Revista de Microbiologia (1999) 30:104-106
ISSN 0001-3714

TOXIC METABOLITES FROM CULTURE FILTRATE OF *FUSARIUM OXYSPORUM* AND ITS EFFECTS ON CUCUMBER CELLS AND PLANTLETS

Itamar Soares de Melo[1]*; Everaldo Piccinin[2]

[1]EMBRAPA Meio Ambiente, Jaguariúna, SP, Brasil. [2]Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, SP, Brasil

Submitted: April 24, 1998; Returned to authors for corrections: September 29, 1998; Approved: April 08, 1999

SHORT COMMUNICATION

ABSTRACT

Resistance of cucumber plantlets to culture filtrate of *Fusarium oxysporum* is correlated with resistance of single cells from callus. Single cells and plantlets of two cultivars of cucumber were incubated with culture filtrates. Rapid cell death occurred, as assessed by the stain fluorescein diacetate. More cell death ocurred in the cells of the cultivar *Aodai* than in to cells of the cultivar *Caipira,* which presented high level of resistance. Maximum toxic activity of culture filtrates was attained after 21-25 days of growth of the fungus.

Key words: *Fusarium oxysporum*, single cells, cucumber, toxic metabolites

Fusarium oxysporum causes wilt and is a major pathogen of cucumber in greenhouse condition in São Paulo State, Brazil. In young seedlings the cotyledons lose their green color, droop, and wither. In old plants, leaves wilt during the day for several successive days, and then wilt permanently. There is no resistance in commercial cultivars under those conditions and little is known of the inheritance of resistance. The use of resistant cultivars is the most efficient way to control the fungus.

Although effective, the currently used root inoculation and soil infestation with the pathogen for screening procedures are laborious, time consuming and some times permit many scapes.

Correlation of resistance to a parasite and resistance to its toxins is a necessary prerequisite for such use of phytotoxins. (1, 3, 5, 6, 7) Phytotoxins are useful tools for selection techniques in callus cultures and seedlings.

In this paper, the killing of seedlings and single cells of cucumber has been used to detect phytotoxic activity of *F. oxysporum in* two cultivars, *Aodai* and *Caipira.* The cultivar *Caipira* has been considered to be more resistant than *Aodai* by farmers.

Culture of the fungus (isolated from infected cucumber plants) was maintained on PDA slopes (potato-dextrose-agar) at 28ºC and stored at 8ºC. The fungus was grown on a defined liquid medium, Czapek-Dox. The medium was adjusted to pH 5.5. For bulk production of culture filtrate, 200 ml of medium in an 1 L erlenmeyer flask was inoculated with three mycelial plugs taken from the ege of 7-day old cultures grown on PDA. The flasks were incubated on a rotary shaker (150 rpm at 28ºC). At

* Corresponding author. Mailing address: EMBRAPA Meio Ambiente. Caixa Postal 69. CEP 13820-000. Jaguariúna. SP. Brasil.

harvesting the mycelium was collected and filtered through whatman 3 mm filter paper. The broth cultures were then filter sterilized by passing them, under vaccum, through millipore filter (pore diameter of 0.2 µm).

Callus was initiated from leaves of two cultivars of cucumber (*Aodai* and *Caipira*) on Murashige and Skoog (MS) medium supplemented with 11.40 mg/ml NAA and 20 g/L sucrose. Cell suspensions were initiated from the rapidly growing callus cultures of both cultivars on MS supplemented with 20 g/L sucrose and three different combinations of hormones. Medium 1 with 11.40 mg/L NAA, Medium 2 with 5 µM 2.4-D and 5 µM BAP and Medium 3 with 5 µM NAA and 5 µM BAP.

Toxic metabolites from *Fusarium oxysporum* were produced and interacted *in vitro* with two cultivars of cucumber: *Aodai* and *Caipira*.

A time course experiment for the accumulation of toxic metabolites was performed. Culture filtrates of different ages were assayed for toxic activity with three week old plantlets of the two cultivars.

Seven day old cell suspensions of cucumber were interacted with 21 day old toxic metabolites from *Fusarium oxysporum*. Fusaric acid (5-Butylpicolinic acid) (Sigma) was included in the experiments as control. Cell viability was estimated by epifluorescence microscopy, using fluorescein diacetate staining.

Cucumber seeds of both cultivars were sterilized and planted in an equal mixture of autoclaved soil and vermiculite. Pots of 800 ml, containing the mixture were maintained in greenhouse for three weeks.

Healthy, vigorous seedlings were aseptically removed from the soil. The soil adering to the roots was removed by washing with tap water and root system was immersed in different concentrations of toxic metabolites or in distilled water.

Disease incidence was evaluated in terms of severity of wilt as compared with control plants.

Maximum toxic activity of culture filtrate of *F. oxysporum* was attained after 14-25 days of growth of the fungus (Table 1). Therefore, 25 days old culture was used in all subsequent experiments. In this trial, it was observed that the cultivar *Caipira* presented a good level of resistance to *F. oxysporum* when compared to cultivar *Aodai*. Percent of wilt increased with age of *F. oxysporum* culture filtrates.

The severity of wilt in *Aodai* and *Caipira* plantlets was shown to be concentration dependent (Table 2). The autoclaved culture filtrate produced the same level of disease symptoms as the non-autoclaved culture filtrate; indicating the presence of heat resistant toxic metabolites.

Table 1. Capacity of *Fusarium oxysporum* culture filtrates to cause wilt in cucumber plantlets.

Age of culture (days)	Cultivars wilt	
	Caipira	***Aodai***
25	0.7	2.8
21	0.5	2.3
14	0.3	2.0
8	0.3	1.4
PD (control)	0.0	0.0
PD and FA	1.1	1.6
W and FA	0.3	0.6

PD = Potato Dextrose Media W = water FA = Fusaric acid

[1]Wilt scored on a 0-4 scale; 0 indicating no wilt and 4 indicating severe wilt. Symptom score is a mean of 17 plants.

Table 2. Relationship of different concentrations of toxic metabolites from *Fusarium oxysporum* to cause wilt in *Aodai* and *Caipira* cultivars of cucumber.

	Symptoms	
Concentration %	***Aodai***	***Caipira***
100	3.42	0.85
75	3.00	0.71
50	2.71	0.14
25	2.28	0.14
10	2.71	0.10
50 autoclaved	2.85	0.14
100 autoclaved	3.00	1.00

Symptoms score is expressed as a mean of 17 plants.

Growth of cucumber cells was good on medium 3 and produced cells of a single nature in comparison to media 1 and 2. Hence, cucumber cell suspensions were maintained on medium 3. Seven days old cell suspensions were interacted with 21 days old toxic metabolites from *F. oxysporum*. More cell death occurred in the cells of the cultivar *Aodai* in comparison to cells of the tolerant cultivar *Caipira* (Table 3). This cultivar could be included in breeding programs to introduce resistance in commercial cultivars.

Table 3. Interaction of 21 days old culture filtrates of *Fusarium oxysporum* with 7 days old cell suspension of the cucumber cultivars, *Aodai* and *Caipira*.

	% Cell Death	
	Aodai	***Caipira***
21 days culture filtrate	23.6	8.4
Czapek Dox (control)	2.5	3.0

Note: 3 repetitions with 10 readings in each repetition.

The advantages of using cells and protoplasts over whole plants for assaying toxic have been discussed in detail elsewhere (2, 4, 9). An effective screening procedure has to be amenable for testing a large number of plants, and it should be simple, relatively rapid, and significantly differential. All assays evaluated satisfied these criteria and could be used in breeding programs. The action of the culture filtrate on cell suspensions of cucumber, closely reflect the action of the filtrate in plantlets suggesting a role fungal extracellular toxic compounds in the disease.

RESUMO

Metabólitos tóxicos de filtrado de cultura de *Fusarium oxysporum* e seus efeitos em células e plântulas de pepino

Plântulas de pepino e células isoladas obtidas de calos, das cultivares *Aodai* e *Caipira* foram incubadas com filtrado de cultura de *Fusarium oxysporum*, em condições assépticas. As reações de murchamento das plântulas frente à ação do filtrado evidenciaram que as cultivares *Aodai* e *Caipira* se comportaram como suscetível e resistente, respectivamente. Após avaliação da reação de células isoladas, sob microscópio acoplado com epifluorescência, utilizando-se de acetato de fluoresceína, discriminou-se a porcentagem de células mortas. A cultivar *Aodai* se comportou como extremamente suscetível e a *Caipira* como resistente.

Estes resultados sugerem que compostos tóxicos extracelulares produzidos pelo patógeno servem para utilização em "screening" de cultivares, como também para seleção de células sobreviventes quando submetidas ao filtrado tóxico do fungo com vistas à obtenção de regenerantes resistentes.

Palavras-chave: *Fusarium oxysporum*, células isoladas, pepino, metabólitos tóxicos.

REFERENCES

1. Behnke, M. Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of resistant plants. *Theor. Appl. Genet.* 55:69-71, 1979.
2. Breiman, A. and Gallun, E. Plant protoplasts as tool in quantitative assays of phytotoxic compounds from *Phytophthora citrophthora*. *Physiol. Plant. Pathol*. 19:181-191, 1981.
3. Byther, R.S.; Steiner, G.W. Use of helminthosporoside to select sugarcane seedlings resistant to eye spot disease. *Phytopathology* 62:466-476, 1972.
4. Gendlof, E.H.; Scheffer, R.P.; Somerville, S.C. An improved biossay for victorin based on the use of bat protoplasts. *Physiol. Mol. Plant. Pathol*. 31:421-427, 1987.
5. Kuo, M.S.; Yoder, O.C.; Scheffer, R.P. Comparative specifity of the toxin of *Helminthosporium carbonum and H. victoriae. Phytopathology* 60:365-368, 1970.
6. Martern, V.; Strobe, G.; Shepard, J. Reaction to phytotoxins in potato population derived from mesophyl protoplasts. *Proc. Nac. Acad. Sci*. USA 75:4935-4939, 1978.
7. Michael, S.H. and Carr, A.J.H. Use of culture filtrates as a rapid technique for screening lucerne for resistance to *Verticillium albo-atrum. Trans. Br. Mycol. Soc*. 49:133-138, 1966.
8. Panton, C. A. The breeding of lucern, *Medicago sativa*, for resistance to *Verticillium albo-atrum* IV. Evidence for the presence of a toxic substance in culture filtrate of the causal organism and its effects on plants. *Acta Agric. Scand.* 17:59-77, 1967.
9. Shohet, S. and Strange, R.S. Use of isolated cells and protoplasts to detect phytotoxic activity in cultures of *Phytophthora drechsleri* f.sp. *cajani*. *Physiol. Mol. Plant. Pathol*. 34:345-359, 1989.