RELAÇÃO ENTRE SELEÇÃO *IN VITRO* E EM PLÂNTULA PARA RESISTÊNCIA À *Bipolaris sorokiniana* EM TRIGO

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RESUMO – Doze genótipos de trigo (*Triticum aestivum* L.) foram avaliados *in vivo* e *in vitro* para resistência à helmintosporiose, moléstia induzida pelo fungo *Bipolaris sorokiniana*, com o objetivo de verificar a existência de uma correlação entre a resposta apresentada por calos e por plântulas . A partir de dois isolados foram obtidos filtrados tóxicos para serem adicionados ao meio de cultura onde se desenvolveram os calos de trigo; também foram produzidas suspensões de esporos para serem pulverizados a plântulas. A reação dos calos foi quantificada através do crescimento daqueles expostos aos filtrados em comparação aos que não receberam este tratamento. A resposta das plântulas foi medida pela porcentagem de lesões necróticas presentes nas folhas. Foi verificado que um menor crescimento de calo correspondeu a uma maior porcentagem de lesões em plântulas.

Palavras-chave: Bipolaris sorokiniana, Triticum aestivum, filtrados tóxicos, cultura de tecidos.

RELATIONSHIP BETWEEN *IN VITRO* AND SEEDLING SELECTION FOR RESISTANCE TO SPOT BLOTCH (*Bipolaris sorokiniana*) IN WHEAT

ABSTRACT – Twelve wheat (*Triticum aestivum* L.) genotypes were assessed, *in vitro* and *in vivo*, for resistance to spot blotch induced by *Bipolaris sorokiniana*, with the objective of verify correlation between calli and seedling responses. Toxic filtrates were obtained from two fungal isolates and added to the culture medium where the wheat callus developed; spore suspensions were also produced and applied to wheat seedlings. The callus reaction was quantified by the difference between growth of those exposed to the filtrates and the untreated cheks. The response of the seedlings was measured by the percentage of necrotic lesions present on the leaves. A smaller callus growth corresponded to a higher percentage of lesions on the seedling leaves.

Key words: Bipolaris sorokiniana, Triticum aestivum, toxic filtrates, tissue culture.

INTRODUCTION

The spot blotch of wheat induced by *Bipolaris sorokiniana* diminishes seed germination, increases premature seedling mortality, dries the aerial photosynthetic parts, prevents the formation of cariopsis, develops lesions in the form of stains on the leaves and glumes, and rot in the roots and stalk base resulting in yield reduction (CHRISTENSEN, 1925). The fungus is found in the plant tissues, and in the soil (MERONUK and PEPPER, 1968). The pathogen multiplies mainly in the residues of the host species and on the wild and cultivated grasses, carrying inoculum from one year to another (DIEHL, 1982; REIS, 1982). Reaction to spot blotch fungus depends on the pathogen specialization (MEHTA, 1981,b), moisture during incubation (LUZ, 1982), and temperature (LUZ and BERGSTROM, 1986). *B. sorokiniana* produces toxins which are essential for spot blotch development in rye, wheat and oat seedlings (LUDWING, 1957). These toxins act as an inhibitor of the electron transfer process in the oxidative phosphorylation in the mitochondria, affecting cellular respiration (TANIGUCHI and WHITE, 1967).

The use of tissue culture allows easier analysis of the host-pathogen interaction that may help breeders to select disease resistant cultivars. A great advantage of this technique is the control of the variable effects of the environment which is reduced in field studies caused by the variation between locations and yearly climatic fluctuations. Furthermore, *in vitro* cultures allow the use of a great number of genotypes in a relatively small space. An *in vitro* selection combining tissue culture

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with the use of toxins or pathogen toxin filtrates may be successful if the disease resistance is expressed at a cellular level (DAUB, 1986). Thus, the resistance mechanisms should not depend on organized structures or organized tissues, such as flowers or cuticle (MEREDITH, 1984). The manifestation of resistance or susceptibility should be easily identifiable among the individuals exposed to selection. Furthermore, the existence of a correlation between resistance to a pathogen and resistance to its toxin is a fundamental requirement for the use of phytotoxins in *in vitro* cultures (BEHNKE, 1980).

The use of *B. sorokiniana* toxic filtrates applied to young wheat and oat calli has been shown as an efficient approach to identify resistant genotypes, by avaliation of callus growth (CRISTALDO, 1993; BARBIERI, 1995; HANDEL, 1996). This study was developed to test the correlation between the *in vitro* and *in vivo* responses of 12 wheat cultivars to *B. sorokiniana*.

MATERIAL AND METHODS

Twelve wheat genotypes, selected for their known resistance or susceptibility to the spot blotch pathogen, were assessed in field trials during several years. The fungal isolates were obtained from wheat grains from the cultivars IAC 5 -Maringá and Trigo BR 35 infected with *B. sorokiniana* and showing the most characteristic symptom of the disease: a black point.

In vitro selection

The toxin filtrates were obtained from the colonies of the purified isolates using the methodology described by ALAM (1989) (Figure 1).

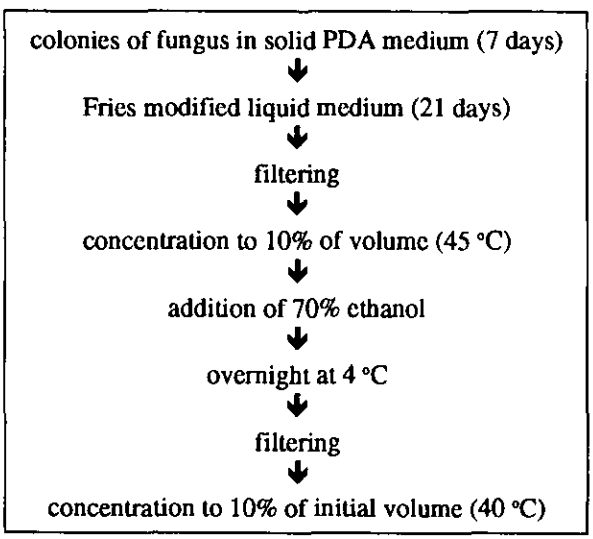

FIGURE 1 – Scheme for obtaining *B. sativum* toxin filtrates from the isolates IAC 5 - Maringá and Trigo BR 35

Immature wheat embryos were used as controls. After being removed, they were placed on Petri dishes containing the callus induction medium MS (MURASHIGE and SKOOG, 1962) with 2.0 mg/l of 2.4-D (dichlorophenolacetic acid), 3% saccharose and 0.8% carrageen. The calli were kept in this medium for four weeks at a temperature of 25 °C ± 1 °C. After this period, they were cut in pieces of approximately 1.0 mm and transferred to the MS maintenance and callus growth medium with 0.5 mg/l of 2.4-D, 3% saccharose and 0.8% carrageen, containing toxin filtrates of the fungus in the ratios of 1:8 or 1:16. The plates were placed under the light and temperature conditions mentioned above. The calli were measured twice: when placed in the growth and maintenance medium and after four weeks. The difference between these two measurements was considered as the measurement of callus growth.

Seedling selection

The fungal isolates Trigo BR 35 and IAC 5-Maringá were placed in Petri dishes containing PDA medium covered with a damp filter paper. The plates were kept at 24 °C ± 2 °C in the dark, for three weeks.

The conidial suspension was prepared by adding 1 ml of sterilized distilled water to the plates containing the spores and agitating until the spores were detached from the paper surface. This concentrated conidial suspension was diluted to obtain the concentration used in inoculation studies. Dilutions were carried out in an Erlenmeyer flask with 400 ml of sterilized distilled water. The suspension was added until reaching a concentration 1 x 10^5 conidia per ml of solution. A drop of the emulsifying agent Tween 20 for each 200 ml of suspension was added. The standardization of the inoculum concentration was done by counting the conidia in a Neubauer chamber (MATSUMURA, 1991).

This experiment was conducted in a growth chamber. The 12 wheat genotypes were sown, after disinfestation, in plastic vases containing sterilized vermiculite. Germinated seeds were thinned to four seedlings per vase and were watered periodically with the nutritive solution (4.2 g of $MgSO_4 7H_2O$; 1.4 g of K_2HPO_4; 5.8 g of KNO_3 and $Ca(NO_3)2.4H_2O$, diluted in 10 liters of water) and kept in the growth cabinet at a temperature of 20 ± 1° C, with a 12 hour photoperiod, until reaching the stage of three expanded leaves. At this stage, the vases were put in a damp chamber (metal cabinet completely closed with plastic) and inoculated with the spore suspension from Trigo BR 35 and IAC 5-Maringá isolates, while the controls were treated with sterilized distilled water (LINDEN, 1989; MATSUMURA, 1991).

A continuous 10 pounds pressure jet spray obtained with a compressor motor was used to ensure uniform inoculation. The plants were sprayed until their leaves were completely covered with the suspension. After

inoculation, half of the plants were kept in these chambers for 18 hours and the other half for 36 hours under relative humidity near to saturation point (± 100%) at a temperature of 24 ± 2° C. The incubated plants were then transferred to the growth chamber at the same temperature and ± 70% relative humidity, for six days, when the infection indexes were scored (MATSUMURA, 1991).

Assessment of the disease intensity was carried out by considering the percentage of necrotic lesion on the second leaf of each seedling (JAMES, 1971).

Experimental Design

The experiment with the callus and the toxin filtrates was carried out in an 3 x 12 x 3 factorial design; three toxins (Trigo BR 35 toxin, IAC 5-Maringá toxin and control), 12 genotypes and three replications. Each replication was made up of a plate with 10 calli. The analysis was done considering the mean of each replication.

The test with seedlings and fungal spore suspension was in a 2 x 2 x 12 x 3 factorial design: two isolates (Trigo BR 35 and IAC 5-Maringá), two incubation periods (18 and 36 hours of nearly 100% relative humidity) and 12 genotypes in three replications. Each replication was made up of three vases with four seedlings. The evaluations (% of infected leaf area) were made only on the second leaf of each plant. The mean of each replication was used in the analysis without transformation of the data.

RESULTS AND DISCUSSION

The results were obtained from the analysis of two experiments; one using *B. sorokiniana* toxin filtrates in tissue culture, and the other carried out in the growth chamber with seedlings and spore suspension from the same isolates.

The first provided data on the genetic variability among the wheat genotypes using fungal toxin filtrates, while the second allowed an seedling assessment of trait resistance or susceptibility to the spot blotch. Correlations between the responses at *in vitro* callus and seedling reaction were established.

Genotype response to the toxin action

The genotypes were distinct in their callus production potential and had different response in the presence of toxins from different origins (such as IAC 5-Maringá and Trigo BR 35). The genotypes sensitive to the presence of toxins showed different reactions to the Trigo BR 35 and IAC 5-Maringá toxins, while the resistant genotypes had a similar response to both toxins.

The growth measurement of the calli from 12 genotypes treated with the IAC 5-Maringá and Trigo BR 35 toxins at 1:16 dilution were significantly different among each other and in relation to the control treatment. The toxins delayed the callus growth, where the genotypes BH 1146, Mitacoré and IA 815 were the most affected, producing callus growth 50% inferior in size, when compared with the callus of the same genotypes without toxin. CEP 76146, CNT 1, line 290 and line 291 developed callus growth varying between 60 and 80%, while IA 7968, LD 7813 and line 293 had callus growth greater than 90% compared to the treatment without toxins. On the other hand, the callus development of line 289 and line 294 were similar to those observed on the callus without toxins (Table 1). The genotypes were classified in four distinct groups. Genotypes 289 and 294 were considered resistant; IA 7968, LD 7831 and 293 as moderately resistant; genotypes CEP 76146, CNT 1, 290 and 291 as moderately susceptible and BH 1146, IA 815 and Mitacoré as susceptible (Table 1).

TABLE 1 – Response of 12 wheat genotypes to 1:16 dilution toxins from *Bipolaris sorokiniana* isolated from IAC 5-Maringá and Trigo BR 35

Genotype	Callus growth					
	control (mm)	with IAC 5 toxin (mm)	%** of control	with BR 35 toxin (mm)	%** of control	host class***
BH1146	1.67 g*	0.69 g	42	0.36 g	22	S
Mitacoré	2.44 ef	1.13 f	47	0.77 gf	31	S
IA815	2.33 f	1.20 f	51	0.95 f	41	S
291	3.42 bc	2.50 cd	73	2.44 bc	71	MS
290	2.85 de	2.10 e	74	1.89 e	67	MS
CEP76146	1.24 g	1.02 f	82	0.93 f	75	MS
CNT1	3.10 cd	2.54 cd	82	1.99 de	64	MS
293	2.89 de	2.63 c	91	2.40 bcd	83	MR
LD7831	2.51 ef	2.27 de	91	2.27 cde	90	MR
IA7968	3.02 cd	2.76 c	92	2.74 b	91	MR
289	3.63 ab	3.66 b	100	3.65 a	100	R
294	4.02 a	4.07 a	100	3.91 a	97	R

C.V.(%) = 5.8

* measurements followed by the same letter did not differ significantly at 5% by Tukey's test

** growth percentage in relation to the treatment without toxin

*** phenotypic classification of the host according to scale established for percentage callus growth relative to the control without toxin: R (resistant) > 95%, MR (moderately resistant) = 80% to 94%, MS (moderately susceptible) = 60% to 79%, and S (susceptible) < 60%

No interaction among the tested genotypes and the toxins was observed. Trigo BR 35 toxin, however, caused a more intense reaction than the IAC 5-Maringá toxin showing differences in aggressiveness among the isolates. The difference in aggressiveness of these isolates was clearly visible among moderately susceptible and susceptible genotypes, especially in CNT 1 and BH 1146, than among the resistant or moderately resistant genotypes. Furthermore, the resistant and moderately resistant genotypes did not have differentiated response to the toxins of the same isolates.

Callus growth treated with the 1:8 dilution was lower than that observed in the 1:16 dilution. All genotypes, those with a resistance, susceptible or intermediate reaction, drastically reduced the growth of their callus obtained in the 1:16 dilution. The only exception was CNT 1 where callus size remained unchanged in both dilutions. Besides smaller calli, the genotypes developed calli with a lot of necrosis. The toxin dilution of 1:8 did not allow genotype classification among host reaction (resistance and/or susceptibility) groups. Furthermore, genotypes IA 7968 and LD 7813, considered moderately resistant at 1:16 dilution had callus growth close to genotypes considered susceptible, such as IA 815 and Mitacoré, especially in the presence of the Trigo BR 35 toxin. Genotypes 289, 293, 290, CEP 76146 and BH 1146, with reactions ranging from resistant to moderately susceptible with 1:16 dilution behaved the same at 1:8 dilution. However, CNT 1 had similar reaction for both 1:8 and 1:16 dilutions and along with genotype 294 were considered the most resistant genotypes in the experiment (Table 2).

Significant differences were detected in the means of the two dilutions. There were also distinct effects among the toxin within each dilution, with Trigo BR 35 toxin having a more drastic effect than IAC 5-Maringá toxin (Table 2). As in the case of 1:16 dilution, no interaction was observed among the toxins and the genotypes of 1:8 dilution level. However, the toxins showed their differences in aggressiveness, with the toxin from Trigo BR 35 isolate being more aggressive than that extracted from the IAC 5-Maringá isolate. Lack of genotype x isolate interaction is indicative of absence fo fungal specialization among isolates studied.

TABLE 2 – Percentage of callus growth exposed to two dilutions (1;8 and 1:16) of two toxin filtrates (IAC 5 Maringá and Trigo BR 35) relative to the growth of toxin free control

Genotypes	1:16 dilution		1:8 dilution	
	IAC5-Maringá isolate	BR 35 isolate	IAC5-Maringá isolate	BR 35 isolate
BH1146	42	22	53	23
Mitacoré	47	31	11	6
IA815	51	41	9	9
291	73	71	-	-
290	74	67	23	21
CEP67146	82	75	41	40
CNT1	82	64	63	46
293	91	83	47	32
LD7831	91	90	26	11
IA7869	92	91	16	13
289	100	100	30	21
294	100	97	35	31

Evaluation of seedling response

The seedling reaction caused by inoculation with the conidial suspension of the IAC 5-Maringá and Trigo BR 35 isolates demonstrated significant differences among genotypes at both levels of incubation (18 and 36 hours) (Table 3).

Seedlings incubated for 18 hours, under high relative humidity conditions, allowed classification of genotypes in two categories: resistant, such as 294 or susceptible, such as IA 815 and Mitacoré (Table 3). On the other hand, 36 hours of incubation period allowed identification of more than two classes. The isolates IAC 5-Maringá and Trigo BR 35 allowed the ranking of the tested genotypes in the categories: resistant (294, 293 and CNT 1); moderately resistant (289, 290 IA 7968 and CEP 76146); moderately susceptible (BH 1146 and LD 7831), and susceptible (IA 815 and Mitacoré). The differences in reaction were more intense in the presence of the Trigo BR 35 isolate.

TABLE 3 – Percentage of seedling infection on 12 wheat genotypes after 18 and 36 hours incubation period, inoculated with two *B. sorokiniana* isolates

Genotypes	IAC 5- Maringá		*BR 35*	
	Isolate	suspension	isolate	suspension
	18 horas*	36 horas*	18 horas*	36 horas*
BH1146	1.2 bc**	16.6 bcd	2.9 b	20.0 c
CEP76146	0.6bc 7.5	de 3.1	b 13.3	cd
CNT1	0.4 bc	5.7 de	0.2 b	10.8 de
IA7968	1.1 bc	8.3 bc	0.2 b	17.1 c
IA815	15.0 a	26.7 b	23.3 a	36.6 b
LD7831	2.7 bc	17.1 bcd	3.2 b	18.3 c
Mitacoré	17.7 a	40.0 a	26.2 a	50.4 a
289	3.5 bc	15.0 cd	3.2 b	15.0 c
290	5.4 b	15.0 cd	5.5 b	13.0 cde
293	0.6 bc	1.7 e	0.3 b	1.5 e
294	0.2 c	1.5 e	0.2 b	2.3 de
means	b*** 4.4	b 14.1	a 6.2	a 18.00

C.V.(%) = 23.6
* percentage of seedling infection on the second leaf
** means followed by the same letter did not differ significantly at 5% by Tukey's test
*** measurements preceeded by the same letter did not differ significantly at 5% by Tukey's test

Relationship between *in vitro* and seedling selection

A correlation analysis to check the existence of a possible relationship among the genotypes selected by the *in vitro* toxins and those classified by isolate conidial suspension was carried out (Table 4).

Significant and negative correlation coefficients (0.47 to -0.68) were observed between the toxin dilution and the incubation period under high relative humidity. Thus, a bigger callus growth was related to a lower seedling infection. The highest correlation (-0.68) was observed for the 1:8 dilution and 36 hours of incubation period. On the other hand, the correlation coefficient detected for the 1:16 dilution and 36 hours of incubation (-0.51) was similar to the coefficient of the 1:8 dilution and 18 hours of incubation (-0.52) (Table 4).

TABLE 4 – Correlation matrix between callus in 1:8 and 1:16 dilutions of *B. sorokiniana* toxins and the percentage of seedling infection assessed after 18 hours and 36 hours of incubation period

	1:16 dilution	1:8 dilution	18 hours	36 hours
1:16 dilution	1.00			
1:8 dilution	0.87*	1.00		
18 hours	-0.47*	-0.52*	1.00	
36 hours	-0.51*	-0.68*	0.85	1.00

P(0.05)

The experiments carried out *in vitro* and *in vivo* showed a small but significant and negative correlation, based on callus growth in toxin filtrate and seedling infection measured in percentage of lesions. Other parameters, such as number and size of lesions, and chlorosis were not considered. The latter is an important factor to determine the degree of susceptibility (MEHTA, 1981,a). Thus, genotypes with few and small lesions, without chlorosis, would be considered resistant, while the genotypes with the same number of lesion but with pronounced chlorosis would belong to a distinct class. However, the significance of the correlations indicated that there were correspondence between the *in vitro* response of the callus growth and the seedling reaction to infection.

CONCLUSIONS

In vitro selection of wheat callus for resistance to spot blotch, using fungal toxin filtrates, was shown to be a relatively simple and highly feasible technique. Considering the elimination of the pathogen in the

filtrate under controlled environmental conditions, the assessment criteria of the callus exposed to a 1:16 dilution of the toxin filtrates allowed an accurate screening of the genotypes according to their seedling reaction to the pathogen.

A smaller callus growth corresponded to a higher percentage of lesions on the seedling leaves, determining a negative correlation among *in vitro* and *in vivo* selection. The existence of this correlation allows, therefore, the use of phytotoxins of *B. sorokiniana* as possible selection procedure in wheat callus tissue to determinate its resistance to the disease.

REFERENCES

ALAM, B.K. **Genetic regulation of host-selective toxin production;** characterization of necrosis minus mutant of *Pyrenophora tritici-repentis* (DIED.) Drech. which has lost the ability to produce host-selective toxins in wheat. Columbia: University of Missouri, 1989. 128 p. Thesis (Ph.D), University of Missouri, 1989.

BARBIERI, R.L. **Genética da resistência ao *Helminthosporium sativum* em trigo:** uso de filtrados tóxicos em cultura de tecidos. Porto Alegre:UFRGS, 1995. 47 p. Dissertação (Mestrado) – Genética e Biologia Molecular, Departamento de Genética, Instituto de Biociências, UFRGS, 1995.

BEHNKE, M. General resistance to late blight of *Solanum tuberosum* plants regenerated from callus resistant to culture filtrates of *Pyrenophora infestans*. **Theoretical and Applied Genetics**, Berlin, v. 56, p. 151-152, 1980.

CHRISTENSEN, J. J. Physiologic specialization and mutation *in Helminthosporium sativum*. **Phytopathology**, St. Paul, v. 15, p. 785-795, 1925.

CRISTALDO, R.M.L.O. **Uso de filtrados tóxicos para avaliar a resistência ao fungo Helminthosporium sativum em trigos hexaplóides in vitro**. Porto Alegre: UFRGS, 1993. 129 p. Tese (Doutorado) – Fitotecnia, Departamento de Plantas de Lavoura, Faculdade de Agronomia, UFRGS, 1993.

DAUB, M. Tissue culture and the selection of resistance to pathogens. **Annual Review of Phytopathology**, Stanford, v. 23, p. 159-181, 1986.

DIEHL, J. Podridão comum. In: TRIGO no Brasil. Campinas: Fundação Cargill, v.2, cap. 12, p. 501-508,1982.

HANDEL, C.L. **Avaliação *in vitro* da resistência à helminthosporiose em aveia através do uso de filtrados tóxicos do fungo e do inseticida Methomyl**. Porto Alegre: UFRGS, 1996. 69 p. Dissertação (Mestrado) – Fitotecnia, Departamento de Plantas de Lavoura, Faculdade de Agronomia, UFRGS, 1996.

JAMES, W.C. An illustrated series of assessment keys for plant diseases their preparation and usage. **Canadian Plant Disease Survey,** Ottawa, v. 51, n. 2, p. 20-73, 1971.

LINDEN, A.R. **Alterações na patogenicidade, morfologia e atividade isoesterásica de *Helmintosporium sativum* decorrentes de passagens sucessivas por duas cultivares de trigo.** Porto Alegre: UFRGS, Faculdade de Genética, UFRGS, 1989. 116 p. Dissertação (Bacharelado) – Genética, Departamento de Genética, Instituto de Biociências, UFRGS, 1989.

LUDWING, R. A. Toxin production by *Helminthosporium sativum* P.K. & B. and its significance in disease development. **Canadian Journal of Botany**, Otawa, v. 35, p. 291-303, 1957.

LUZ, W.C. Influência do período de umidificação pós-inoculação na reação de cultivares de trigo a mancha foliar (*Cochliobolus sativus*). **Fitopatologia Brasileira**, Brasília, v. 7, p. 111-115, 1982.

LUZ, W.C.; BERGSTROM, G.C. Temperature-sensitive development of spot blotch in spring wheat cultivars differing in resistance. **Fitopatologia Brasileira**, Brasília, v. 11, p. 197-204, 1986.

MATSUMURA, A.T. **Variabilidade intraespecífica quanto a patogenicidade, características de cultura e padrão isoesterásico em populações naturais de *Bipolaris sorokiniana* (*Helminthosporium sativum*).** Porto Alegre: UFRGS, 1991. 262 p. Tese (Doutorado) – Genética, Departamento de Genética, Instituto de Biociências, UFRGS, 1991.

MEHTA, Y. R. Produção de conídios, período de esporulação e extensão da lesão por *Helminthosporium sativum* nas folhas bandeiras de trigo. **Pesquisa Agropecuária Brasileira**, Brasília, v. 16, n. 1, p. 77-99, 1981,a.

MEHTA, Y. R. Identification of races of *Helminthosporium sativum* of wheat in Brazil. **Pesquisa Agropecuária Brasileira**, Brasília, v. 16, n. 3, p. 331-336, 1981,b.

MEREDITH, C.P. Selecting better crops from cultured cells. IN: STADLER GENETICS SYMPOSIUM 16., 1986, Columbia. **Gene manipulation in plant improvement.** New York: Plenum Press, 1984. p. 149-172. 1984.

MERONUCK, R.A.; PEPPER, E.H. Clamidospore formation in conidia of *Helminthosporium sativum* **Phytopathology**, St. Paul, v. 58, p. 866. 1968.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth biossays with tobacco tissue cultures. **Physiologia Plantarum**, Copenhagen, v. 15, p. 473-497, 1962.

REIS, E. M. Levantamento de plantas cultivadas, nativas e invasoras hospedeiras de fungos causadores de podridões radiculares em cereais de inverno e em outras culturas. **Summa Phytopathologica**, Jaguariúna, v. 8, p. 134-140, 1982.

TANIGUCHI, E.; WHITE, G.A. Site of action of the phytotoxin helminthosporal. **Biochemistry and Biophysical Research Communications,** New York, v. 28, p. 879-885, 1967.