Journal of Tropical Agriculture 34 (1996) : 115-120

SCREENING OF BLACK PEPPER *(PIPER NIGRUM* L.) CALLICLONES FOR *PHYTOPHTHORA* FOOT ROT RESISTANCE / TOLERANCE

M. R. Shylaja, G. Sreekandan Nair and James Mathew
College of Horticulture, Kerala Agricultural University, Vellanikkara 680 654, Trichur, India

Abstract: Calliclones of different black pepper cultivars viz., Kalluvally, Cheriakanyakkadan, Balankotta, Karimunda and Panniyur-1 were regenerated from calli, screened against toxic metabolite(s) of *Phytophthora capsici.* The regenerants derived from screened and unscreened calli were further tested for resistance / tolerance to *P. capsici* using different methods of screening viz., natural screening (keeping in infected field), screening by electrolyte leakage method and screening by artificial inoculation with culture disc of *P. capsici.* None of the regenerated calliclones was found to be completely resistant to the disease in natural screening. However, some of the regenerants derived from unscreened calli exhibited higher degree of tolerance to the disease revealing the possibility of exploiting somaclonal variation for *Phytophthora* foot rot disease screening in black pepper. The calliclones of Cheriakanyakkadan recorded greater degree of tolerance to the disease as compared to others.

Key words: Black pepper, calliclones, *Phytophthora* foot rot, *Piper nigrum* L., screening.

INTRODUCTION

Black pepper *(Piper nigrum* L.), the king of spices is affected by the dreadful *Phytophthora* foot root disease caused by *Phytophthora capsici.* The disease is prevalent in all pepper growing tracts of the country causing considerable crop losses and none of the cultivated types is resistant to the disease. Many of the valuable genotypes are lost from the gene pool every year due to this serious malady. Moreover, the variability with respect to disease resistance is limited in cultivated types and the conventional breeding programmes so far carried out for evolving resistant types were found unsuccessful. The present investigations were hence aimed at exploiting somaclonal variation in black pepper and screening the regenerated calliclones for resistance / tolerance to *Phytophthora* foot rot disease.

MATERIALS AND METHODS

In vitro callus screening was attempted in five black pepper cultivars viz., Panniyur-1, Karimunda, Kalluvally, Cheriakanyakkadan and Balankotta using concentrated culture filtrate (CCF) containing toxic metabolite(s) of *P. capsici* as reported by Shylaja *et al.* (1994). The callus mediated organogenesis reported by Nazeem *et al.* (1990) was used to produce regenerants from screened and unscreened calli. The regenerated calliclones were further subjected to screening by the pathogen since the toxic metabolite(s) produced by the pathogen is of non-specific in nature. Different methods were used for screening regenerated calliclones viz., natural screening for resistance to *P. capsici* by keeping in infected field, screening by inducing electrolyte leakage with CCF and screening by artificial inoculation of culture disc of *P. capsici.*

Natural screening for resistance to P. capsici by keeping in infected field

Black pepper nursery area severely infected with *P. capsici* was utilized for the study and screening was done during July-August, 1994. One row of healthy plants was kept in the middle of two rows of infected plants. Plants regenerated from screened and unscreened calli and plants of different age groups were compared for the development of symptoms.

Screening by inducing electrolyte leakage with concentrated culture filtrate

Leaf tissues of the clones produced from screened and unscreened calli of the different cultivars were collected at random and prepared for electrolyte leakage studies as reported by Vidhyasekaran *et al.* (1986). The electrolyte leakage values recorded by tissues from both the sources were compared.

Screening by artificial inoculation of culture disc of P. capsici

The screening and scoring technique as reported by Kueh and Khew (1980) for *Phytophthora* foot rot in black pepper was used with slight modification in scoring. From the two months old regenerants established in the glass house, leaves of the same maturity were inoculated with 5 mm culture disc of *P. capsici*.

The average diameter of the lesions developed 48 h after inoculation was observed and based on the average diameter of the lesion formed, the clones from screened and unscreened calli of different cultivars were grouped into five classes as shown below:

Class	Lesion diameter, cm
1	< 0.5
2	0.5-1.0
3	1.1-1.5
4	1.6-2.0
5	> 2.0

Each class was given a score based on a scale ranging from 1 to 5 where 1 represented average lesion diameter < 0.5 cm; 2 = 0.5-1.0 cm; 3 = 1.1-1.5 cm; 4 = 1.6-2.0 cm; and 5 = > 2.0 cm. Lesion diameter score (LDS) was calculated by multiplying the percentage of plants coming under each class with the score given for the class. Based on LDS the variation in intensity of lesion development among calliclones was evaluated.

RESULTS AND DISCUSSION

Natural screening for resistance to P. capsici by keeping in infected field

The plants kept for natural screening took infection and wilted completely within a fortnight showing that none of the calliclones was completely resistant to the disease. When the effect of callus screening on the development of symptoms was observed, it was found that the unscreened plants registered slightly lower rate of wilting as compared to the screened ones, but the difference was not statistically significant (Table 1).

There was no significant difference observed between the calliclones of different age groups in the number of plants wilted due to infection on each day (Table 2). All the plants wilted within a period of 10 days in plants of age groups, 3-6 months and 2-3 months. On the other hand, plants of the age group 1-2 months took 14 days for complete infection and wilting. This was because the rainfall and RH during the period was low and there was total absence of rain for 3 days during the period of study.

Since none of the regenerated calliclones showed complete resistance to the disease, the tolerance level of the clones was tested by electrolyte leakage method.

Screening the calliclones by inducing electrolyte leakage with concentrated culture filtrate

Comparison of electrolyte leakage values from screened and unscreened sources showed that there was no significant difference in cultivars like Karimunda, Kalluvally and Balankotta. But in the cultivar Cheriakanyakkadan leakages from the two sources showed highly significant variation recording a higher leakage of 63.5 µmho in clones derived from screened calli and 51.42 µmho in clones derived from unscreened calli (Table 3).

Screening the regenerated calliclones by artificial inoculation of culture disc of P. capsici

The tolerance level of the calliclones to the disease was assessed based on the intensity of lesion development.

The screened and unscreened regenerated calliclones when compared for lesion development irrespective of the cultivars, the clones from unscreened calli were found to be more tole-

Table 5. Response of black pepper cultivars to intensity of lesion development in regenerated calliclones

Class based on lesion diameter, cm	Cultivars			
	Kalluvally*		Cheriakanyakkadan *	
	Percentage of plants	LDS **	Percentage of plants	LDS **
< 0.5	3.64	3.64	5.63	5.63
0.5-1.0	34.54	69.08	52.11	104.22
1.1-1.5	32.73	98.19	29.58	88.74
1.6-2.0	27.27	109.08	9.86	39.44
>2.0	1.82	9.10	2.81	14.05
Total LDS	-	289.09	-	252.08

*No. of calliclones screened : Kalluvally - 55; Cheriakanyakkadan - 71; **Lesion diameter score (LDS) calculated by multiplying the percentage of plants in each group with the score. Score was worked out based on a scale 1 to 5, where I - < 0.5 cm lesion diameter, 2 - 0.5-1.0 lesion diameter, 3 - 1.1-1.5 cm lesion diameter, 4 - 1.6-2.0 cm lesion diameter, 5 - > 2.0 cm lesion diameter

plants using different techniques of cell culture and screening the whole plants for resistance to *Phytophthora* foot rot. The high amount of somaclonal variation in the cultivar Kalluvally and the high tolerance level observed in Cheriakanyakkadan to *P. capsici* should be exploited in further studies.

ACKNOWLEDGEMENT

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

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Table 3. Effect of callus screening on electrolyte leakage from regenerated black pepper calliclones

Cultivars	Mean electrolyte leakage, µmho		Y value	Probability
	Screened	Unscreened		
Karimunda	82.42	73.85	1.5810	0.4250
Cheriakan-yakkadan	63.50	51.42	2.7047	0.0221
Kalluvally	72.33	79.50	0.7630	0.4100
Balankotta	63.42	61.60	0.8244	0.3800

Table 4. Effect of callus screening on intensity of lesion development in regenerated black pepper calliclones

Class based on lesion diameter, cm	Screened*		Unscreened**	
	Percentage of plants	LDS ***	Percentage of plants	LDS ***
< 0.5	4.20	4.20	4.23	4.23
0.5-1.0	26.57	53.14	45.07	90.14
1.1-1.5	42.65	127.95	32.39	97.17
1.6-2.0	21.68	86.72	16.20	64.80
>2.0	4.90	24.50	2.11	10.55
Total LDS	-	296.51	-	266.89

*No. of plants observed for intensity of lesion development - 143; ** No. of plants observed for intensity of lesion development = 142; ***Lesion diameter score (LDS) calculated by multiplying the percentage of plants in each group with the score. The score was worked out based on a scale I to 5, where 1= < 0.5 cm lesion diameter, 2 - 0.5-1.0 cm lesion diameter, 3 -1.1-1.5 cm lesion diameter, 4 - 1.6-2.0 cm lesion diameter, 5=> 2.0 cm lesion diameter

interference of other substances found in the culture filtrate which had no role in disease development also can not he ruled out. Moreover, the possibility of toxic metabolite(s) acting as a pre-disposing factor while *in vitro* screening also should be emphasised in this context for the high intensity of disease reaction observed in screened calli derived clones.

The most striking feature of the present investigation is the better tolerance that exhibited by the clones derived from the unscreened calli to *P. capsici*. The better performance of calliclones produced without *in vitro* screening might be due to the high amount of culture induced variability. The use of culture induced variability as such (somaclonal variation) without resorting to *in vitro* selection was demonstrated by several workers in disease resistance breeding (Heinz and Mee, 1969, Krishnamurthy and Tlaskal, 1974, Bidney and Shepard, 1981, Larkin and Scowcroft, 1983 and Daub and Jenns, 1989). Several investigators compared the results obtained from unselected cultures to those obtained from cultures screened with toxins or pathogens (Brettel *et al.*, 1980; Sacristan, 1982; Ling *et al.*, 1985 and Latunde-Dada and Lucas, 1988). In all these cases, resistant plants were isolated in the absence of *in vitro* selection.

Another observation in the present study is the high variability in Kalluvally calliclones and the high level of tolerance observed in Cheriakanyakkadan. The increased variation observed in Kalluvally as compared to Cheriakanyakkadan might be due to the effect of genotype. Daub and Jenns (1989) reported that the genotype of the parents decided the variability in tobacco somaclones. Another reason for the high rate of somaclonal variation in Kalluvally may be the high proliferation rate observed in the cultivar as compared to others. Smith and Drew (1990) also observed that cultures proliferated at excessive rates show more variation than those grown at moderate rates. The lower leakage of electrolytes from leaf tissues and the lower lesion development score observed in Cheriakanyakkadan showed the high tolerance of the cultivar to *Phytophthora capsici*.

The results of the present study paved way for the exploitation of somaclonal variation for screening for resistance/tolerance to *Phytophthora* foot rot. The highly tolerant plants observed in the unscreened population revealed the possibility of exploiting the vast amount of somaclonal variation as such without resorting to *in vitro* selection. So the future thrust should be given for the production of variant

Table 1. Effect of callus screening on development of symptoms in regenerated calliclones (natural screening)

Treatment	No. of plants kept	Plants wilted on each day, %										
		4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th
Screened	186	3.23	24.73	20.43	9,14	6.99	4.84	10.22	9.14	2.15	5.38	3.76
Unscreened	126	3.97	22.22	16.67	7.94	10.32	6.35	10.32	7.14	2.38	7.14	5.55

't' value for comparison of screened vs unscreened - 0.1286; Probability - 0.8989

Table 2. Effect of age of the regenerated calliclones on development of symptoms (natural screening)

Age group	No. of plants kept	Plants wilted on each day, %										
		4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th
3-6 months	43	9.30	37.20	20.93	13.95	6.98	4.65	6.98	-	-	-	-
2-3 months	91	5.49	31.87	36.26	8.79	8.79	3.30	5.49	-	-	-	-
1-2 months	178	1.12	16.29	9.55	7.30	8.43	6.74	13.48	14.61	3.93	10.67	7.87

-rant to *P. capsici*. Majority of the plants regenerated from screened calli came in the third class giving an average lesion diameter of 1.1 to 1.5 cm while majority of the plants from unscreened calli came in the second class having less average lesion diameter of 0.5-1.0 cm. The overall lesion diameter score when compared, it was found that the regenerants from the screened group registered a score of 296.51 which was 11.10 per cent higher than that recorded in regenerants from unscreened calli (Table 4).

The intensity of lesion development when compared in two cultivars namely Kalluvally and Cheriakanyakkadan it was found that Cheriakanyakkadan registered higher (5.63) percentage of plants showing lesion diameter less than 0.5 cm as compared to Kalluvally (Table 5). Similarly majority of the plants of Cheriakanyakkadan came in the second class having average lesion diameter ranging from 0.5 to 1.0 cm while most of the plants of Kalluvally came in the second and third classes having higher lesion diameter 0.5 to 1.0 cm and 1.1 to 1.5 cm. The overall lesion diameter score recorded for Kalluvally was 289.09 which was 14.68 per cent higher than that recorded for Cheriakanyakkadan (252.08).

From the results of the screening studies conducted it could be seen that in all the three methods of screening, the plantlets derived from screened calli showed higher intensity of disease as compared to unscreened ones. The results also demonstrated the differential expression of CCF resistance between cultured cells and regenerated whole plants. The regenerants produced from resistant calli were found to exhibit higher intensity of disease. In culture, the calli induced from the cultivar Kalluvally showed the lowest callus necrosis, lowest leakage of electrolytes and thereby highest resistance to CCF (Shylaja *et al.*, 1994). But in the regenerated calliclones, Kalluvally was found to be more susceptible to the disease as compared to Cheriakanyakkadan. Differential expression of toxin resistance in cultured cells and whole plants was reported to be one of the major drawbacks of *in vitro* screening system (Brettel and Ingram, 1979; Daub, 1986; Brar and Vidhyasekaran, 1990; Bulk, 1991). The lack of correlation in disease reaction between the cultured cells and regenerants obtained in the present studies might be primarily due to non-specific toxic metabolite(s) employed in the screening work which was reported to be the secondary determinant of the disease. The

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