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REACTION OF SOME GRAIN SORGHUM CULTIVARS TO DOWNY MILDEW DISEASE INFECTION CAUSED BY *Peronosclerospora sorghi* .

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ABSTRACT

This work was carried out in disease nursery at Gemmeiza Agricultural Research Station, El-Gharbeia Governorate, Egypt in 2009 and 2010 growing seasons. Five grain sorghum cultivars i.e. Isis, Horas, Zosar, Giza 15 and Giza 113 were evaluated for downy mildew disease resistance caused by *Peronosclerospora sorghi*. Effect of downy mildew disease on grain yield and leaves chemical components was also studied. The tested grain sorghum cultivars were varied significantly in their response to downy mildew disease. The cultivars Isis and Zosar were showed as highly resistant. Horas cv. was classified as moderately susceptible. While, Giza 15 and Giza 113 were classified as susceptible. The highly resistant cultivars Isis and Zosar showed the highest grain yield, in addition the leaves of these varieties contained the highest level of total nitrogen, crude protein , phosphorus and potassium compared with the susceptible cultivars. The enzymatic activity of peroxidase and polyphenoloxidase and also, phenolic compounds contents in the grain sorghum cultivars leaves were the highest in case of the highly resistant cultivars. The leaves of susceptible cultivars showed the lowest contents of enzymatic activity and phenolic compounds. Results showed a positive correlation between the resistance degree and grain yield and / or chemical components.

Keywords: Grain sorghum, downy mildew, *Peronosclerospora sorghi*, peroxidase, polyphenoloxidase, phenolic compounds

INTRODUCTION

Grain sorghum (*Sorghum bicolor* L. Moench), is one of the most important cereal and forage crops in Egypt and all over the world . It is the fourth most important cereal crops after wheat, rice and maize . More than 35% of grain sorghum is grown directly for human consumption . The rest is used primarily for animal feed, alcohol production, and ect Awika and Rooney (2004)) . Sorghum downy mildew (SDM) caused by *peronoscelrospora sorghi* (Weston and Uppal S.G. Shaw) is a destructive disease of sorghum, grain sorghum and maize . Soil-borne oospores are the major source of inoculum, and yield loss is directly related to oospores initiated infection (Frederiksen (1980)) . Genetic resistance is the most efficient and environmentally sound way to control the SDM disease (Kamala *et al.,* 2002 and Barbosa *et al.,*2005) . SDM controlled by using the resistance commercial hybrids, lines, genotypes and cultivars (*Yeh* and Frederiksen, 1980; EL-Moghazy, 2003; and *Barbosa et al*. 2005 . EL-Sherbeni *et al.* (2008) showed that Zosar and shandaweel-1 grain sorghum cvs. were highly resistant to downy mildew disease and gave the highest yield, while Giza 15 was being highly susceptible and gave the lowest green yield .

The use of resistant genotypes is the most efficient method to control downy mildew disease caused by *P. sorghi* of sorghum and maize (**Barbosa** *et al.,*2006) . The increase in Phenolic compounds and oxidative enzymes activity gave an increase in the capacity of plant defense against disease infection process and disease development . In the resistant grain sorghum cultivars to downy mildew disease, Phenolic compounds and/ or oxidative enzymes were higher than in the susceptible one (EL-Sherbeni *et al.,* 2008) . The main objective of this work was to evaluate grain sorghum cultivars for resistance to *P. sorghi* in the infection field during 2009 and 2010 growing seasons .

MATERIALS AND METHODS

The Present study was carried out at Gemmeiza Agricultural Research Station, A.R.C. during 2009 and 2010 growing seasons .

Experimental design:

This experiment was performed in the disease nursery field prepared for evaluation against downy mildew disease, in a completely randomized block design with three replications . Cultivars i.e. Isis, Horas, Zosar, Giza 15 and Giza 113 were planted in a double row plots, separated by two rows of the highly susceptible variety of sudan grass (Sordon 79), Planted 21 days before, to act as spreader rows .

Disease assessment:

Evaluations of disease incidence were performed three times one, two and three monthes after planting during the seasons by counting the number of plants which developed infection .

Grain sorghum cultivars were placed in one of four categories: highly resistant, moderately resistant, moderately susceptible and susceptible . All cultivars with less than 6% downy mildew incidence were classified as highly resistant, those in the 6-10% range were considered as moderately resistant, cultivars with 11-20% were classified as moderately susceptible, and those with more than 20% incidence were classified as susceptible (Barbosa *et al.,* 2005) . The infection percentage of downy mildew disease was recorded as incidence of the total plant (D.I) using the following equation:

$$\text{Disease incidence (D.I)\%} = \frac{\text{No. of infected plants}}{\text{No. of total plants}} \times 100$$

Chemical analysis:

Leaves of each cultivar were collected from plants at 55 days after sowing to determine chlorophyll contents, total nitrogen, crude protein, phosphorus, potassium, phenolic compounds content and oxidative enzymes assay the activity of peroxidase and polyphenoloxidase .

a) chlorophyll contents:

Chlorophyll a, b and a+b were determined according to Moran and porath (1980) using N, N-dimethyl formamide .

b) Nitrogen, protein, phosphorus and potassium:

Leaves from each cultivar were dried to costant weight . The dried leaves were grounded to fine powder . Amount of 0.2 gm of the fine powder was digested using sulphuric acid and perchloric acid (5:1 V/V, respectively) then the solution was used to determine nitrogen, phosphorus and potassium content as follows:

i) Total nitrogen and crude protein were determined using Kjeldahl method **according to Chalmers (1984).The total protein of the samples was** calculated as crude protein by multiplying the nitrogen content by 5.75 .

ii) Phosphorus and potassium contents was determined using ammonium molybdate and a flme photometer, respectively according to Chapman and Pratt (1961) .

c) Phenolic compounds:

Total and free phenols were determined in leaves of grain sorghum plants, using the colorimetric method of folin at 520 nm, as described by Snell and Snell (1953) Conjugated phenols were determined by substracting free phenols from the total phenols . The results are expressed as milligram per gram fresh weight of plants (mg/g fresh weight) .

d) Oxidative enzymes activity:

Peroxidase and polyphenoloxidase activity were determined in the extraction grain sorghum leaves, as described by Maxwell and Bateman (1967) .

i) Peroxidase activity was spectrophotometrically by measuring the oxidation of pyrogallol in the presence of H_2O_2 at wave length of 425 nm, according to Allam and Hollis (1972) .

ii) Polyphenoloxidase activity was determined using spectrophotometric procedure at 495 nm, as described by Matta and Dimond (1963).

Statistical analysis:

Field experiment excuted throughout the present investigations was designed according to Randomized complete block method . Least significant differences (L.S.D) and Duncan's multiple range tests were applied for comparing means (Duncan, 1955) .

RESULTS AND DISCUSSION

A field trial was carried out to screen the available grain sorghum varieties for their susceptibility to infection with downy mildew disease caused by *P. sorghi.* This experiment was done at Gemmeiza Agricultural Research Station in 2009 and 2010 growing seasons. Results (Table 1) show that, the two cultivars i.e. Isis and Zoser were shown to be highly resistant. The first cultivar Isis showed 0% infection . Zosar showed less incidence (1.76%). Horas cv. was classified as moderately susceptible (18.26%). While Giza 15 and Giza 113 were classified as susceptible. But Giza 15 cv. exhibited the highest disease incidence (60.22%).

Data also revealed that, the highly resistant cultivars gave the highest grain yield per plot compared with the other cultivars . The same trend was observed in both seasons of evaluation as shown in Table (1) Combimed data over seasons show that, the highly resistant cvs. Isis and Zosar showed the highest grain yield (6.17 and 5.84 kg/plot, respectively). While Giza 15 cv. gave the lowest yield (1.87 kg/plot). Data also showed that, the highest disease incidence correlated with the lowest grain yield.

Other investingators obtained similar results about the relationship between the genes for resistance to *P. sorghi* and differential Sorghum lines (Barbosa *et al*.,2005 and2006) .EL- sherbemi *et al* .,(2008) found that, Zosar and Shandaweed 1- grain sorghum cultivars were highly resistant, while Giza 15 cultivar was highly susceptible to downy mildew disease caused by *P. sorghi* . Also, showed that Zosar cv. gave the highest yield per feddan followed by Dorado. More researches were carried out to study the relationship between downy mildew incidence (*P. sorghi*) and grain yield. Graig *et al* ., 1989 ; Bock and Jeger , 1996 and EL- sherbeni *et al.,* 2008 reported that, the relationship between the incidence of downy mildew disease *(P. sorghi)* and grain yield was negatively correlated **.**

Table (1): Response of five grain sorghum cultivars for downy mildew infection and grain yield in 2009 and 2010 growing seasons .

Cultivars	Disease incidence %			·Scale	Grain yield/ plot "kg"		
	2009	2010	Mean		2009	2010	Mean
Isis	0.00[d]	0.00[d]	0.00[e]	H.R.	6.24[a]	6.09[a]	6.17[a]
Horas	17.97[c]	18.54[c]	18.26[c]	M.S.	3.72[b]	3.46[b]	3.59[b]
Zosar	1.69[d]	1.83[d]	1.76[d]	H.R.	5.82[a]	5.85[a]	5.84[a]
Giza 15	58.43[d]	62.00[a]	60.22[a]	S.	1.99[c]	1.74[d]	1.87[d]
Giza 113	35.80[b]	37.50[b]	36.65[b]	S.	2.70[c]	2.52[c]	2.61[c]
L.S.D at 0.05	3.86	3.27	1.65		0.91	0.56	0.46

Means followed by the same letter in a column do not differ significantly according Duncan's multiple range test at 5% level

- **where**

H.R.: Highly resistant **M.S.: Moderately susceptible**
S.: Susceptible

Data in Table (2) show that, downy mildew infection decreased chlorophyll contents in grain sorghum leaves. Chlorophyll a+b and a were the highest in the highly resistant cultivar Isis 6.64 and 5.27 mg/dm^2 followed by Zosar cv. 6.62 and 5.09 mg/dm^2 respectively, In the case of chlorophyll b, Zosar cultivar leaves contained the highest value (1.53 mg/dm^2), followed by Isis (1.37 mg/dm^2). Leaves of Giza 15 and Giza 113 cultivars, contained the lowest values of chlorophyll a, b and a+b. Data showed that chlorophyll a, b and a+b were higher in the highly resistant cultivars than the susceptible ones. The same trend was observed in both seasons Table(2).

Table (2): Effect of downy mildew disease (*P. sorghi*) on the concentrations of chlorophyll in leaves of some grain sorghum cultivars during 2009 and 2010 growing seasons.

Cultivars	Chlorophyll (a+b) mg/ dm^2			Chlorophyll (a) mg/ dm^2			Chlorophyll (b) mg/ dm^2		
	2009	2010	Mean	2009	2010	Mean	2009	2010	Mean
Isis	6.78[a]	6.49[a]	6.64[a]	5.37[a]	5.17[a]	5.27[a]	1.41[a]	1.32[a]	1.37[a]
Horas	3.53[b]	3.41[b]	3.47[b]	2.81[b]	2.77[b]	2.79[b]	0.72[b]	0.64[b]	0.68[b]
Zosar	6.77[a]	6.46[a]	6.62[a]	5. 18[a]	5.00[a]	5.09[a]	1.59[a]	1.46[a]	1.53[a]
Giza 15	2.38[c]	2.04[c]	2.21[c]	1.86[c]	1.64[c]	1.75[c]	0.52[b]	0.40[c]	0.46[b]
Giza 113	3.17[b]	3.27[b]	3.22[b]	2.51[bc]	2.65[b]	2.58[b]	0.66[b]	0.62[b]	0.64[b]
L.s.D at 0.05	0.76	0.78	0.62	0.75	0. 56	0.65	0.34	0.23	0.24

Means followed by the same letter in a column do not differ significantly according Duncan's multiple range test at 5% level.

Similar results were obtained by Kumhar *et al*., 1990 and Yadav *et al.,* 1998. Sadoma, 2003 and EL-Sherbeni *et al*., 2008, they reported that *P. sorghi* infection decreased the content of chlorophyll . Decline in chlorophyll content due to chloroplast structural modification by the fungus such as dilation of the whole chloroplast, separation of grana and accumulation of starch granules which have a direct bearing on the photo synthetic capacity of the chloroplast (Raghavendra *et al.,* 2007) .

Data given in Table (3) revealed that total nitrogen, crude protein, phosphorus and potassium were increased significantly in the highly resistant Isis cv. (3.85, 22.11, 0.74 and 3.76,% respectively) followed by Zosar cultivar. While, the susceptible cultivar Giza 15 contained the least percentages of total nitrogen (2.15%), crude protein (12.36%) and phosphorus (0.43%). In the case of potassium percentage, Horas cultivar leaves contained least amount (1.46%), compared with other cultivars. The result in 2010 growing seasons were in the same trend of 2009. These results were in the same direction those obtained by EL-Kafrawy *et al.,* 2001 and EL-Sherben: *et al.,* 2008 . Luthra *et al*., (1988)they found that, downy mildew infected leaves of Lucerne varieties resistant to *peronospora trifoliorum* had a higher amounts of nitrogen and phosphorus than the susceptible varieties. Boyer (1995) mentioned that, reducing protein synthetic activity could decrease the synthesis of metabolites and enzymes responsible for disease resistance.

Table (3): Effects of downy mildew disease *(P. sorghi)* infection on total nitrogen, crude protein, phosphorus and potassium contents in leaves of grain sorghum cultivars during 2009 and 2010 growing seasons .

Cultivars	Total nitrogen %			Crude protein %			Phosphorus %			Potassium %		
	2009	2010	Mean	2009	2010	Mean	2009	2010	Mean	2009	2010	Mean
Isis	3.82[a]	3.87[a]	3.85[a]	21.97[a]	22.25[a]	22.11[a]	0.75[a]	0.73[a]	0.74[a]	4.01[a]	3.50[a]	3.76[a]
Horas	2.78[b]	2.73[c]	2.76[c]	15.99[b]	15.70[c]	15.83[c]	0.49[b]	0.51[b]	0.50[b]	1.47[c]	1.44[c]	1.46[c]
Zosar	3.57[a]	3.56[b]	3.57[b]	20.53[a]	20.47[b]	20.50[b]	0.69[a]	0.67[a]	0.68[a]	3.57[a]	3.43[a]	3.50[a]
Giza 15	2.19[c]	2.11[d]	2.15[e]	12.59[c]	12.13[d]	12.36[e]	0.45[b]	0.40[c]	0.43[b]	2.24[b]	2.11[b]	2.18[b]
Giza 113	2.55[b]	2.47[c]	2.51[d]	14.66[b]	14.20[c]	14.46[d]	0.51[b]	0.47[bc]	0.49[b]	2.28[b]	2.44[b]	2.36[b]
L.s.D at 0.05	0.36	0.27	0.21	2.07	1.55	1.21	0.15	0.08	0.10	0.60	0.73	0.64

Means followed by the same letter in a column do not differ significantly according Duncan's multiple range test at 5% level.

Data obtained in Table (4) show the effect of *P. sorghi* on phenolic compounds (total, free and conjugated phenols) in grain sorghum cultivars leaves . Results showed that, total, free and conjugated phenols contents were the highest in variety Isis (8.24, 4.97 and 3.23mg/g fresh weight, respectively) followed by, Zosar cultivar. While, Giza 15 and Giza 113 cultivars contained the lowst amount values of total, free and conjugated phenols. Combined data over two seasons show that downy mildew disease was significantly decreased by increasing phenolic compounds contents in grain sorghum leaves. This differences in total phenol content between the grain sorghum cultivars tested might be due to different resistant degree of these cultivars . These results are in agreement with these found by Shetty and Ahmed, 1980; Basarkar *et al.,* 1992; EL-Moghazy, 2003 and Ravischankar *et al.,* 2007 . They reported that amounts of phenolic compounds were higher in the resistant maize and/ or sorghum than in susceptible one.

Table (4): Phenolic compounds in leaves of some grain sorghum cultivars under infection with downy mildew disease in 2009 and 2010 growing seasons .

Cultivars	Total phenols mg/g fresh weight			Free phenols mg/g fresh weight			Conjugated phenols mg/g fresh weight		
	2009	2010	Mean	2009	2010	Mean	2009	2010	Mean
Isis	8.23[a]	8.25[a]	8.24[a]	4.87[a]	5.06[a]	4.97[a]	3.36[a]	3.19[a]	3.28[a]
Horas	5.07[b]	4.86[c]	4.97[c]	2.21[b]	2.15[c]	2.18[c]	2.86[a]	2.71[a]	2.79[b]
Zosar	7.75[a]	7.56[b]	7.66[b]	4.86[a]	4.61[b]	4.65[b]	3.07[a]	2.95[a]	3.01[ab]
Giza 15	3.24[c]	2.98[d]	3.11[e]	1.49[c]	1.38[d]	1.44[e]	1.75[b]	1.60[b]	1.68[c]
Giza 113	3.67[c]	3.42[d]	3.55[d]	1.80[c]	1.65[d]	1.73[d]	1.87[b]	1.77[b]	1.82[c]
L.s.D at 0.05	0.53	0.54	0.40	0.36	0.35	0.23	0.63	0.56	0.49

Means followed by the same letter in a column do not differ significantly according Duncan's multiple range test at 5% level .

Data in Table (5) show that, the tested cultivars showed significantly difference in oxidative enzymes (Peroxidase and Polyphenoloxidase) activties in grain sorghum leaves . Isis and Zosar cvs . showed the highest activity of peroxidase (2.77 and 2.41, respectively) and polyphenoloxidase (1.10 and 0.97, respectively). While the contrast was the least in Giza 15 (1.40 of peroxidase and 0.42 of polyphenoloxidase).These results indicated that, the highly resistant cultivar contained high level of peroxidase and polyphenoloxidase activities than the susceptible cultivars in both seasons . Weber *et al.* (1967) reported that peroxidase and polyphenoloxidase activity increased in resistant sweet potato varieties inoculated with *ceratocystis fimbriata* than susceptible varieties . Ride (1983) reported that increase in peroxidase activity enhance lignifications in response to infection which may restrict the penetration of the pathogen . The role of the oxidative enzymes in this case may be attributed to oxidize the phenolics to more fungitoxic compounds such as quinons which limit the fungal activity (Misaghi, 1982 and Reuveni; *et al.,* 1991) .

Table (5): Oxidative enzymes activity of the tested grain sorghum cultivars in 2009 and 2010 growing seasons.

Cultivars	peroxidase activity unit/g fresh weight			Polyphenoloxidase activity unit/g fresh weight		
	2009	**2010**	**Mean**	**2009**	**2010**	**Mean**
Isis	2.87[a]	2.67[a]	2.77[a]	1.11[a]	1.09[a]	1.10[a]
Horas	1.75[b]	1.68[c]	1.72[c]	0.70[b]	0.68[c]	0.69[c]
Zosar	2.42[a]	2.40[b]	2.41[b]	0.96[a]	0.98[b]	0.97[b]
Giza 15	1.47[b]	1.32[d]	1.40[d]	0.45[c]	0.39[d]	0.42[d]
Giza 113	1.64[b]	1.48[cd]	1.56[cd]	0.48[c]	0.46[d]	0.47[d]
L.S.D at 0.05	0.48	0.24	0.32	0.21	0.10	0.10

Means followed by the same letter in a column do not differ significantly according Duncan's multiple range test at 5% level .

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رد فعل بعض أصناف الذرة الرفيعة للإصابة بمرض البياض الزغبى المتسبب عن الفطر بيرونوسكليروسبوراسورجاى

عبد الناصر بدوى بدوى السيد ، محمد طه صادومة و حسام الدين محمد فتحى عوض
قسم بحوث أمراض الذرة والمحاصيل السكرية والأعلاف النجيلية – معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر .

أجرى هذا البحث فى حقل الأمراض المقام بمحطة البحوث الزراعية بالجميزة - محافظه الغربيه- مصر خلال موسمى 2009، 2010. تم تقييم خمسة أصناف من الذرة الرفيعة (إيزيس ، حورس ، زوسر ، جيزة 15، جيزة 113) من حيث المقاومة لمرض البياض الزغبى المتسبب عن الفطر بيرونوسكليروسبوراسورجاى وايضا تم دراسة تأثير الإصابة بمرض البياض الزغبى على محصول الحبوب والمكونات الكيماوية فى الأوراق وأظهرت الأصناف المختبرة من الذرة الرفيعة اختلافاً معنوياً فى استجاباتها للإصابة بالمرض حيث كانت الأصناف إيزيس وزوسر عالية المقاومة والصنف حورس متوسط الحساسية بينما كانت الأصناف جيزة 15، جيزة 113 من الأصناف الحساسة. كما أعطت الأصناف عالية المقاومة أعلى محصول للحبوب بالإضافة لاحتواء أوراقها على أعلى مستوى من الكلورفيل والنتروجين الكلى والبروتين الخام والفوسفور والبوتاسيوم مقارنة بالأصناف الحساسة . كان النشاط الإنزيمى للبيروكسيديز والبولى فينول أوكسيديز وكذلك المركبات الفينولية فى الأوراق أعلى ما يمكن فى حالة الأصناف العالية المقاومة، أما الأصناف الحساسة فكانت تقل فى محتوى أوراقها من المركبات الفينولية وكذلك النشاط الإنزيمى . أظهرت النتائج وجود علاقة طردية بين درجة المقاومة لمرض البياض الزغبى ومحصول الحبوب وكذلك المكونات الكيماوية فى الأوراق .

قام بتحكيم البحث

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