

RESPONSE OF BLACK PEPPER (*PIPER NIGRUM* L.) VARIETY PANNIYUR-1 AND CULTIVARS TO INDIRECT ORGANOGENESIS

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Abstract: Indirect organogenesis was attempted in the black pepper variety Panniyur-1 and four cultivars viz. Karimunda, Kalluvally, Balankotta and Cheriakanyakkadan from explants of axenic seedlings. The response of variety/cultivars at various stages of morphogenesis was studied in detail. The response suggests the manipulation of media specifically to each variety / cultivar to achieve the maximum morphogenetic potential.

Key words: Black pepper, indirect organogenesis, morphogenesis, *Piper nigrum* L.

INTRODUCTION

Phytophthora foot rot disease caused by *Phytophthora capsici* is the most dreadful disease affecting black pepper in all pepper growing tracts. Since the available germplasm is susceptible to the disease, to exploit somaclonal variation, indirect organogenesis was attempted in black pepper as per the procedure reported by Nazeem *et al.* (1990). The black pepper variety Panniyur-1 and four different cultivars viz. Karimunda, Cheriakanyakkadan, Balankotta and Kalluvally were made to regenerate by indirect organogenesis. The response of variety / cultivars at various stages of morphogenesis was studied in detail.

MATERIALS AND METHODS

Calli were induced on stem and leaf segments of *in vitro* seedlings of black pepper variety Panniyur-1 and four cultivars viz. Karimunda, Cheriakanyakkadan, Balankotta and Kalluvally. The explants were surface sterilised with 0.1% $HgCl_2$ for 3 min and washed free off the sterilant and inoculated to MS (Murashige and Skoog, 1962) medium supplemented with sucrose 3%, IAA and BAP each at 1.0 mg l^{-1} . After three weeks of incubation, the induced calli were subcultured to the medium of the same composition for proliferation. Comparative performance of the different variety / cultivars for callus induction, percentage of callusing and callus proliferation were observed. Callus index (CI) was worked out as $CI = PxG$ where P is the percentage of callus initiation and G is the growth score. Maximum score of three was given for those calli putting forth good growth covering the entire surface of the media.

The cream nodular calli induced in the modified MS medium ($1/2$ MS supplemented with IAA and BAP each at 1.0 mg l^{-1}) were subcultured to the same medium for further proliferation and regeneration of shoots. The response of variety / cultivars was compared with respect to shoot regeneration. The induced shoots of the different variety / cultivars were subcultured to the same modified MS medium for further proliferation of shoots. The response was compared with respect to shoot proliferation in the third subculture.

The proliferated shoots of the different variety / cultivars were transferred to the elongation medium ($1/2$ MS supplemented with IAA 0.1 mg l^{-1} and BAP 0.2 mg l^{-1}) for the 4th, 5th and 6th subcultures. The response of variety / cultivars to recovery of rootable shoots was compared. The rootable shoots were transferred to rooting medium ($1/2$ MS supplemented with 2% sucrose and IBA 1.0 mg l^{-1}) after giving a pulse treatment with IBA 1000 mg l^{-1} prepared in absolute alcohol. The response of different variety / cultivars was compared with respect to percentage of rooting, days taken for root induction, length, number and thickness of roots.

RESULTS AND DISCUSSION

The variety / cultivars differed significantly in the days taken for callusing, percentage of callusing and callus proliferation (Table 1). The highest percentage of callusing was recorded for Kalluvally (92.22) followed by Karimunda (85.92), both of them were found to be on par. The lowest callusing was exhibited by Panniyur-1 (55.55), which was found

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to be on par with Cheriakanyakkadan (66.66). The callus growth in Panniyur-1 was found to be poor. Panniyur-1 exhibited the lowest growth score of 1.50 while the highest growth score of 2.73 was registered by Kalluvally followed by Balankotta (2.20). The highest callus index was observed for Kalluvally followed by Karimunda (171.84) and Balankotta (167.02). Panniyur-1 registered the lowest callus index of 83.33 followed by Cheriakanyakkadan (133.32). The days taken for callusing (14.00 to 20.33) varied significantly among the variety / cultivars. Early callusing was observed in Kalluvally (14.00) followed by Karimunda (15.33). Panniyur-1 showed late callusing (20.33).

The variety / cultivars differed significantly in the days taken for shoot induction and percentage of shoot regeneration (Table 2). Karimunda showed highest regeneration potential giving shoot induction in 66.66 per cent of the cultures folio-wed by Kalluvally in 60.00 per cent of the cultures. Panniyur-1 registered the lowest regeneration of shoots giving 33.33 per cent, which differed significantly from all other cultivars. With respect to the days taken for shoot induction, early induction was observed in the cultivar Karimunda (33.33 days) while late induction was observed in Panniyur-1 (60.00 days). Other cultivars studied viz. Kalluvally, Balankotta and Cheriakanyakkadan took more or less the

Table 1. Response of black pepper variety / cultivars to callusing and callus growth

Variety/cultivars	*Callusing (%)	Growth score	Callus index	Days for callus initiation
Panniyur-1	55.55 (0.842)**	1.50	83.33	20.33 (4.505)
Karimunda	85.92 (1.190)	2.00	171.84	15.33 (3.915)
Cheriakanyakkadan	66.66 (0.959)	2.00	133.32	16.00 (3.995)
Kalluvally	92.22 (1.295)	2.73	251.76	14.00 (3.737)
Balankotta	75.92 (1.059)	2.20	167.02	16.00 (3.996)
CD (0.05)	0.163			0.386
SEm ±	0.052			0.125

Cultured period: 3 weeks; *Average of three replications; ** Values in parentheses represent transformed values
Medium: ½ MS supplemented with IAA and BAP 1.0 mg l⁻¹

Table 2. Response of black pepper variety / cultivars to shoot regeneration from calli

Variety / cultivars	Days for shoot induction	Cultures showing regeneration of shoots in second subculture (%)
Panniyur-1*	60.00	33.33
Karimunda	33.33	66.66
Kalluvally	43.00	60.00
Balankotta	45.66	55.55
Cheriakanyakkadan	47.33	53.70

Culture period: 3 weeks; *The percentage of regeneration in third subculture (due to the late induction of shoots)

Table 3. Response of black pepper variety / cultivars to shoot proliferation

Variety / cultivars	*No. of shoots further proliferated in third subculture	Highest number noted in third subculture
Karimunda	16.58 (4.066)**	25
Kalluvally	18.16 (4.255)	38
Balankotta	11.33 (3.357)	14
Cheriakanyakkadan	15.66(3.946)	28
Panniyur-1***	3.91 (1.968)	6
CD (0.05)	0.216	
SEm ±	- 0.076	0.3

Culture period: 3 weeks; Container: big culture tubes (380 x 200 mm); * Average of 12 observations; ** Values in parentheses represent transformed values; *** No. of shoots proliferated in the fourth subculture (due to late induction of shoots)

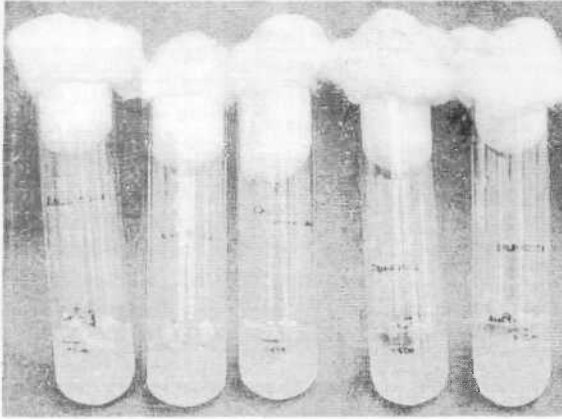


Plate 1. Calli induced from leaf segments of black pepper
(From left to right: Kalluvally, Karimunda, Cheriakanyakkadan, Panniyur 1 and Balankotta)

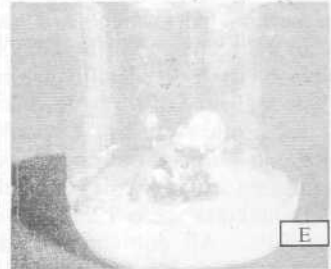
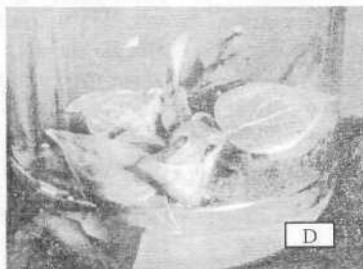
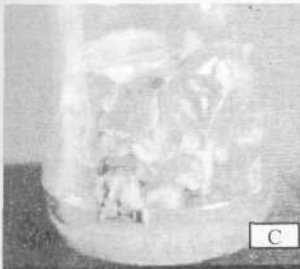
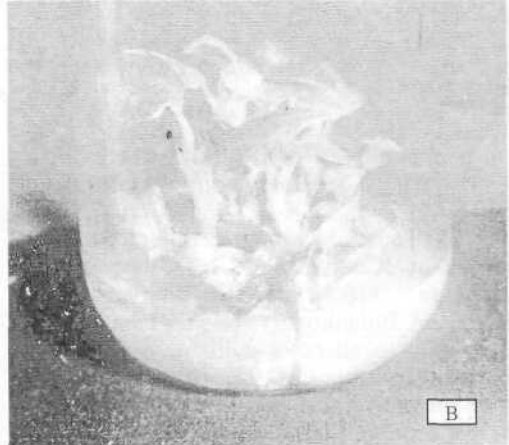
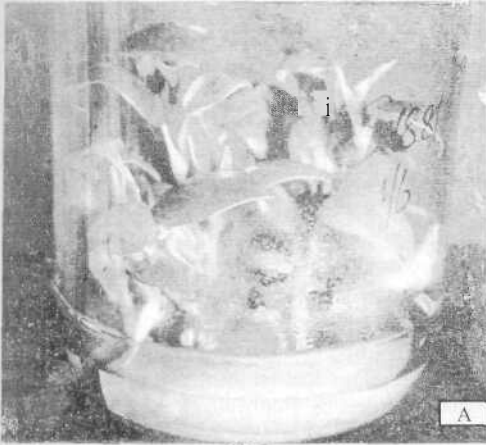


Plate 2. Shoot regeneration and production of rootable shoots in black pepper
(A. Kalluvally, B. Balankotta, C. Cheriakanyakkadan, D. Karimunda, E. Panniyur 1)

Table 4. Response of black pepper variety / cultivars to the recovery of rootable shoots

Variety / cultivars	Number of rootable shoots*			Highest no. noted in sixth subculture
	Fourth subculture	Fifth subculture	Sixth subculture	
Karimunda	1.25 (1.470)**	2.91 (1.939)	5.00 (2.439)	8.0
Kalluvally	2.50 (1.852)	8.00 (2.991)	12.00 (3.600)	15.0
Balankotta	1.16 (1.443)	7.08 (2.833)	9.33 (3.211)	12.0
Cheriakanyakkadan	1.92 (1.689)	7.16 (2.852)	10.25 (3.349)	13.0
Panniyur-1	0	1.0 (1.390)	2.08 (1.734)	3.0
CD (0.05)	0.251	0.230	0.184	
SEm ±	0.084	0.081	0.065	

Culture period: 3 weeks; *Average of 12 observations; ** Values in parentheses represent transformed values
Medium: ½ MS supplemented with IAA 0.1 and BAP 0.2 mg l⁻¹

Table 5. Response of black pepper variety / cultivars to rooting and root growth

Variety / cultivars	Rooting (%)	*Days for root initials to appear	*No. of roots	*Length of roots (cm)	*Thickness of roots (mm)
Kalluvally	100	12.75 (3.707)**	15.83 (3.947)	2.75	1.02
Cheriakanyakkadan	100	13.50 (3.806)	17.66(4.007)	3.04	1.27
Balankotta	100	13.83 (3.850)	13.66 (3.660)	2.79	1.08
Karimunda	100	13.16(3.762)	12.25 (3.468)	2.64	1.12
Panniyur-1	100	15.00 (3.998)	8.75 (2.949)	2.19	0.90
CD (0.05)		0.086	0.376	0.543	0.151
SEm ±		0.030	0.132	0.191	0.053

Culture period: 1 month; *Average of 12 observations; ** Values in parentheses represent transformed values
Medium: ½ MS supplemented with sucrose 2% and IBA 1.0 mg l⁻¹

same period (43.00 - 47.33 days) for induction of shoots. The variety / cultivars differed significantly in the rate of shoot proliferation (Table 3). The highest shoot proliferation was recorded by Kalluvally (18.16) followed by Karimunda (16.58), Cheriakanyakkadan (15.66) and Balankotta (11.33). Panniyur-1 was inferior to all other cultivars registering lowest proliferation of 3.91. However, Karimunda and Cheriakanyakkadan were on par with respect to proliferation of shoots.

Comparative performance of variety / cultivars to the recovery of rootable shoots is presented in Table 4. Kalluvally registered the highest recovery of rootable shoots in all subcultures followed by Cheriakanyakkadan. The mean number of rootable shoots in 6th subculture for Kalluvally, Cheriakanyakkadan and Balankotta were 12, 10.25 and 9.33 respectively. All cultivars except Balankotta and Cheriakanyakkadan differed significantly with respect to recovery of rootable shoots. The lowest recovery of rootable shoots was recorded by Panniyur-1 followed by Karimunda. The five variety / cultivars studied

recorded 100 per cent rooting (Table 5). All the cultivars except the variety Panniyur-1 took 12.75 to 13.83 days for the root initials to appear while Panniyur-1 took 15 days. The variety / cultivars studied showed highly significant variation in root number. The highest root number was recorded by Cheriakanyakkadan (17.66) closely followed by Kalluvally (15.83). The lowest number of roots (8.75) was recorded by Panniyur-1.

Panniyur-1 differed significantly from all other cultivars with respect to root length, all other cultivars being on par. The highest mean root length of 3.04 cm was recorded by Cheriakanyakkadan followed by Balankotta (2.79 cm), Kalluvally (2.75 cm) and Karimunda (2.64 cm). Panniyur-1 registered the lowest root length (2.19 cm). The mean root thickness in different cultivars varied significantly and ranged from 0.9 to 1.27 mm. The highest root thickness was observed in Cheriakanyakkadan which differed significantly from all other variety / cultivars. The influence of genotypes in the *in vitro* response had been observed by several workers (Reghu-

nath, 1989; Brandt, 1992 & 1994 and Das, 1995a & b). The reason for the genotypic difference in the *in vitro* performances can be attributed to the differences in endogenous cytokinin and / or auxin in the genotype (Looney *et al.*, 1988 and Sossountzov *et al.*, 1988) as well as differences in the *in vitro* uptake of exogenous cytokinins (Marino, 1988). The differences in the *in vitro* uptake of exogenous cytokinins lead to differences in the endogenous balance of auxin and cytokinin (Alvarez *et al.*, 1989 and Gronroos *et al.*, 1989) and hence the difference in response. Since explants from axenic seedlings of different variety / cultivars were subjected to indirect organogenesis, the response observed in the present study may vary from the true varietal response. However, the difference in response observed between variety / cultivars in the present study may be due to the difference in auxin / cytokinin balance of the genotypes. Hence, it can be concluded from the present study that media should be standardised specifically for each variety / cultivar to achieve the maximum morphogenetic potential.

ACKNOWLEDGEMENT

This paper forms a part of the Ph.D. (Hort.) thesis of the senior author submitted to Kerala Agricultural University, Thrissur, India

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