IDENTIFICATION OF RACES OF HELMINTHOSPORIUM SATIVUM OF WHEAT IN BRAZIL[1]

Y.R. MEHTA[2]

ABSTRACT - Ninety-six monoconidial isolates of *H. sativum* were obtained from 41 municipal regions comprising five different states and as many as 51 different cultivars. Initially, 45 out of 96 isolates were tested against 40 wheat cultivars in the seedling stage; and based on the reaction pattern, a set of thirteen differential wheat cultivars was established. This set of differentials was further used to test the rest of the 51 isolates and a total of 32 different races were identified. The identified races were given serial numbers. Twenty-three races were identified from Paraná, 4 from São Paulo, 3 from Rio Grande do Sul, 1 from Mato Grosso and 1 from Brasilia, DF. Race 9 was most predominant in the states of Paraná, São Paulo and Rio Grande do Sul.

Index terms: *Helminthosporium sativum*, race identification, wheat.

IDENTIFICAÇÃO DE RAÇAS DE HELMINTHOSPORIUM SATIVUM DO TRIGO NO BRASIL

RESUMO - Noventa e seis isolados de monoconídios de *H. sativum* foram obtidos de 41 municípios abrangendo cinco estados e 51 cultivares de trigo. Inicialmente, dum total de 96 isolados 45 foram testados em 40 cultivares de trigo na fase de plântulas, e, baseado no tipo de reação, estabeleceu-se um conjunto de treze cultivares diferenciais. Tal conjunto foi posteriormente usado para testar os restantes 51 isolados e permitiu identificar um total de 32 raças diferentes. Estas raças identificadas foram designadas por seqüência numérica. No Paraná, detectaram-se 23 raças, em São Paulo 4, no Rio Grande do Sul 3, Mato Grosso 1 e finalmente para DF. - Brasília 1 (uma). A Raça 9 foi predominante nos Estados do Paraná, São Paulo e Rio Grande do Sul.

Termos para indexação: *Helminthosporium sativum*, identificação de raças, trigo.

INTRODUCTION

Leaf blight (spot blotch) of wheat caused by *Helminthosporium sativum* is not reported to be a serious disease in most part of the world. In recent years, in Brazil it has become a threat to wheat cultivation, especially in the state of Paraná. Severe epidemics of this disease occurred in Paraná during 1972, 1976, 1979 and 1980, causing heavy yield losses (Mehta et al. 1980, Mehta 1978). Chemical control of this disease is rather difficult since no specific fungicide is so far available to achieve a reasonably good control. Till today, Maneb or Mancozeb are the only fungicides that are generally used against this disease. Generally speaking, in Brazil such fungicides are applied soon after the appearance of the first symptoms of the disease, or, in other words, 55 - 60 days after sowing in case of late maturing varieties, and 45 - 50 days after sowing in case of early maturing varieties. A total of three applications are given with an interval of fifteen days. Experiences during the past few years indicate that under the severe epidemic conditions even three applications of such fungicides with a dose of 2,5 kg/ha in 200 l of water (with a stiker) does not give a satisfactory control, which means that the disease is not checked bellow 50% infection level at the critical growth stage 83 (Mehta et al. 1978). Hence, breeding for resistance against this disease deserves top priority.

Some sources of resistance against this disease are already available, like the cultivars PAT 7219, BH 1146, Horizon, LD 7831 and PF 72707. The resistance is being incorporated in the desirable cultivars and the advanced lines. However, knowledge regarding the existence of races of *H. sativum* is very much lacking. For the breeding program it is very important to first of all identify the different races so that the sources of resistance effective against most of the races could be identified and finally such a wide spectrum resistance could be incorporated in desirable cultivars. It is of little or no use to incorporate resistance against one or two races when a number of virulent races exist in a particular region. Due to lack of knowl-

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2 Engº, Agrº, Ph.D., IAPAR, Caixa Postal, 1331, CEP 86.100 - Londrina, PR.

edge regarding races, it is not yet known if the aforesaid cultivars possess resistance against one or more races. There exist very few reports about the race identification of *H. sativum*.

The first attempts to identify the races of *H. sativum* were made as early as 1922 (Christensen 1925). Christensen (1925) reported that there existed at least 37 races of this fungus based on morphological and pathological characters. He made tests by growing plants in inoculated soil, which is more laborious, especially when a large number of isolates are to be tested. Moreover, no standard set of differential cultivars has so far been established. Hamilton et al. (1960), while working with seven isolates of *H. sativum* in seedling blight tests on four barley varieties, although observing variation in virulence among the isolates, concluded that no differential response of the test varieties existed. On the other hand, Clark & Dickson (1958), while working with *H. sativum* on barley, reported that the isolates differed significantly in pathogenicity. Wood (1962) strongly believed the existence of physiologic races within *H. sativum*. He reported that the isolates of this fungus differed strikingly in their parasitic capabilities, irrespective of the plant source or the geographical region from which they came. He further reported that the progenies from a single conidium differed strikingly in pathogenicity. This finding further complicates the problem in race identification, added to other problems like the constant and rapid mutations, saltation and the lack of a standard differential set of cultivars. In the present investigation attempts were made to identify the races of *H. sativum* by establishing a preliminary set of differential cultivars.

MATERIALS AND METHODS

Ninety-six leaf samples infected with *H. sativum* were obtained from 41 locations and 51 cultivars (Table 1). Out of 96 samples 56 were from Paraná, 24 from São Paulo, 13 from Rio Grande do Sul, 2 from Mato Grosso and only one from Brasilia, DF. Monoconidial cultures from all the samples were obtained, and the inoculum was increased on autoclaved sorghum seeds in Erlenmeyer flasks and incubated at room temperature (Joshi et al. 1969). For this purpose sorghum seeds were thoroughly washed and soaked overnight in distilled water, later all the water was decanted and the seeds were autoclaved. A small quantity of the monoconidial culture inoculum on Potato Dextrose Agar (PDA) was added to the flask. The flasks were agitated twice a day to avoid the formation of mycelium and to promote a heavy amount of sporulation. Simultaneously, the isolates were maintained in test tubes with PDA in a refrigerator. To avoid saltation, mutations, and the loss of pathogenicity, the cultures were also maintained on small filter paper disks (5 mm in diameter). For this purpose, the filter paper disks were cut, sterilized in a hot air oven, and put into the flasks containing heavy sporulation on sorghum seeds. The flasks were shaken for a few minutes so as to allow the conidia to adhere on the disks. Later the disks were removed and were preserved, in dry and sterilized test tubes in a refrigerator at about 5°C. In such a way the cultures were stored in the form of dry conidia, and whenever needed a single disk was used to remultiply the culture on autoclaved sorghum seeds, or PDA. Besides, a few sorghum seeds with heavy sporulation were also preserved in a similar way as were the paper disks so as to establish the best way of conservation of original cultures without losing their morphological and physiological characters.

During three weeks abundant sporulation was obtained on autoclaved sorghum seeds. Fifty grams of such seeds were taken in a beaker with 200 ml of sterilized distilled water and shaken thoroughly. The conidial suspension was filtered through a cheese cloth. The conidial suspension was diluted by adding more water or else the conidial concentration was increased by using more seeds, so as to always obtain a concentration of about 56,000 conidia per ml of water. For every 200 ml of conidial suspension a drop of sticker "Sandovit", manufactured by Sandoz S.A., was added and was used for inoculation. Whenever necessary, the inoculum from original monoconidial cultures was increased in standard petri dishes containing PDA. After sufficient sporulation was obtained, a conidial suspension was made, as explained earlier, and the plants were automized.

Forty cultivars were grown in groups of fifteen, in plastic trays (55 cm x 30 cm x 12 cm) with sterilized soil. One row of each cultivar with ten plants was obtained and a total of twelve cultivars were sown per tray. Twenty days after sowing, the seedlings were inoculated uniformly, using a small automizer and a pressure pump. The plants were incubated in the dark for sixteen hours at about 20°C and at near saturated humidity. Later, the plants were incubated for seven days at 20°C in a walk-in-cold chamber with fluorescent lamps with alternated cycles of twelve hours light and twelve hours darkness. The relative humidity was not controlled; it varied from 60 to 90. Readings on infection were taken seven days after inoculation using four categories of infection (Table 2, and Fig. 1). After observation, such plants, along with the soil, were autoclaved before throwing away.

In such a way, 40 cultivars were tested against 45 different single conidial isolates (randomly chosen) in

TABLE 1. Sources of monoconidial isolates of *Helminthosporium sativum*.

Plant source	Nº of cultivars	Nº of isolates	Nº of municipal regions	States of origin
Wheat leaves	16	54	20	Paraná
Wheat leaves	18	23	8	São Paulo
Wheat leaves	6	8	5	Rio Grande do Sul
Wheat leaves	2	2	1	Mato Grosso
Wheat spikes	1	1	1	Paraná
Wheat spikes	3	3	1	Rio Grande do Sul
Wheat seeds	1	1	1	Brasília, DF
Triticale leaves	1	1	1	São Paulo
Rye leaves	1	1	1	Paraná
Barley leaves	2	2	2	Rio Grande do Sul
Totals	51	96	41	5

order to establish a differential set of cultivars having the ability to differentiate the races. The rest of the 51 isolates were tested only on the differential set of cultivars to further identify the races. Before establishing a differential set of cultivars, the test was repeated in part and similar results were obtained. This was done to ensure the validity of the testing procedure. Races were identified based on their infection pattern on differential cultivars. Though plants were examined individually, the overall reaction of infection type on ten plants per cultivar was noted. Minor differences (up to three) only between the reaction type "S" and "MS" within the isolates were ignored and such isolates were grouped under a single race.

RESULTS, DISCUSSION AND CONCLUSIONS

Fourty cultivars were tested against 45 monoconidial isolates, and, based on their reaction pattern, thirteen cultivars could be selected having capacity to distinguish the races. Such cultivars formed a set of differentials and were tested against the rest of the 51 isolates. In such a way, a total of 96 monoconidial isolates were tested in groups of fifteen isolates in a series of experiments and a total of 32 races were identified, as shown in Table 3. The races thus identified on seedlings were given serial numbers. Some of the races thus identified also differed from each other when tested on adult plants, indicating thereby, the validity of the present testing techniques (Mehta 1981).

Only one isolate from rye leaves was tested which did not infect any of the wheat cultivars used in the present investigation. Wood (1962) also reported that none of the isolates of *H. sativum* from rye infected wheat. The isolates from barley lost their ability to sporulate during the process of maintenance and hence could not be tested. Out of the 32 races identified, 23 were from Paraná, 4 from São Paulo, 3 from Rio Grande do Sul, 1 from Brasilia, DF., and 1 from Mato Grosso. The simple fact that almost 70% of the races identified were from Paraná indicates the importance of *H. sativum* in this state. Of course the number os isolates tested from other states was much lower than that of Paraná.

Race 9 was the most predominant in the states of Paraná, São Paulo and Rio Grande do Sul. Apparently, some of the isolates differed in their degree of virulence even within the highly susceptible class "S". More rigorous classification of the infection types may lead to distinguish more races.

Considering that Brazil is a big country, the number of samples tested may be considered to be small, but it was considered representative at least for the states of Paraná and São Paulo. A relatively large number of cultivars were tested to establish a differential set of cultivars. The differential set of thirteen cultivars was considered satis-

TABLE 2. Reaction types of *Helminthosporium sativum* on wheat seedlings observed 7 days after inoculation.

Category \ Reaction type	R (Resistant)	MR (moderately resistant)	MS (moderately susceptible)	S (susceptible)
Lesions	Absent, or very few and small necrotic spots	Present	Present	Present
N°. of lesions	Less than 5 per leaf	More than 5 per leaf	Many per leaf	Many per leaf and coalesce
Lesion size	Less than 1 mm x 2 mm	Less than 1 mm x 5 mm	More than 1 mm x 5 mm	More than 1 mm x 5 mm
Chlorosis	Absent	Very little or no chlorosis	Lot of chlorosis	Lot of chlorosis and lesions and the chlorosis coalesce
% of necrotic leaf area	Almost nill	Very little	Less than 50% in all the leaves	At least over 50% in at least one leaf per plant

FIG. 1. Reaction types of *Helminthosporium sativum* on wheat seedlings observed seven days after inoculation. R - Resistant, MR - Moderately Resistant, MS - Moderately Susceptible, and S - Susceptible.

factory since when the experiments were repeated time and again, similar results were obtained especially during the first three-four months of isolations. Nevertheless, when the experiments were once again repeated after about eight-ten months of monoconidial isolations, in most of the cases the reaction pattern of the races on the differential cultivars was altered. This indicates that soon after the races are identified the race cultures should be used for testing resistance in varieties and segregating populations. Repeated transfers and storage of race cultures over a period of three - four months may lead to alterations in their pathogenic capabilities. Undoubtedly, more races could be identified in future by using a larger number of samples per state and by adding some other cultivars or hosts like rye, oats and barley. The present investigation indicates

TABLE 3. Reaction pattern of different races of *H. sativum* on 13 differential wheat cultivars.

Cultivar \ Race	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
BH 1146	MR	MR	S	MS	S	S	MS	S	S	MS	S	MS	MS	S	S	MS
IAC 5	MR	MR	MS	MS	S	MS	MR	MS	S	MS	MS	S	MS	MS	MR	MR
IAS 62	MS	MR	S	MS	S	MS	MS	MS	S	MS	S	S	S	S	MS	MS
HORIZON	MR	MR	S	MS	S	S	S	S	S	MR	S	S	S	S	S	MS
JUPATECO	MR	MR	MS	MS	S	MR	MR	MR	S	MR	MS	MR	MR	S	MR	MR
MR 74503	MR	MR	S	MS	S	MS	MR	MR	S	MR	MR	MS	S	R	MR	
PARAGUAY 214	MR	R	MR	MR	MR	MS	MR	MS	S	MR	S	MS	MS	MR	S	MR
PAT 7219	MR	R	MS	MS	S	S	S	S	S	MR	MS	MS		MR	MS	MR
PF 72707	R	MR	MS	MS	S	S	MR	MS	S		MS	MS	MS	S	MS	MR
PF 7673	MR	MS	S	MS	S	MS	MS	MS	S	MS	MR	MS	MS	MS	MR	MR
SEL. LONDRINA	MR	MR	MS	MS	MS	MR	MR	MS	S	MS	MS	MS	S	S	MR	MR
SONORA 64	MR	MR	MS	MS	S	MS	MR	MR	S	MR	S	MS	S	S	MR	MR
SEL. TIFTON	S	R	MR	R	R	MS	S	MR	S	R	MS	R	S	MS	S	S

17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
S	MR	S	MS	S	MS	S	S	MS	R	MS	MS	S	S	MS	MS
S	MS	S	S	MS	S	MS	S	MS	R	MS	MS	S	S	S	MS
S	MS	S	S	MS	S	MS	S	MS	R	MS	S	S	S	S	S
S	MR	S	S	S	S	MS	S	S	R	MS	S	S	MS	S	S
S	MR	MS	S	MS	S	S	S	MS	R	MR	S	MS	MS	S	MS
S	MS		MS	MS	S	S	MS		R	MS	S	S	MS	S	S
S	MR	MS	MS	MR	MS	MS	MS	MR	R	MR	S	MS	MS	MS	MS
S	MR	MS	MS	MS	MS	MS	S	MS	R	MR	S	MS	S	S	S
MS	MR	MS	MS	S	S	MS	S	MS	R	S	MS	MS	S	MS	S
MS	S	S	MS	MS	S	MS	MS	S	R	MR	MS	MS	S	S	MS
MS	MS	MS	MS	MR	MR	MS	MS	MS	R	MR	MS	MS	S	MS	S
S	MS	S	MS	MS	S	S	S	MR	R	MS	MS	MS	S	MS	MS
S	S	S		S	MS	S			R	MS	S	S	S	MS	MS

OBS: R - Resistant, MR - Moderately resistant, MS - Moderately suscetible and S - Susceptible.
For details, consult the text. Blank columns indicate that no reading was taken because of poor germination or no germination at all.

that the races of *H. sativum* could be identified and used towards the search for a broad spectrum resistance (resistance against a wide number of races). On the other hand, it is true to incorporate such a resistance in agronomically desirable cultivars is not an easy task.

One of the problems in race identification in *H. sativum* is the constant and rapid mutations of the fungus and the maintenance of the isolates in the sporulation forms. Christensen (1925), in his detailed studies, especially on mutants of *H. sativum*, reported that the mutants differed from the parents in rate of growth, color, zonation, amount of aerial mycelium and also in pathogenicity. In his later publication, Christensen (1929) studied the influence of temperature on the frequency of mutation and stated that, in general, the greatest number of mutants appeared at temperatures between 25°C. Considering this fact, all the isolates during the present studies were maintained at about 5°C on filter paper disks thinking that mutations could be avoided. Using this technique most of the isolates could be successfully maintained over a period of ten months in conidial form. Indeed, in a few cases the isolates when recultured on PDA, using a single paper disk, formed mutants and lost their ability to produce conidia and pathological capabilities. Maintenance of isolates on autoclaved sorghum seeds was not satisfactory, since a large number of isolates formed mutants and/or saltation. The best period of keeping the cultures in sporulation form on sorghum seeds in Erlenmeyer flasks was between three to four weeks.

The present work is considered as a preliminary attempt to identify the races of *H. sativum* by establishing an infection pattern scale, and a set of differential cultivars. It is well known that after some time the identified races change or even loose their pathogenic capability, as it has already happened during the present investigation. However, if the infection pattern scale and the set of standard differential cultivars are well established, then the races could be identified at any time by using fresh monoconidial isolates.

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