PATHOGENIC AND BIOLOGICAL VARIABILITY OF *MACROPHOMINA PHASEOLINA* (Tassi) Goid. ISOLATES IN DIFFERENT AREAS OF SUNFLOWER CULTIVATION IN ITALY.

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SUMMARY

Macrophomina phaseolina (Tassi) Goid, agent of charcoal rot of sunflower, is the most important disease of this crop everywhere in Italy. 64 isolates were collected in 12 sunflower growing areas in four climatic environment from Northern to Southern Italy to study the variability of the pathogen from the same host.

Colony characteristics of all isolates on synthetic media were homogeneous with high production of sclerotia and poor mycelium; isolates growth rate and colony shape at 30 °C were also homogeneous. Chromogenicity of cultures in tube varied from light yellow to deep red. Sclerotia diameter varied from 72.8 μm to 127.8 μm; difference between sclerotia size of 64 isolates was significant.

Pathogenicity of isolates was tested in growth chamber on 2-wk seedlings with unwounded stem base inoculation method, using cornmeal sand inoculum. Severity was assessed 4 and 8 days after inoculation.

Isolates were grouped into three classes of pathogenicity: highly virulent, virulent and poorly virulent.

Pathogenicity, sclerotia size and chromogenicity were not related to climatic areas.

The variability of sunflower strains found in this work did not appear, in a first examination, a fungal response to environmental pressure. The great variability in pathogenicity in all the climatic areas suggest good adaptation to the host by *Macrophomina*.

These are first results of a detailed study on relationship between environmental factors and variability of sunflower isolates of *M. phaseolina*.

* Planning, executing and writing of the report;

** Planning of the work and supervision;

*** Executing laboratory work.

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid., the causal agent of charcoal rot, is the most important stem rot parasite of sunflower in Italy (Zazzerini *et al.*, 1985), in Spain (Jeménez-Diáz and Blanco López, 1983) and in the warmer regions of the world (Diangra and Sinclair, 1978; Luciano and Davreux, 1967; Bouhot, 1967; Sackston, 1957).

The anamorphic fungus can attack a wide range of hosts encompassing 75 plant families and more than 500 species, including legumes, vegetables, fruits and many other economically important crops (Diangra and Sinclair, 1978; Holliday and Punithalingam, 1970).

Although only one species is recognised within the *Macrophomina* genus (Sutton, 1980), a great variability has been observed among isolates obtained from different hosts (Person *et al.*, 1986 and 1987; Chan and Sackston, 1970).

Pearson *et al.* (1986 and 1987) found host preference for *Macrophomina* isolates from maize, soybean and other cultivated host species. They divided isolates from different hosts into three physiologically distinct classes on the basis of chlorate sensitivity and nitrogen source utilization (Pearson *et al.*, 1987). Different isolates from the same host may also vary, Diangra and Sinclair (1973) found a great variability in the pathogenicity and morphological and physiological characteristics among isolates from soybean obtained in different states of U.S.A.

Macrophomina isolates from cotton, coming from a great growing area in India, were divided in two races by Sulaiman and Patil (1968) on the basis of pathological and biological tests.

Cytological studies partially explain the great variability of the fungus: brown old hyphal cells of *Macrophomina* are uninucleate, but young hyphal cells and hyphal apices are usually multinucleate (Knox-Davies, 1966). Hyphal anastomosis has been observed (Knox-Davies, 1967) and probably during hyphal fusion, after mitotic segregation and recombination, heterokaryosis occurs (Punithalingam, 1983).

Many authors also maintain that water-stressed plants are more susceptible to charcoal rot than plants with a normal water supply (Blanco-López and Jeménez-Diáz, 1983; Ghaffar and Erwin, 1969), others maintain that metabolic products produced by the hosts, in response to stress or to physiological variations, may influence disease severity (Ilyas and Sinclair, 1974 Pearson *et al.*, 1987; Wyllie and Calvert, 1969).

M. phaseolina is a very frequent sunflower disease in Italy. In 1991, charcoal rot was found on 64 out of 70 sunflower crops visited at harvest, in northern, middle and southern Italy. Numerous isolates of *Macrophomina* from infected sunflower stems, were collected from different growing areas for a detailed study of the relationship between environmental factors and variability of isolates from the same host species.

This is the first report of this study and it describes the variability in pathogenicity and morphological characteristics of *M. phaseolina* isolates.

MATERIALS AND METHODS

M. phaseolina isolates were collected at harvest, between 20 August and 10 September 1991 in twelve sunflower growing areas, located in twelve Italian provinces, chosen in four climatic areas of Italy (tab. 1) according to the Agroclimate Atlas of Europe (Thran and Broeckuizen, 1965). Samples were collected in fields at least 6 Km apart and from the main infected areas of the fields.

Isolations were made from lower stems of 64 samples; the tissues cut into three to four 4 cm-pieces were washed in running water for 5 minutes and the surface disinfected in 0.5 % sodium hypoclorite for 3 minutes. Then fragment of tissues were cut and placed in Petri dishes containing PDA-SP (Potato Dextrose Agar + 100 mg/l of Streptomicyn sulfate and 50 mg/l of Penicillin) and incubated at 30 °C in the dark. The cultures were stored at 4 °C on tubes containing PDA.

Morphology of colonies was recorded on PDA and on OA (Oatmeal Agar) after an incubation of 20 days at 30 °C. Cultures were grown on PDA with day light and on OA with 12 h photoperiod of near UV light.

Chromogenicity was recorded on 1 month-old cultures in tubes containing 5 ml of PDA, stored at room temperature with day light. Isolates were divided into homogeneous chromogenicity groups.

Sclerotia size was measured under the microscope (250:1), recording the major diameter of three samples of ten sclerotia taken from the periphery of 4-day-old colonies on PDA stored at 25 °C.

Pathogenicity was tested on 2-wk-old sunflower seedlings grown in 96-hole polistyrene grids at 24-26 °C in a growth chamber with a photoperiod of 12 h (Cold white 2000 Philips and Grow lux Sylvania) and R.H 60 %. Inoculum were grown on 100 g cornmeal sand medium (Riker and Riker, 1936) with five 4-mm agar disks of 6-day-old colonies and stored for four days at 30 °C in the dark (Jeménez-Diàz and Blanco-Lòpez, 1983). Unwounded stem base inoculations (Chan and Sackston, 1969) were made with the isolates and the control on 4 seedlings each in a randomised complete block design with three replications. The temperature of the growth chamber after inoculation was 28-30 °C. Disease severity was assessed 4 and 8 days after inoculation on a 0-100 scale:

0 no symptoms
10 brown discoloration of lower stem
40 dark brown necrotic lesions around the entire stem base
55 dark brown necrotic lesions on lower 1/3 of the stem
85 dark brown necrotic lesions on lower half of stem
100 dead seedling.

Statistical analysis was with the M-STAT program, data were subjected to factorial analysis of variance ($P \geq 0.05$), to Tukey's test and to Correlation analysis.

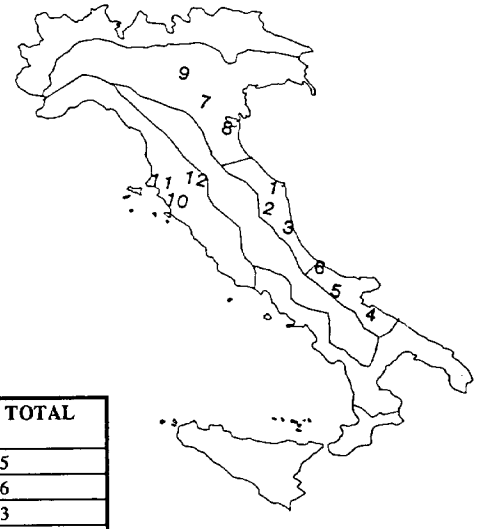
RESULTS

Cultures of sunflower isolates of *M. phaseolina*, compared under different growing conditions, did not show appreciable differences under the visual examination. Mycelia, with regular growth, reached the edge of petri dishes in 4 days ; first sclerotia were superficially produced 24-48 hours after inoculation; 6-7 days later sclerotia were observed on and within the media giving a typical grey-black colour to cultures. White-grey aerial mycelium was scarcely produced by some isolates and collapsed in old cultures. Stereomicroscopic observation of 15-day-old cultures revealed slight differences between sclerotia formation.

Chromogenicity of the cultures, stored for one month in tube, varied: there was an equal frequency of isolates with neutral colour, grey, yellow, violet, light red, medium red, deep red; chromogenicity was not related to the climatic areas.

Climate Areas (Provinces)	Rainfall	Summer	Winter
1, 2, 3 (AN, AP, MC ,TE)	wet area, from 550 to 1400 mm/year	from warm (22-26°C) to very hot (>32°C)	mild (+4 +6°C)
4, 5, 6 (FG, CB)	occasionally dry, from 300 to 1050 mm/year	from warm (22-26°C) to very hot (>32°C)	tepid (+6 +8°C)
7, 8, 9 (BO, RA, MN)	wet area, from 550 to 1400 mm/year	from warm (22-26°C) to very hot (>32°C)	cold (-1 +2°C)
10, 11, 12 (GR, LI, SI)	wet area, from 550 to 1400 mm/year	from warm (22-26°C) to very hot (>32°C)	tepid (+6 +8°C)

Tab. 1 - Sunflower growing areas grouped in four climatic environments.
(From Agroclimate Atlas of Europe by Thran and Broeckuizen, 1965)



Growing areas	hightly virulent	virulent	powerly virulent	TOTAL
1	III		II	5
2	IIIII	I		6
3	III			3
4	IIII	I	II	7
5	IIIII	I	IIII	10
6		III		3
7	I		I	2
8	IIIIII	IIII	II	13
9		I		1
10	II	IIII	II	8
11	I		II	3
12	I	I	I	3
	32	16	16	64

Tab. 2 - Distribution of *Macrophomina phaseolina* isolates collected in twelve sunflower growing areas.

Sclerotia diameter varied from 72.8 μm to 127.8 μm with an average of 102.2 μm. Difference between the sclerotia size of 64 isolates was significant ($P \geq 0.05$). The distribution frequency of sclerotia diameter was continuous. Sclerotia size was not homogeneous within isolates coming from the same climatic areas, but showed a random variation.

All isolates were able to produce symptoms on seedlings, but virulence was significantly different both 4 days and 8 days after inoculation. The pathogenicity index after 4 days varied from 0 to 88.7, whereas after 8 days it varied from 7.5 to 100. The pathogenicity index after 4 days was significantly correlated with that after 8 days, isolates producing no symptoms after 4 days caused only stem discoloration or light necrotic lesion on stem base after 8 days.

It was possible to divide the isolates into three classes on the basis of the pathogenicity index recorded after 8 days:

- highly virulent (100 - 61),
- virulent (60 - 45),
- poorly virulent (5 - 44).

Some only slightly virulent isolates did not produce necrosis, but only brown discolorations. The pathogenicity index of isolates was not related to climate area. No significant correlations were observed between sclerotia size and the pathogenicity index. There was no correspondence between sclerotia density in culture and virulence.

DISCUSSION AND CONCLUSION

Italian isolates of *M. phaseolina* from sunflower appear to be very variable, nevertheless some factors like culture characteristics appear to be homogeneous. Various researchers report differences in cultural characteristics of isolates from the same host (Diangra and Sinclair, 1973a; Khare *et al.*, 1970). Sulaiman and Patil (1968) distinguish cotton isolates in two races: first one, more virulent, producing abundant mycelia *in vitro*, and a second one producing more sclerotia and less mycelium. The culture morphology may be an homogeneous characteristic of *Macrophomina* sunflower isolates in Italy, while the different sclerotia density produced in cultures rich in nutrients such as PDA and OA appear to be attributable to the nutritional stage of the fungus and not to the virulence of isolates (Chan and Sackston, 1973).

Variability in chromogenicity, sclerotia size and pathogenicity may reflect the polykaryotic character of mycelium (Punithalingam, 1983), and the probable presence of heterokaryotic nuclei, even if nuclei fusion and the formation of heterokaryotic nuclei has not yet been directly observed (Knox-Davies, 1967). Pearson *et al.* (1986; 1987) showed a different metabolic adaptation to the host by maize and soybean strains of *M. phaseolina*. The variability of sunflower strains found in this work did not appear, in a first examination, a fungal response to environmental pressures. Future studies of the physiological and biological characteristics should thoroughly examine adaptation to external factors by sunflower strains. The great variability in pathogenicity in all the climatic areas suggests good adaptation to the host by *Macrophomina* in Italy.

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