MORPHOLOGY AND MYCELIAL COMPATIBILITY OF SCLEROTINIA SCLEROTIORUM AND CHICKPEA VARIETAL REACTION

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Abstract

Variability among 8 isolates of Sclerotinia sclerotiorum associated with stem rot of chickpea , collected from six governorates of Egypt namely Assiut , Minia , Beni-Suef , Beheira , Gharbia and Kafr El-Sheikh was studied . The isolates varied in their aggressiveness, with Gharbia isolate being the highest 70.0 %, while Assiut isolate was the lowest (5.0 %). Also, the results indicated that environmental conditions and geographic location have some effects on aggressiveness of isolates, growth of mycelium and size of sclerotia. Also the isolates varied in their mycelial growth rate, type of mycelium, weight and size of sclerotia and color of substratum. Variability among isolates on the basis of their mycelial compatibility was also observed for 28 combinations. More than half (60.71 %) showed compatible reactions. Four types of vegetative compatibility reactions were defined as gap, line-gap, barrage, and compatible reaction types. Aggressive isolates were less compatible with other isolates and their mycelium was fast growing and bate the mycelium growth of other isolates and be tried in bio control tests.

INTRODUCTION

Stem rot (Sclerotinia wilt or white mold) caused by *Sclerotinia sclerotiorum* is found in all economically important food and feed legumes (Pratt & Knight, 1984). The pathogen is associated with root rot / wilt disease complex of chickpea. Its occurrence, however, is increasing in both incidence and severity on chickpea in the Mediterranean region (Anon., 1996). The initial infection occurs in late winter or early spring, and the fungal mycelia grow within and between plants. Patches of dead plant enlarge and coalesce through spring and cause major losses in stands (Bolton *et al.*, 2006). The fungus survives from one crop season to the next through sclerotia. Overwintering sclerotia may germinate during the summer or may stay dormant for many years (Adams & Ayers, 1979). Abou-Zeid *et al.* (1997) reported that the most frequently isolated pathogens from the roots and basal stems of diseased samples of chickpea, collected from of Beheira and Assiut governorates were *S. sclerotiorum*, *F. solani*, *F. moniliforme*, *F. oxysporum*, *Fusarium* spp., and *R. solani*. Studies of variation within the population in a geographical region are important and help interpret the changes occurring in the pathogen.

The purpose of the present study was to determine the variability in cultural morphology, type of mycelium, mycelial growth rate, size and weight of sclerotia and mycelial compatibility or incompatibility among *S. sclerotiorum* isolates collected from different locations of Egypt, as well as the reaction of certain plant entries.

MATERIALS AND METHODS

1. Field survey

A survey of the stem rot disease in chickpea was carried out in six governorates (Beheira, Kafr El- Sheikh, Gharbia, Beni-Suef, Minia and Assiut) in January and February of 2008. The symptoms of stem rot disease were expressed and the percentage of infected plants was recorded. Twenty random diseased plants were collected from each field for laboratory isolation.

2. Isolation and identification

Stems of diseased plants showing typical symptoms of stem rot were washed carefully in running tap water. The infected plant portions were cut into small pieces, surface sterilized in 2 % sodium hypochlorite for 3 min., then washed twice in sterilized distilled water. The sterilized pieces were dried between two sterilized filter papers and directly placed on PDA medium, then incubated at 25C° for 7 days. Emerged fungi were purified using hyphal tip and single spore techniques, then identified based on their morphological characters (Kohn, 1979; Abida *et al.* 2008).

3. Pathogenicity test

Pathogenic differences of eight isolates of *Sclerotinia sclerotiorum* were determined according to Mazen (1995) on chickpea cv. Giza 88. Sterilized plastic pots (25-cm-diam.) filled with sterilized light loam soil separately infested with the isolates tested at the rate of 5 % (w / w). The inocula were prepared by growing the fungus on autoclaved sorghum grains in 500 ml bottles at 25C° for 15 days and mixed with the sterilized soil . Ten seeds of Giza 88 cv. were sown in each pot. Four replicates were used for each treatment and the control. Disease was recorded as percentages after six weeks.

Varietal reaction

A - Under greenhouse conditions:

Fourteen chickpea entries obtained from the Germplasm Unit of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were tested for their reaction against eight isolates of *S. sclerotiorum* under greenhouse

conditions at Giza Research Station . Ten seeds of each cultivar were sown in each pot (30 cm. in diam. of four replicates), 7 days after soil infestation and host reaction was categorized as follows:

R: Resistant = 0-10 % stem rot. M: Moderately resistant = 11-30 % stem rot. S: Susceptible = 31-50 % stem rot. HS: Highly susceptible = > 50 % stem rot.

B - Under field conditions:

The field experiments were conducted during 2008 and 2009 seasons at El-Gharbia, El-Behira and Beni-Suef to evaluate the reaction of tested plant entries under natural infection. Each treatment was replicated four times in plots of 1 / 200 feddan. All agricultural practices for chickpea production were followed. Disease reaction to *S. sclerotiorum* was recorded after 6 weeks.

5. Morphological characters of S. sclerotiorum isolates

Single sclerotial cultures of these isolates were kept on PDA medium. Isolates were subjected to detailed morphological and cultural characteristics viz., radial colony growth (mm), type of mycelium, size and mean weight of sclerotia / dish. Single sclerotia were placed on PDA medium with four replicates for each isolate. Incubation was made at 24 ± 2 C°. Data of radial colony growth were taken 5 days after inoculation, while weight and size of sclerotia of each isolate were recorded after 25 days (Abida *et al.*, 2008).

6. Vegetative pairings

The isolates were paired in all possible combinations on PDA medium. Reactions were recorded as incompatible when an apparent line of demarcation was observed between the confronting paired isolates. Each pairing was performed at least four times. Mycelial discs (5-mm-diam.) taken from the edge of an actively growing colony (3 to 4 day old) of each isolate were placed at 40 mm apart on opposite side of Petri dishes (90 mm diam.) and incubated for 12 days at 24 ± 2 C°. Vegetative compatibility was determined based on the reactions along the interaction zone between paired colonies (Figure 1) according to Powell & Vargas (2001).

RESULTS AND DISCUSSION

1 - Field survey

Plants showing stem rot were found in all surveyed governorates; however, the average percentages of infection varied from one governorate to another (Table 1). Percentage of infection was high in Gharbia (33.7 %) followed by Beheira (30.0%), while Assiut showed the lowest infection percentage (14.3%). Also, data indicated the environmental conditions effect on percentage of infection. Similar results were found by Welty and Busbice (1978) who reported that occurrence of S. sclerotiorum crown and stem rot was associated with seasonal variation, location and the cultivars tested. Also, Mazen (1995) found that S. sclerotiorum disease incidence on legumes was high in Beheira, Kafr El-Sheikh, Gharbia and Sohag governorates, while low incidence was found in Giza governorate. These results indicated that different locations manifest different disease incidence, which may be due to variations in environmental conditions and / or different populations of the pathogen as well as the cropping sequence in each location. The fungus., however, has a wide host range as well as worldwide distribution on numerous crops (Purdy, 1979; Boland & Hall, 1994). In this respect, Abou-Zeid et al. (1997) reported that the S. sclerotiorum disease has been found in high incidence in Egyptian fields planted with faba bean, lentil and chickpea .

Table 1. Average percentages of chickpea plants infected by stem rot in five governorates of Egypt, during 2008 season .

Governorate	No. of surveyed fields	Percentage of infected plants
Beni-Suef	2	20.0
Gharbia	4	33.7
Assiut	2	14.3
Beheira	3	30.0
Kafr El- Sheikh	4	15.8
Minia	2	15.0
Total	17	****

2 - Isolation and identification of the pathogens

The most important fungi isolated from diseased chickpea samples were *S. sclerotiorum, F. oxysporum* and *R. solani* (Table 2) . The overall average percentage of occurrence were: 62.5, 16.1 and 10.3 %, respectively, while the occurrence of other fungi such as *Fusarium* sp. and *Macrophomina phaseolina*. were 6.7 % 4.2 %. *S. sclerotiorum*, the causal pathogen of stem rot was found in all governorates with high frequency compared with the other fungi. Also, data showed the environmental

conditions effect on the frequency of isolates. These results are similar to those reported previously by Abou-Zeid *et al.* (1997) and Mazen, (1995) where the highest disease incidence was found in Beheira, Kafr El- Sheikh, Gharbia and Sohag, while it was low in Giza governorate.

Table 2. Identity and occurrence frequency of stem rot fungi isolated from chickpea plants , collected from 5 governorates during 2008 season .

	Infection	Frequency of fungi occurrence %					
Governorate	(%)_	Α	В	C_	D	E	
Beni-Suief	20.0	50.0	33.3	0.0	16.7	0.0	
Gharbia	33.7	58.3	0.0	25.0	16.7	0.0	
Assiut	14.3	83.3	0.0	0.0	0.0	16.7	
Beheira	30.0	64.3	7.1	0.0	14.3	14.3	
Kafr El-Sheikh	15.8	63.6	27.3	0.0	0.0	9.0	
Menya	15.0	57.1	28.6	0.0	14.3	0.0	
Mean (%)		62.8	16.1	4.2	10.3	6.7	

A = S. sclerotiorum, B = F. oxysporum f.sp. ciceri,

C = M. phaseolina , D = R. solani & E = Fusarium spp.

3 - Pathogenicity test

Data in Table (3) showed considerable variations among isolates. Isolate no. (7) was the highest in virulence followed by isolate no. (2) on cv. Giza 88, while isolate no.(8) was the lowest in virulence since they resulted in 70.0 %, 63.3 % and 5.0 % disease incidence, respectively. Abou-Zeid *et al.* (1997) reported that stem rot caused by *S. sclerotiorum* is the most important disease on chickpea in Egypt and showed that isolate aggressiveness may be affected by their geographic origin. In this respect, Atallah *et al.* (2004) found variation in virulence among isolates of *S. sclerotiorum* collected from different geographical areas in Columbia Basin of Washington State. The fungus is associated with root rot / wilt complex of chickpea and is increasing in both incidence and severity on chickpea grown in the Mediterranean region (Anon., 1996). Therefore, more attention showed be given in developing more tolerant or resistant cultivars.

Table 3. Pathogenicity test of eight S. sclerotiorum isolates on Giza 88 chickpea cultivar, during 2009 season .

Governorate	isolate No.	Location	Disease (%)
Beheria	1	North El -Tahrir	20.0
Kafr El- Sheikh	2	Sakha	63.3
Menya	3	Manfalot	13.3
Beni-Suief	4	Sids	10.0
Beheira	, 5	Zarzora	15.0
Gharbia	6	Gemmeiza 1	26.7
Gharbia	7	Gemmeiza 2	70.0
Assiut	8	El –Kosia	5
L.S.D. at 5%			11.4

4 - Varietal reaction

A- Under greenhouse conditions

Data presented in Table (4) showed that the entry ICC-x8500498-P-PBN-SH was more resistant to infection than the other 14 entries tested, followed by entry ICC-94927. However, the entries tested possessed different levels of resistance to the eight fungal isolates. Isolate no. 7 was the most aggressive since it infected all entries with only two being moderately resistant, while isolates no.3,4, and 8 were the least aggressive. Also, the entries showed differential reaction. Cultivation of resistant varieties is the ideal approach for the control of the disease; however, no resistant varieties against this disease has been identified so far. Erect type cultivars can better withstand the disease and management can also minimize the crop losses. Stable resistance could not be achieved due to the prevalence of virulent isolates of *S. sclerotiorum* as reported by Sharma *et al.*, (2002). However, the most resistant entry in this study could form a good base for breeders.

Table 4. Reaction of eight S. sclerotiorum stem rot isolates on 14 chickpea entries, under greenhouse conditions, 2009 season.

		Isol	ates no	o. and	diseas	se reac	tion	
Chickpea Entries	1	2	3	4	5	6	7	8
ICC-12442	М	s	М	R	s	М	S	R
ICC-14432	М	HS	М	М	s	M	HS	M
ICC- 89303	S	S	М	R	S	HS	S	М
ICC- 90217	М	s	М	M	S	S	HS .	R
ICC-91108	S	S	М	s	s	S	HS	М
ICC-91128	М	HS	М	R	М	М	S	М
ICC-92034	· M	S	М	S	М	М	S.	R
ICC- 93104	М	S	М	R	S	S	S	R
ICC- 94927	R	М	R	М	R	М	S	М
H-86-20	М	М	М	R	М	S	S	R
ICC-x8500498-P-PBN-SH	R	R	R	R	R	R	М	R
ICC-x850622-BH-ISH-BH	М	S	М	М	М	М	S	М
ICCL-91125S	HS	М	М	R	S	S	М	М
90250	М	М	М	М	HS	М	S	R
Giza 88 cv.	М	HS	М	М	HS	S	HS	R

R: Resistance

= 0 - 10 % stem rot .

M: Moderately resistant

= 11-30 % stem rot.

S-: Susceptible

= 31 - 50 % stem rot.

HS: High susceptible

> 50 % stem rot .

B- Under field conditions

The behavior of the entries tested with respect to their reaction to infection (%) in three governorates was recorded (Table 5). Levels of infection of different entries were highest in Gharbia followed by Beheira . This variation could be due to environmental conditions, being more conducive in Gharbia and less conducive in Beni-Suef. These results confirmed the finding of Welty and Busbice (1978), who reported that occurrence of *S. sclerotiorum* crown and stem rot disease in alfalfa was associated with seasonal ecological variation, location and the cultivars used. Again, the entry ICC-x 8500498-P-PBN-SH showed the least disease incidence in the three governorates.

Table 5. Evaluation of 14 chickpea entries to stem rot caused by S. sclerotiorum, under field conditions in three governorates of Egypt during 2009 season.

		Infection (%)	in
Entry	Gharbia	Beheira	Beni-Suef
ICC-12442	8.00	6.25	4.5
ICC-14432	19.25	17.00	15.25
ICC- 89303	11.75	10.50	8.50
ICC- 90217	13.00	12.75	9.75
ICC-91108	21.25	18.75	15.75
ICC-91128	8.50	6.75	5.00
ICC-92034	15.25	13.25	10.75
ICC- 93104	19.75	17.50	15.00
ICC- 94927	5.75	5.00	3.00
H-86-20	7.50	7.00	4.75
ICC-x8500498-P-PBN-SH	4.00	3.50	2.25
ICC-x850622-BH-ISH-BH	12.50	11.00	9.00
ICCL-91125S	8.00	6.00	5.25
90250	10.50	9.25	6.25
Giza 88 cv.	16.75	15.75	13.50

L.S.D. at 5 % for :

Cultivar (C) = 0.23

Governorate (G) = 2.00

C x G = 0.34

5 - Morphological characters of S. sclerotiorum isolates

Clear differences among S. sclerotiorum isolates in type of mycelium, semblance, pigments in substratum, size, weight of sclerotia, pigment and mean radial growth were observed (Table 6). Mycelium of Beni-Suef isolate was scanty, semi submerged, light gray, while size and weight of sclerotia were greater than the other isolates. Mycelium of Kafr El-Sheikh isolate, however, was abundant, cottony lobed. white and the size and weight of sclerotia were less than the other isolates. Environmental conditions at each location may affect the growth of the fungus and weight and sclerotial size. Also, the disease incidence levels were found to be highest in Gharbia followed by Beheira and was least in Assiut. The results indicated the environmental conditions and geographic location effect on abundance of mycelium, size of sclerotia and aggressiveness of isolates. The mycelium of aggressive isolates was faster growing and abundant in most cases. These results are in agreement with Mazen (1995), who reported differences in the characters of different Sclerotinia isolates, isolated from different geographic locations of Egypt. Several workers recorded variation in size of sclerotia among different isolates of the fungus (Mirza et al, 1985). Similar results were reported by Ziqin et al., (2008) and Abida et al., (2008).

Table 6. Cultural characteristics of eight S. sclerotioum isolates obtained from six governorates and grown on PDA for 7days at 22 C°.

descent	ti	Teologia No.	Two of myrella	Semblance	Colony colour	Average dlameter and	neter and	M . R . G. * (mm) day)	G. *) day)
GOVERNAGE	72000	Todate No.				weight of sclerotia/ plate	rotia/ plate	3	7
Benl-Suef	Sids	4	Scanty	Semi- submerged	Light gray	> 3 mm	0.27 g	37	78
Gharbia	Gemmeiza 1	9	Abundant moderate	Cottony	White to very light gray	1- 2 mm	0.14 g	14	84
Gharbia	Gemmeiza 2	7	Semi moderate	Semi fluffy	White to light gray	2 < mm	0.11 g	40	85
Assiut	El-Kosia	Ħ	Semi moderate	Smooth	Gray white	2 – 3 mm	0.21 g	35	73
Minla	Manfalot	m	Semi scanty	Semi submerged	Light gray	2-3 mm	0.18 g	35	75
Kafer El-Sheikh	Sakha	2	Generally abundant	Cottony- lobed	White	1 < mm	0.06 g	41	87
Beheira	Zarzora	ın	Abundant	Cottony- smooth	White	1- 2 mm	0.16 g	40	82
Beheira	North El-Tahrir	8	Moderate abundant	Smooth	Light gray	2 < mm	0.09 g	38	79

* M.R.G. = Mean Radial Growth.

6 -Vegetative pairings

The combination between each isolate and the others formed a thin band of living or dead mycelia (Fig.1). There were 28 possible pairings of the 8 isolates and 17 combinations showed compatible reaction, (60.71 % of all the combinations) where mycelia of the two isolates intermingled at the zone of interaction. Based on mycelial compatibility, 11 vegetative incompatibility reactions (39.29 %) were found among all the isolates .The isolates no.4, 8, 1, and 3 were compatible with most of the isolates, and among these isolates no. 1 and 8 were the highest compatible with others, (Table 7). The isolates no.6 and no.7 were the least compatible with others.

Four types of vegetative compatibility reaction were observed among the isolates tested. They are: a- gap, b- line gap, c- barrage and d- compatible reaction types. The gap reaction was characterized by a wide gap (3-8 mm) between the two paired colonies and two dark lines on the back of the PDA plate (Fig. 1A). The line gap reaction was characterized by narrow (1 mm) gap along the interaction zone, which looked dark with one or two brown lines on the back of the PDA plate (Fig. 1B). The barrage reaction did not form gap or line gap at the interaction zone and formed a thick white mycelial barrage about (3-5 mm)wide between the two paired colonies and dark zone on the back of the PDA plate (Fig. 1C). In compatible reaction, two paired colonies merged uniformly, with a slight mycelial thickening along the interaction zone, and no dark or brown line appeared on the back of the PDA plate (Fig 1D). In all the antagonistic reactions, sclerotia and mycelium were not formed at the interaction zone area. Sclerotia were formed only in the border of the lytic zone of the two isolates. Also, it showed that isolates no. 2, 6, and no. 7 were more aggressive, of fast and abundant mycelium growth and less compatible with other isolates and bate mycelial growth of other isolates, so it can be used in bio control and programs of breeding.

One hundred and sixteen isolates of *S. homoeocarpa*, the causal organism of dollar spot of turfgrass, were assessed for vegetative compatibility in culture and four types of reactions were observed by Deng *et al.* (2002). Population variability of *S. sclerotiorum*, the causal organism of stem rot of soybean, was determined by mycelial compatibility grouping and isolate aggressiveness comparisons (Kull *et al.* 2004). Variability among 16 isolates of *S. sclerotiorum*, the causal pathogen of stem rot of chickpea on the basis of their mycelial compatibility was observed and 120 combinations, more than half, showed compatible reactions between pairs of isolates. In all the antagonistic reactions, sclerotia were not formed at the interaction zone (Abida *et al.* 2008). After hyphal contact, hyphal death could be observed in the thick

mycelial zone within 2-3 days for the gap reaction, 3-5 days for the line-gap reaction, and 6-8 days for the barrage reaction. Hyphal death eventually led to the formation of wide (gap) or narrow (line-gap) reaction along the interaction zone, but no line or gap appeared in the barrage reaction up to 14 days after inoculation (Deng *et al.*, 2002).

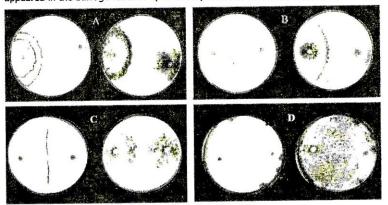


Fig.(1): Vegetative compatibility reaction types in *Sclerotinia sclerotiorum*: (A) Gap reaction, (B) Line-gap reaction, (C) Barrage reaction and (D) Compatible reaction. In each figure, the left is the bottom and the right is the top of PDA culture after 10 - 12 days of incubation at $22C^{\circ}$.

A = Isolate no. 7 and no.5,

B = Isolate no.8 and no. 6,

C = Isolate no. 2 and no. 5 and

D = Isolate no 2 and no. 3.

Table 7. Mycelial compatibility among 8 isolates of Sclerotinia sclerotiorum associated with stem rot disease of chickpea .

Isolates	2	3	4	5	6	7	8
1	****				****		
2				****	****	****	****
3				****		****	
4				.,	****		
5						****	
6						****	
7							

**** = Incompatible.

..... = Compatible .

Further studies should be conducted to uncover the possibility of using isolates showing the highest incompatible reactions for bio control purposes.

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المورفولوجى و التوافق الميسليومي للإسكلروتينيا اسكلروتيورم ورد فعل بعض اصناف الحمص

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تم الحصول على ٨ عزلات مختلفة من الفطر اسكاروتينيا اسكاروتيورم والمصاحبة لعفن ساق الحمص من ٦ محافظات مختلفة وهي: أسيوط – المنيا – بنى سويف – البحيرة – الغربية – كفر الشيخ. وقد ثبت اختلاف العزلات في قدرتها المرضية حيث شوهد أن عزلة الغربية كانت ذات قدرة مرضيه عالية ٧٠% بينما عزلة أسيوط كانت الأضعف ٥ % . أظهرت النتائج أن الظروف البيئية والموقع الجغرافي يؤثران على النسبة المئوية للمرض والقدرة المرضية للعزلات وسرعة نمو المسيليوم وحجم الاسكاروشيا. أيضا اختلفت العزلات في شكلها المورفولوجي من حيث سرعة النمو المسيليومي – نوع الميسيليوم – وزن وحجم الأجسام الحجرية – ولون المزرعة. وأثبتت النتائج المتوافق بين كل عزلتين كانت اكبر من النصف ٢٠,٠١ %. تم الحصول على اربعة انواع من التفاعل التفاعلات هي: الجاب – اللاين جاب – الباراج وتفاعل الالتحام (التوافق) . تم نقارنة أنواع التفاعل ونوع التفاعل (توافق – عدم توافق) والصفات المورفولوجية حيث لوحظ أن العزالات رقم (٢٠٦٠) قوية وسريعة وغزيرة النمو الميسليومي واقل توافقا مع العزلات الأخرى وتضعف من نموها الميسيليومي ولهذا يمكن تجربة استخدام هذه العزلات في المقاومة الحيوية.