SHORT COMMUNICATION

USE OF CULTURE FILTRATES OF *PYRENOCHAETA LYCOPERSICI* IN TESTS FOR SELECTING TOLERANT VARIETIES OF TOMATO

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SUMMARY

Pyrenochaeta lycopersici, the causal agent of corky root, is one the most important pathogens on tomato plants and other vegetable crops in protected and field crops all over the world. The present study was aimed at evaluating the effectiveness of an *in vitro* test in which culture filtrates of *P. lycopersici* and tomato seedlings were used for selecting tolerant plants. The method allowed assessment of plant response to the disease and quick screening for tolerance. The rapid appearance of symptoms on seedling rootlets suggests that *P. lycopersici* may release toxic compounds into the liquid medium during its growth.

Key words: *Pyrenochaeta lycopersici*, corky root, cultural filtrates, *in vitro* test, precocious selection, resistance.

Pyrenochaeta lycopersici (Schneider *et* Gerlach) is the causal agent of the corky root, a serious disease affecting tomato and other vegetable crops in greenhouse and field crops. Corky root occurs in some tomato growing areas in Italy (Ciccarese and Amenduni, 1991; Fiume, 1995) and abroad (Davet, 1973; Granges, 1981; Campbell *et al.*, 1982; Shishkoff and Campbell, 1990). The disease is commonly reported to be responsible for yield losses higher than 50%, especially in protected crops (Gullino *et al.*, 1995).

Infected plants show brown corky lesions, often fissured, appearing in bands around the roots. The taproot and stem base may eventually turn brown and rot. Plants grow slowly, may look older, and show symptoms like those of drought. Fruit size and yield are reduced (Matta, 1976).

The control of corky root is based on cropping practices and soil solarization alone (Mihira *et al.*, 1999) or integrated by application of calcium cyanamid (Fiume, 1995, 1996; Fiume and Parisi, 1995). Unfortunately, many growers find soil solarization too laborious to balance its beneficial effects. Methyl bromide and other fumigants, such as metham sodium, methyl isothiocyanate or dazomet, are very effective against *P. lycopersici* and other soil-borne fungal pathogens, but treatments are expensive, require special machinery and well-trained personnel and cause environmental pollution. Methyl bromide, in particular, is recognized as an ozone-depleting chemical (Albritton and Watson, 1992), and its use is more and more restricted. Promising results have been obtained by biological control based on VA-mycorrhizas, alone or integrated with *Bacillus subtilis* Berliner and nitrogen and potassium fertilizers (Bochow and Abou-Shaar, 1990).

Plant resistance may be the most effective and long lasting control strategy against *P. lycopersici*. The known source of resistance to corky root, the *pyl* gene, exhibits incomplete penetrance and expressivity, therefore, new genetic sources are needed (Kim *et al.*, 1988; Levine *et al.*, 1994; Wu *et al.*, 1995). A program of tomato genetic improvement for resistance to *P. lycopersici* is in progress at our Institute. Genetic resistance was transferred by crossing wild and cultivated tomato species into fresh market cultivars suitable to Italian areas. Screening for resistance or tolerance in segregating progeny was made by artificial inoculations of plantlets with the pathogen or growing plants in naturally infested soil (Restaino *et al.*, 2000).

Rapid selection of resistant or tolerant plants under controlled conditions is the crucial step for screening. Therefore, in the present work tomato genotypes showing different responses to corky root, from susceptibility to tolerance, were used for investigating the reliability of an *in vitro* test based on the use of culture filtrates of *P. lycopersici*.

The isolate of *P. lycopersici* was obtained from infected roots of diseased tomato plants with typical symptoms of corky root and maintained on potato dextrose agar (PDA).

A 9-mm-diameter disk of inoculum, from 20-day-old colonies on PDA, was inoculated in 150 ml of potato dextrose broth (PDB) in 250 ml-Erlenmeyer flasks that was kept horizontal at 21±1°C under static conditions. The inoculum disk was placed on the bent end of a glass rod to enable the flotation of the growing fungal colony. Aliquots (8 ml) of the cultural filtrate were collected after 7, 15 and 30 days under a laminar-flow cabinet and transferred into test tubes (18 x 3 cm).

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Plants of the susceptible cv Arletta and of genotypes of the genus *Lycopersicon*, named A1, P14 and P31, showing responses to corky root in the field ranging from susceptibility to tolerance were used. Tomato seedlings were obtained from seeds previously immersed in 10% calcium hypochlorite for 10 min, washed 3 times in sterile water and germinated on humid absorbent paper in Petri dishes at 25±1°C. Single seedlings at the cotyledon stage were placed with their rootlets in the culture filtrate in test tubes. The treatments compared were: 1) sterile water; 2) PDB medium; 3-5) cultural filtrates collected after 7, 15 or 30 days of fungal growth. Test tubes were kept at 25±1°C, with 12 hours lighting per day.

Symptom severity was assessed after 1, 2, 3 and 4 days by visual estimation of symptoms on rootlets and the use of the following empirical scale: 0 = growing rootlets with no visible symptoms; 1 = necrosis localized on the rootlet apex and extended over less than 25% of the rootlet surface; 2 = no growth of rootlet with necrosis on 26-50% of its surface; 3 = necrosis on 51-75% of the rootlet surface; 4 = necrosis on 76-100 of the rootlet surface and seedling death. Use of the scale allowed calculation of the McKinney Index, according to the formula:

$$I = \frac{\sum (f \cdot v)}{N \cdot X} \cdot 100$$

where: f = number of seedlings in each class; v = class value; N = number of observed seedlings; X = highest value of the empirical scale.

All the treatments were replicated 10 times. Data were statistically analyzed by variance analysis and Duncan's Multiple Range test.

Plants cv Arletta showed necrosis on rootlets just after one day of exposure to *P. lycopersici* filtrates (Table 1). The age of *P. lycopersici* cultures and exposure time of tomato seedlings to culture filtrates played important roles. Filtrates obtained after 15 and 30 days of fungal growth caused very severe symptoms on seedling roots of the susceptible cv Arletta. When seedlings were exposed to cultural filtrates for 3 and 4 days, symptoms on roots were particularly severe. The seedlings exposed for 4 days to filtrates from 30-days old cultures of *P. lycopersici* showed the most severe necrotic symptoms or died.

The method yielded promising results for screening tolerant plants to corky root by exposing tomato seedlings for 3 days to culture filtrates from 15-day-old *P. lycopersici* cultures. Exposure to culture filtrates caused McKinney Indexes of 60, 40, and 65% for the three tomato genotypes A1, P14 and P31, respectively (Table 2). The responses of tomato genotypes to filtrates were similar to those observed in preliminary field trials. Hence, the method described seems a valid and rapid laboratory test for evaluating the response of tomato plants to corky root. The rapid brown rot caused by culture filtrates of *P. lycopersici* on tomato seedlings suggest that the pathogen secretes phytotoxic substances during its growth.

Table 2. Responses (McKinney Index) of three *Lycopersicon* genotypes to culture filtrates from 15-day-old *P. lycopersici* cultures [a].

Treatments	Tomato genotypes		
	A1	P14	P31
Water	0.0 B	2.5 B	0.0 B
PDB medium	0.0 B	0.0 B	2.5 B
Culture filtrate	60.0 A	40.0 A	65.0 A

[a] Values followed by the same letter, in each column, do not differ significantly at P≤0.01.

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Table 1. Reactions of tomato seedlings cv Arletta to filtrates from *P. lycopersici* cultures of different ages [a].

Treatments	McKinney Index (%)			
	1 day	2 days	3 days	4 days
Water	0.0 D	2.5 D	2.5 C	0.0 C
PDB medium	2.5 D	0.0 D	0.0 C	2.5 C
Culture filtrate (7 days)	15.0 C	30.0 C	52.5 B	50.0 B
Culture filtrate (15 days)	27.5 B	52.5 B	80.0 A	85.0 A
Culture filtrate (30 days)	60.0 A	75.0 A	85.0 A	100.0 A

[a] Values followed by the same letter, in each column, do not differ significantly at P≤0.01.

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