10}	MS +4.5 mg/l BAP + 1.0 mg/l IAA	19.33 (26.08)	95.33 (77.52)	93.33 (75.03)	69.33 (56.37)
SIM_{11}	MS +5.0 mg/l BAP + 1.0 mg/l IAA	18.67 (25.59)	88.67 (70.32)	89.33 (70.93)	65.56 (54.06)
	Mean (%)	6.85 (15.17)	53.82 (47.18)	52.30 (46.32)	37.66 (37.85)
		SE (m)±	**CD at 1%**		
	Media combination	1.414214	5.304787		
	Explants	0.738549	2.770335		
	Interaction	2.44949	9.188161		

TABLE 4. Average number of leaves per explants

Sr. No.	Combination details	Explants			Mean (%)
		Berries (%)	Shoot apex (%)	Nodal segment (%)	
SIM_1	MS medium (control)	0.00	0.00	0.00	0.00
SIM_2	MS+0.5 mg/l BAP + 1.0 mg/l IAA	0.00	0.00	0.00	0.00
SIM_3	MS+1.0mg/l BAP + 1.0 mg/l IAA	0.00	0.00	0.00	0.00
SIM_4	MS +1.5 mg/l BAP + 1.0 mg/l IAA	0.00	0.00	0.00	0.00
SIM_5	MS +2.0 mg/l BAP +1.0 mg/l IAA	0.00	1.00	1.00	0.67
SIM_6	MS+2.5mg/l BAP +1.0 mg/l IAA	0.00	1.00	1.67	0.89
SIM_7	MS +3.0 mg/l BAP +1.0 mg/l IAA	0.00	2.00	2.67	1.56
SIM_8	MS +3.5 mg/l BAP +1.0 mg/l IAA	0.00	2.67	3.67	2.11
SIM_9	MS +4.0 mg/l BAP +1.0mg/l IAA	0.00	3.67	4.33	2.67
SIM_{10}	MS +4.5 mg/l BAP + 1.0 mg/l IAA	0.00	4.67	5.33	3.33
SIM_{11}	MS +5.0 mg/l BAP + 1.0 mg/l IAA	0.00	3.33	4.00	2.44
	Mean (%)	0.00	1.67	2.06	1.24
		SE (m)±	CD at 1%		
	Media combination	0.100504	0.376995		
	Explants	0.052486	0.196879		
	Interaction	0.174078	0.652974		

2. Per cent shoot induction

Per cent shoot induction response of three different explants observed on 11 media combinations are presented in Table 3. There was significant difference for percent shoot induction among explants, medium and their interaction.

2.1 Effect of explants

Among the three explants, maximum per cent shoot induction observed in shoot apex (53.82%) irrespective of media followed by nodal segment (52.30%) and berries (06.85%).(Table 3). Mean of percent shoot induction ranged from 6.85% to 53.82% in all explants (Plate 2).

2.2. Effect of media

The significant differences were observed between percent shoot induction in different media. The media SIM_{10} (MS +4.5 mg/l BAP + 1.0 mg/l IAA) showed maximum (69.33%) shoot induction followed by media SIM_9 (67.11%), media SIM_{11} (65.56%) and media SIM_8 (60.67%). The media SIM_3 showed significantly lowest (13.56 %) shoot induction.

3. Number of leaves per explants

There was significant difference observed for number of leaves among explants, medium and interaction.

3.1 Effect of explants

Among the three explants, maximum number of leaves was observed in nodal segment (2.06) irrespective of media. Shoot apex showed minimum number of leaves (1.67) (Table 4). Mean number of leaves per explants was range in between 1.67 to 2.06.

3.2 Effect of media

The significant difference was observed between shoot induction medium. SIM_{10} (MS + 4.5 mg/l BAP + 1.0 mg/l IAA) showed highest 3.33 number of leaves per explants followed by SIM_9 (2.67), SIM_{11} (2.44), SIM_8 (2.11) and SIM_7 (1.56). SIM_5 (MS +2.0 mg/l BAP +1.0 mg/l IAA) showed lowest 0.67 number of leaves per explants (Table 4).

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