Journal of Tropical Agriculture 35 (1997) : 33-37

# EFFECT OF TOXIC METABOLITE(S) OF *PHYTOPHTHORA CAPSICI* ON VARIOUS STAGES OF MORPHOGENESIS OF BLACK PEPPER CALLI

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**Abstract:** The effect of toxic metabolite(s) of *Phytophthora capsici* on various stages of morphogenesis of black pepper calli was studied. The survival rate of the calli in toxin medium was influenced by the varieties / cultivars used for the study. Once the calli survived in the toxin medium, the toxic metabolite(s) did not inhibit further growth of the calli. The shoot proliferation and elongation were also not influenced by the metabolite(s) in the media. However, the root growth was affected adversely.

Key **words:** Black pepper, morphogenesis, *Phytophthora capsici*, toxic metabolite.

## INTRODUCTION

*Phytophthora* foot rot disease of black pepper incited by *Phytophthora capsici* is one of the major constraints in black pepper production. Many of the valuable genotypes are lost from the gene pool every year due to this serious malady. Since the conventional breeding programmes so far carried out could not bring about a resistant genotype, exploitation of *in vitro* culture induced variation is of great significance. *In vitro* callus screening using toxic metabolite(s) of *Phytophthora capsici* was hence attempted at the College of Horticulture, Vellanikkara during 1992-1996. The effect of toxic metabolite(s) at various stages of morphogenesis of calli was studied in detail.

## MATERIALS AND METHODS

Calli were induced from stem and leaf explants of *in vitro* raised seedlings of five black pepper cultivars (Kalluvally, Cheriakanyakkadan, Balankotta, Karimunda and Panniyur 1) in modified MS medium (Murashige and Skoog, 1962) supplemented with IAA and BAP. The indirect organogenesis reported in black pepper by Nazeem (1990) was used to produce regenerants, in the different cultivars. *In vitro* production of toxic metabolite(s) of *Phytophthora capsici* was attempted in Ribeiro's medium (Ribeiro, 1978). Concentrated culture filtrate (CCF) of the fungus containing the toxic metabolite(s) was used as the screening agent for *in vitro* callus screening. In preliminary standardisation experiments 7.5 per cent v/v of CCF was fixed as the level of CCF for *in vitro* callus screening.

Concentrated culture filtrate was incorporated to modified MS media at 7.5 per cent v/v and the effect of toxic metabolite(s) on callus growth, callus proliferation, shoot regeneration, shoot proliferation, shoot growth and root growth were studied. Calli / shoots inoculated to modified MS medium to which concentrated Ribeiro's medium was added to the same volume as that of CCF served as control (medium control).

The growth and proliferation of surviving calli in the CCF added medium and medium control were compared by working out the callus growth index (CGI) which was computed as CGI = P1 x G, where P1 is the percentage of surviving calli and G is the growth score given for the calli.

Three to four week old calli were inoculated to the CCF containing modified MS medium and observed for regeneration of shoots for three more subcultures in the medium of the same composition each with an incubation period of three weeks.

For shoot proliferation, already regenerated cultures in CCF free medium were inoculated to the CCF containing medium and observed for proliferation of shoots.

Uniform shoots of the different cultivars were used to study the difference in shoot and root

Table 1. Effect of concentrated culture filtrate (7.5% v/v) on callus growth and proliferation in black pepper cultivars (calli induced from *in vitro* seedling explants)

| Cultivars | Treatments | Surviving calli, % | Average growth score | Callus growth index |
|---|---|---|---|---|
| Kalluvally | CCF medium | 78.89 | 2.87 | 226.41 |
| | Medium control | 100.00 | 2.85 | 285.00 |
| Cheriakanyakkadan | CCF medium | 62.63 | 2.75 | 172.23 |
| | Medium control | 100.00 | 2.80 | 280.00 |
| Balankotta | CCF medium | 57.00 | 2.00 | 114.00 |
| | Medium control | 100.00 | 2.25 | 225.00 |
| Karimunda | CCF medium | 48.40 | 3.00 | 145.20 |
| | Medium control | 100.00 | 3.00 | 300.00 |
| Panniyur 1 | CCF medium | 45.56 | 2.50 | 113.90 |
| | Medium control | 100.00 | 2.50 | 250.00 |

Culture period = 3 weeks; $X^2$ values for comparison of callus growth index of cultivars = 56.412**
Medium = ½ MS supplemented with IAA and BAP 1.0 mg $l^{-1}$

Table 2. Effect of prolonged duration of selection with concentrated culture filtrate on shoot regeneration from Hack pepper calli (calli induced from *in vitro* seedling explants)

| Cultivars | Treatments | Regeneration of shoots, % | | | |
|---|---|---|---|---|---|
| | | 1st culture | 1st subculture | 2nd subculture | 3rd subculture |
| Kalluvally | CCF medium | Nil | Nil | Nil | Nil |
| | Medium control | Nil | 55.55 | - | - |
| Cheriakanyakkadan | CCF medium | Nil | Nil | Nil | - |
| | Medium control | Nil | 52.94 | - | - |
| Balankotta | CCF medium | Nil | Nil | Nil | Nil |
| | Medium control | Nil | 50.00 | - | - |
| Karimunda | CCF medium | 62.50 | - | - | - |
| | Medium control | 64.70 | - | - | - |
| Panniyur 1 | CCF medium | Nil | Nil | Nil | Nil |
| | Medium control | Nil | 27.77 | - | - |

Culture period - 3 weeks in each subculture; Medium - ½ MS supplemented with IAA and BAP 1.0 mg $l^{-1}$

growth in the CCF containing medium and medium control.

## RESULTS AND DISCUSSION

The percentage of surviving calli in CCF incorporated medium was found to be low as compared to the medium control for all the cultivars (Table 1). The cultivar Kalluvally registered higher survival percentage (78.89) followed by Cheriakanyakkadan (62.63), Balankotta (57.00), Karimunda (48.40) and Panniyur 1 (45.56). The higher survival of Kalluvally observed in the CCF added medium might be due to its inherent ability to withstand all stress conditions (Ravindran and Nair, 1983). As regard to the growth of the surviving calli, there was not much difference observed in CCF added medium and the medium control. This showed that once the calli survived in the CCF added medium, toxic

Table 5. Effect of concentrated culture filtrate (7.5% v/v) on root growth in black pepper cultivars

| Cultivars | Treatments | * No. of roots | *Length of roots, cm | *Thickness of roots, mm |
|---|---|---|---|---|
| Kalluvally | CCF medium | 10.83 (3.275)** | 4.058 | 0.642 |
| | Medium control | 10.75 (3.260) | 1.692 | 0.875 |
| | t value | 0.1000 | 19.1394 | 4.6549 |
| | Probability | 0.9213 | 0.0000 | 0.0001 |
| Cheriakanyakkadan | CCF medium | 15.83 (3.906) | 3.300 | 0.617 |
| | Medium control | 14.66 (3,803) | 1.983 | 0.975 |
| | t value | 0.3874 | 8.3315 | 6.5162 |
| | Probability | 0.7022 | 0.0000 | 0.0000 |

Culture period = 40 days; * - Average of 12 observations; ** Values in parentheses indicate transformed values
Medium = ½ MS supplemented with sucrose (2%) and IBA 1.0 mg $l^{-1}$

So it could be assumed that prolonged duration of selection with CCF inhibited the regeneration potential of the calli totally. Poor regeneration of selected callus / cell lines against toxins was reported to be one of the major problems in *in vitro* selection system (Brar and Vidyasekharan, 1990). The shoot regeneration observed in Karimunda might be due to the early induction of shoots observed in the cultivar. Three to five days after inoculation in the CCF added medium Karimunda putforth shoots. So CCF might not have influenced the regeneration in Karimunda. The effect of toxic metabolite(s) on further proliferation of shoots in cultivars like Kalluvally, Karimunda, Cheriakanyakkadan and Balankotta was compared with the medium control using `t' test (Table 3). Observations recorded one month after inoculation showed that there was no significant difference in the proliferation of shoots between the two media. With respect to increment in shoot length and number of nodes produced, no significant differences were observed between the CCF added medium and the medium control in all the cultivars studied (Table 4). In the case of leaf production, Cheriakanyakkadan and Balankotta recorded significantly higher number of leaves in medium control while there was no significant difference in leaf production in other cultivars between the two media studied.

The effect of toxic metabolite(s) on different root characters like root length, number of roots and root thickness was studied in two cultivars *viz.*, Kalluvally and Cheriakanyakkadan (Table 5). The number of roots showed no significant variation in the two media studied while the length and thickness of roots showed significant variation. The roots were found to be thinner and longer in the CCF added medium as compared to the medium control.

The toxic metabolite(s) in the culture filtrate was not found to inhibit the growth of the surviving calli, further proliferation of shoots and shoot growth in the already regenerated cultures. However, CCF had its influence on root growth. The roots were found to be thinner and longer in CCF added medium compared to the medium control. Detailed symptomatology of *Phytophthora* foot rot disease in black pepper by Mammootty (1978) and Sarma and Nambiar (1982) showed that root and collar regions of the vine arc more vulnerable to the attack by the pathogen. In the present *in vitro* studies also, out of the various stages of morphogenesis observed, toxic metabolite(s) had its maximum effect on root growth reducing the thickness of the shoots significantly.

## ACKNOWLEDGEMENT

This paper forms a part of the Ph.D (Hort) thesis of the senior author submitted to the Kerala Agricultural University in 1996.

Table 3. Effect of concentrated culture filtrate (7.5% v/v) on proliferation of shoots in black pepper cultivars

| Cultivars | No. of shoots* proliferated | | t value | Probability ; S/NS |
|---|---|---|---|---|
| | CCF medium | Medium control | | |
| Kalluvally | 7.25 (2.689)** | 7.16 (2.674) | 0.2691 | 0.7903 NS |
| Karimunda | 4.16 (2.034) | 4.00 (1.992) | 0.5631 | 0.5791 NS |
| Cheriakanyakkadan | 4.83 (2.191) | 4.91 (2.211) | 0.2622 | 0.7956 NS |
| Balankotta | 5.00 (2.230) | 4.83 (2.191) | 0.5424 | 0.5930 NS |

Culture period = 1 month; Container = Small culture tubes (15 x 2.5 cm); * Average of 12 observations
** Values in parentheses indicate transformed values; Medium = ½MS supplemented with IAA and BAP 1.0 mg $l^{-1}$

Table 4. Effect of concentrated culture filtrate (7.5% v/v) on shoot growth in black pepper cultivars

| Cultivars | Treatments | *Shoot* length | No. of nodes produced | No. of leaves produced |
|---|---|---|---|---|
| Kalluvally | CCF medium | 0.750 | 1.104 | 1.345 |
| | Medium control | 0.725 | 1.138 | 1.481 |
| | t value | 0.7355 | 0.4318 | 1.6081 |
| | Probability | 0.4698 | 0.6701 | 0.1221 |
| Cheriakanyakkadan | CCF medium | 0.617 | 1.398 | 1.173 |
| | Medium control | 0.650 | 1.406 | 1.478 |
| | t value | 0.8424 | 0.1023 | 3.0731 |
| | Probability | 0.4086 | 0.9195 | 0.0056 |
| Balankotta | CCF medium | 0.325 | 1.242 | 1.104 |
| | Medium control | 0.300 | 1.276 | 1.276 |
| | t value | 0.7609 | 0.4052 | 2.1589 |
| | Probability | 0.4548 | 0.6893 | 0.0420 |
| Karimunda | CCF medium | 0.342 | 1.264 | 1.276 |
| | Medium control | 0.358 | 1.226 | 1.242 |
| | t value | 0.5566 | 0.4152 | 0.4052 |
| | Probability | 0.5834 | 0.6824 | 0.6893 |

Culture period = 1 month; * = Average of 12 observations
Medium = ½ MS supplemented with IAA 0.1 mg $l^{-1}$ and BAP 0.2 mg $l^{-1}$

metabolite(s) did not inhibit further growth of the surviving calli.

Chi-square analysis showed that cultivars differed significantly in callus growth index in CCF added medium and medium control. In CCF added medium, highest callus growth index was observed for Kalluvally (226.41) followed by Cheriakanyakkadan(172.3). Irrespective of the varieties, the medium control registered 73.63 per cent higher callus growth index as compared to CCF added medium.

The effect of toxic metabolite(s) on regeneration of shoots is presented in Table 2. Regeneration was observed only in the cultivar Karimunda while other cultivars could not be made to regenerate even after repeated subculturing in the medium of the same composition.

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