Minufiya J. Agric. Res. Vol. 35 No. 1: 1-19 (2010) "http:// agri.menofia.edu.eg/megla.html"

OCCURRENCE OF THE ANASTOMOSIS GROUP-4 OF COTTON -SHIN CAUSAL ORGANISM PREVAILING IN EGYPT

G. A. Amer, M. Nazim, M. Z. El-Shanawani and M. O. Selim
Agriculture, Botany, Minufiya University, Shibin El-Kom, Egypt
(Received: Nov. 22, 2009)

ABSTRACT: *Sore –shin caused by Rhizoctonia solani Kuhn is a major disease on cotton plants in Egypt .The causal organism attacks the germinating seeds and seedling plants causing damping-off symptoms and consequently reduces the plant stands under field conditions. Different isolates of the pathogen were obtained from infected samples collected from different locations in Egypt. The research work was started by testing the virulence of 21 isolates of the causal organism which proved their virulence against the common cotton cv. Giza 89.Also, the cultural characters of the tested isolates were studied. Test of compatibility among 14 isolates showed the existence of anastomosis group AG-4, in addition to other four anastomosis groups. The DNA analysis and the obtained dendogram confirmed the presence of AG-4 and other 4 groups when compared to a standard isolate of AG-4. the obtained results from the DNA analysis and the ISSR test confirmed the presence of AG-4 in addition to other 4 AGs. Accordingly, greenhouse and/or disease nursery tests for resistance to cotton sore-shin should include the dominant AG-4 and the other AG groups to get effective and durable resistance in cotton plants against sore-shin.*

Key words: *cotton sore – shin, Anastomosis Group, Compatibility, Inter Simple Sequence Repeat.*

INTRODUCTION

Cotton sore-shin caused by *Rhizoctonia solani* Kuhn is considered one of the most important diseases which attack cotton plants in Egypt (Moubasher, 1958; Salem, 1969 and Soleman, *et al.* 1988).The causal organism is a soil-borne fungus which infects a wide number of plant species of field and horticulture crops(Hoa, 1993 and Monga *et al.*, 1994). It produces no spores and produces abundant number of sclerotia (Sneh, *et al.* , 1991 and Ogoshi, 1975).Variation in this fungus usually occurs by asexual means(Carling *et al.*, 2002). Anastomosis phenomenon is common in the population in this fungus, and according to this process the populations of the different species could be divided into different anastomosis groups (Ogoshi, 1996, Paulo,*et.al.* 2003 and Ping,*et al.*,2008).The compatibility among the different isolates of *R. solani* facilitate the determination of the different AGs , which characterized by the same behavior for each group (Kuninaga, 1996).

Isolation and determination of the different AGs are considered the first steps in testing and evaluating the genetic material of cotton to be subjected to the improvement of disease resistance to avoid the loss in the plant stands (Monga, *et al.*, 1994; EL-Akkad Salwa, 1997). In this case, determination and/or identification of AGs on the basis of DNA analysis will be a significant base to determine the fungal variation(Carling and Kuninaga, 1990, Stodart,*et.al.*,2007 and Weerasena,*et.al.*,2004). Therefore, the objectives of this study were to determine: The geographical distribution of AGs isolated from cotton plants, AGs virulence and evaluation of the local genotypes. In addition to identification of AGs by determining the DNA polymorphism using Inter Simple Sequence Repeat (ISSR) test.

MATERIALS AND METHODS

1- Isolation of the causal organism:

Samples of cotton seedlings showing necrotic lesions in the crown area or collar rots were collected from different governorates, i.e. Gharbiya, , Beheira, Minufiya , Dekahliya, Sharkiya , Qalubiya and Kafr el-Sheikh during 2003 growing season. Samples were washed in running tap water and soap several times to remove the adhering debris, cut into small pieces then sterilized by immersing in 1.7% sodium hypochlorite solution, for one minute, rinsing in sterile distilled water and plotted between sterilized filter papers. The sterilized samples were cultured on potato dextrose agar (PDA) medium and incubated at 25°C for 3 -5 days. The hyphal tip technique was followed to obtain pure cultures from the growing fungi (Riker and Riker,1936), then the cultures were maintained on PDA slants and kept at 5°C for further studies.

2- Identification of the isolated fungi:

The obtained pure cultures were identified on the basis of cultural morphological characters and microscopic examination according to the method adopted by Parmeter *et al.*, (1967); Ogoshi (1975); Kuninaga (1980) and Sneh *et al.*, (1991).

3-Virulence of the obtained isolates:

Twenty one isolates of *R. solani* Kuhn, obtained from seven governorates as well as the tester isolate AG-4 (obtained from the Plant Pathology Dept., Fac. Agric., Ain-Shams Univ.) were tested for their virulence against the cotton cv.Giza 89 the common genotype in Lower Egypt. Tests were done in pots 30 cms in sterilized soil.

3.1- Preparation of inocula:

Inocula of the different isolates were prepared by growing each isolate on wheat-bran medium (75g wheat-bran, 25g sand and 100 ml distilled water), packed in polypropylene bags. The bags were inoculated with each particular

isolate and incubated at 25 ^{0}C for 14 day and bags contents were mixed thoroughly every two days.

3.2- Soil infestation:

The sterilized soil was infested with each individual isolate at the rate of 0.15% of the soil weight(one week before sowing) and watered every to days two allow the fungal growth. Ten healthy untreated sterilized cotton seeds of the tested genotypes were obtained from the Cotton Disease Section ARC sown in each pot, covered with thin layer of the same soil and watered as required. Five replicates were used for each fungal isolate. Soil free from inocula was used as a check(control).

3.3- Disease assessment:

Disease incidence was assessed as pre- and post-emergence damping off. The infected plants were recorded after 14 and 45 days from sowing. Survival plants were also recorded after 45 days from sowing.

4. Determination of anastomosis groups:

The obtained isolates were tested for their compatibility to hyphal fusion (anastomosis) with the standard AG-4 (Parmeter *et al.,* 1969) using the modified glass slide technique (Herr and Roberts, 1980). Sterilized glass slides were coated with 1% agar put in sterilized 9 cm Petri dishes contained films of water agar to keep a stable levels of moisture around the slides. Standered disc (6 mm diameter) of three days old of the tester AG-4 was placed 4 cm from the disc of the unknown isolate on the glass slide in Petri dish, then incubated at 25 ^{0}C and examined after 24 hrs and for 12 hrs intervals. When the two isolates showed nearly contact they were stained with 0.5% safranin diluted in lactophenol stain and then covered by cover slip at the contact point. The slides were then separated from the Petri dishes and examined microscopically at the magnification power (40, 100, 200, 400 and 1000 x) to determine the type of hyphal fusion and restrict the compatibility of the tested isolate with the standard isolate of AG-4. Three replicates were used for each isolate.

4.1-DNA analysis of the different isolates of the causal organism:

4.1.1-DNA extraction.

DNA of Rhizoctonia isolates were extracted according to the procedure of Lee and Taylor (1990). Isolates were grown Potato Dextrose Broth medium (PDB) and pieces of mycelia incubated at 25^{0} C for five days by with shaking at 100 rpm. The mycelium was harvested and dried. After grinding the material, 3 ml lyses buffer(100 mM Tris-HCL pH8.00 - 50mM EDTA, pH 8.00 – 0.5 M Na CL and 2% B-mercapto-ethanol), 3 ml H_2O in addition to 1.05 ml of

20% SDS, pH 7.2 were added. Samples were transferred onto water bath 65^0 C for 60minutes with gentle shaking every 20 minute, then 0.72 ml of 3M sodium acetate pH 5.2 was added and samples were stored at 0^0 C for 24 hours. Samples were centrifuged for 10 minutes.

Supernatants were collected and the volume of chloroform were added, centrifuged at 500 rpm for 10 minutes. The aqueous upper phase was transferred a new tubes and chloroform wash was repeated.. Samples were centrifuged and supernatants were transferred into new tube, and DNA was washed carefully with 0.7 X of the volume of cold ethanol (70%) and redissolved in 100 µl Mili-Q autoclaved water.

4.1.2- DNA quantification.

Purity and quantity of DNA were determined by measuring the absorption of UV light at 260 and 280 wave length using spectrophotometer (Jen Way model 6305). Then the DNA samples diluted for final concentration of 20 ng / µl and stored at –20 ^{0}C until use.

4.1.3- DNA marker:

For ISSR technique, a primer of 18 bases was used in this study, synthesized by Operon Technologies and Gibco BRL™ .

4.1.4-Preparation of PCR reactions.

Two X PCR Master Mix from Fermentas ® , Lithuania was purchased and used for PCR reaction. Each reaction contains all necessary reagent (dNTPs 200nm of each and 0.6 unit of Taq DNA polymerase) except primers and DNA template. 75 ng of genomic DNA and 1000 p. mol of the primer were added to a final volume of 50 µl reaction.

4.1.5-Thermocycler program and temperature profiles.

The Thermocycler was programmed by an initial standard denaturation cycle at 94 ^{0}C for seven minutes. The following 45 cycles are composed of denaturation step at 92 ^{0}C for one minute, annealing step at 50 ^{0}C for one minute and elongation step at 72 ^{0}C for two minutes. The final cycle was a polymerization cycle performed at 72 ^{0}C for seven minutes. After DNA polymerization, 5 µl of loading buffer (sucrose 10% w/v, EDTA 20mM and Bromophenol-blue 0.04 % w/v added for each sample.

4.1.6-Gel electrophoresis of PCR product.

The PCR products of each reaction were analyzed by electrophoretic separation in 6% polyacrilamide gel. Gel was stained by ethidium bromide at 0.5 mg/ml. A mixture of 25 µl reagent and 5 µl of loading buffer were loaded into the wells of the gel and electrophoresis was run at 180 volts for 4-5 hours. The resultants ISSR patterns were visualized using UV-transilluminator and photographed.

4.1.7-Genetic relationship using ISSR data:

DNA patterns derived from the ISSR primer were scored with 15 isolates of Data were scored as + and - which stands for the presence and absence of PCR product. The output was analyzed using an agglomerative hierarchical clustering method with complete linkage strategy. Firstly, the data was subjected to analysis to produce a matrix of similarity values according to Jaccard (1908). Secondly,. analysis was conducted on the genetics similarity matrix with unweighted pair group method based on arithmetic average (UPGMA) to develop a dendogram using the computer program NTSYS-pe ver 2.1.

RESULTS AND DISCUSSION

This research work was concerned with cotton sore-shin caused by *R. solani* Kuhn which occurs annually in all growing areas of cotton in Egypt with different magnitude (Soleman *et.al,* 1988). This work was started by collecting infected samples of cotton plants to obtain pathogen isolates of different genetic make up and consequently of different virulence and different anastomosis groups. The results given in table (1) showed that *R. solani* Kuhn was the most dominant fungus when compared to any other fungi(pathogenic or nonpathogenic).The occurrence frequencies of the concerned pathogen ranged from 83.33—94.11% of the isolated fungi. The isolation frequencies were 94.11; 91.66; 91.16; 90.63; 88.88; 86.66 and 83.33% in Kafr El-sheikh ; Qalubiya ;Minufiya ; Gharbiya ; Beheira ;sharkiya and Dekahliya respectively in the descending order .These data confirmed that, the Egyptian soil is very contaminated by *R. solani* causing sore-shin to cotton plant(Soleman *et.al.*,1988). Therefore, this disease and its causal organism should be considered.

Table (1): Frequency of isolation of *Rhizoctonia solani* Kuhn from infected seedlings of cotton plant samples collected from seven governorates in Lower Egypt during 2003 growing season.

Location (Governorate)	No. of samples	Percentage of isolation
Kafr El-sheikh	16	94.11
Qalubiya	11	91.66
Minufiya	30	91.16
Gharbiya	24	90.63
Beheira	8	88.88
Sharkiya	13	86.66
Dekahliya	15	83.33

The dominance of *R. solani* may be mainly due to different factors i.e. the suitable environmental conditions(Carling and Leiner,1990 ; Adams and Butler,1983 and Ritchie, *et.al.*,2006); the nature of soil in cotton growing areas and the wide range of the susceptible hosts(Hoa,1993 and Monga *et al.*, 1994).

Virulence of the obtained isolates:

Twenty one isolates of *R. solani* (which represented by 3 isolates from each governorate) were tested fore their virulence .All testes were compared to a tester isolate. These isolates were tested against the cotton cv. Giza 89 the common cultivar in Minufiya governorate and lower Egypt The occurrence of damping-off was recorded as pre-and post- immergence damping –off at 14 and 45 days from sowing. Data given in table (2) showed that, all isolates were virulent to the tested cotton genotype causing typical symptoms of sore-shin with differences in aggressiveness. Isolate 1,2 and 3 (which obtained from Minufiya governorate) were highly aggressive showing 100% damping-off., also isolate number 8 and 17 were highly aggressive(100% damping-off).Whereas the rest of isolates showed different values of aggressiveness. On the other hand , isolates no. 7, 9 and 14 showed less values of damping-off.

Data presented in Table (3) revealed that all tested cultivars were susceptible to all isolates. Also, significant differences were found between the inoculated and the control treatments which expressed as percentages of pre-and post – emergence dumping of found survival plants %. The cotton cultivar Giza 85 showed the highest pre- emergence damping off. On the other hand, the lowest pre – emergence damping- off was recorded on Giza 86 cultivar. On the contrary, the highest average of survival plants was recorded on cv. Giza 86 .

Furthermore, isolate no. 8 was the most aggressiveness one, which gave 60 , 80 and 70 pre- damping – off on the three cvs. Giza 85, Giza 86 and Giza 89, respectively. The interaction between cultivars and the fungal isolates showed significant variation in the number of died plants in pre and post-emergence damping- off and survivals.

The high incidence of damping-off with isolates No.1,2 and3 may be due to the soil type as mentioned by Garrett (1944) who stated that, the light soil(sandy and / or clay)is favorable for disease incidence. Generally, he also mentioned that *R. solani* spread rapidly in the soil of high amount of fresh organic matter, low moisture content and also on the kind of the previous crop. It should be mentioned that , isolates of high aggressiveness should be included in testing for sore-shin resistance.

Table (2): Virulence of 21 isolates of *Rhizoctonia solani* Kuhn to the cotton cv. Giza 89 as percentages of damping off incidence under greenhouse condition compared to a standard isolate of AG-4.

Isolate No.	Source of isolate	Damping-off (100%)		Survival Plants %
		Per-emergence (14 days after sowing)	Post-emergence (45 days after sowing)	
1	Minufiya	100.0^{a}	0.0^{e}	0.0^{i}
2		100.0^{a}	0.0^{e}	0.0^{i}
3		100.0^{a}	0.0^{e}	0.0i
4	Gharbiya	32.0^{ghi}	28.0^{a}	40.0^{defg}
5		80.0^{abc}	0.0^{e}	20.0^{ghi}
6		44.0^{fghi}	20.0^{abc}	36.0^{efg}
7	Kafr el-Sheik	20.0^{i}	8.0^{abcde}	72.0^{ab}
8		100.0^{a}	0.0^{e}	0.0^{i}
9		16.0^{hi}	26.0^{ab}	58.0^{abcd}
10	Qalubiya	28.0^{ghi}	12.0^{bcde}	$60,0^{abcd}$
11		76.0^{abc}	0.0^{e}	24.0^{gh}
12		70.0^{bcde}	4.0^{de}	26.0^{fgh}
13	Behaheira	50.0^{defg}	4.0^{de}	46.0^{cdef}
14		20.0^{i}	10.0^{cde}	70.0^{ab}
15		90.0^{ab}	4.0de	6.0^{hi}
16	Sharkiya	28.0^{ghi}	12.0^{bcde}	60.0^{abcd}
17		100.0^{a}	0.0^{e}	0.0^{i}
18		56.0^{cdef}	16.0^{abcd}	28.0^{fg}
19	Dakahliya	46.0^{efgh}	4.0^{de}	50.0^{bcde}
20		74.0^{bcd}	0.0^{e}	26.0^{fgh}
21		32.0^{ghi}	4.0^{de}	64.0^{abc}
T*	Standard	28.0^{ghi}	8.0^{abcde}	64.0^{abc}
Control		15.0^{hi}	8.0^{abcde}	77.0^{a}

Control : Naturally infested soil.

Means followed by the same letters are not significantly different according to Duncan's Multiple Range Test($p<0.050$)

* The tester isolate; Anastomosis Group no.4,(AG-4).

Table (3): Reactions of the three cotton cultivars against seven isolates of *Rhizoctonia solani* Kuhn under greenhouse conditions.

Isolate number	Cotton cultivar/ damping-off (%) estimated according to the viable seeds.								
	Giza 85			Giza 86			Giza 89		
	Damping-off %		Survived Plants %	Damping-off %		Survived Plants %	Damping-off %		Survived Plants %
	Pre-	Post-		Pre-	Post-		Pre-	Post-	
1	68.00	6.00	26.00	38.00	34.00	28.00	20.00	26.00	54.00
2	38.00	8.00	54.00	18.00	36.00	46.00	26.00	22.00	52.00
3	42.00	4.00	54.00	18.00	26.00	56.00	20.00	12.00	68.00
5	32.00	12.00	56.00	18.00	32.00	60.00	18.00	12.00	70.00
8	60.00	0.00	40.00	80.0	0.00	20.00	70.0	0.00	30.00
15	24.00	16.00	58.00	26.00	20.00	54.00	24.00	26.00	50.00
17	66.00	6.00	28.00	36.00	42.00	22.00	32.00	08.00	50.00
Control *	14.00	8.00	78.00	08.00	2.00	40.00	06.00	06.00	88.00
L.S.D. at 0.05									
between isolates	17.96	15.14	13.72	17.96	15.14	13.72	17.96	15.14	13.72
between cultivars	10.99	9.27	8.40	10.99	9.27	8.40	10.99	9.27	8.40

* Control: Naturally infested soil.

Identification of *R. solani* isolates:

A- Morphological and cultural characters:

Five parameters i.e. growth rate, zonation, arial hyphae, mycelium type andculture colour were studied to relate theses characters to diagnoses of AG-4 (Table, 4).

The tested isolates of *R. solani* varied in their growth rate (Table 4). Isolate no 21 showed the highest growth rate (29.66 mm/day), while the lowest one was isolate no. 12 (10.33 mm/day), while the other isolates were in between. El-Akkad, Salwa. (1997) and Sherwood (1969) found a remarkable differences between isolates of four anastamosis groups than between isolates of the same AG.

Concerning growth type of mycelial zones, isolates no,s 18, 19, 20 and 21 showed moderate formation of zonation. Isolates no,s 1, 4, 6, 11, 14 and AG-4 formed slight mycelial zonation. No zonation was found with more than 50% of the tested isolates.

Table (4): Morphological characters of *Rhizoctonia solani* Kuhn isolates grown on Potato Dextrose Agar, isolates from different location, Egypt 2003.

Source of isolate	Isolate No.	Growth type				Culture color
		Growth rate (mm/day)	Zonation*	Aerial hyphae**	Mycelium type	
Miunfyia	1	19.33abcde	+	+	Radial	Brown
	2	10.66de	-	++	Appressed	Light brown
	3	19.00abcde	-	+	Fluffy	Light brown
Gharbiya	4	28.33ab	+	+++	Radial	Brown
	5	23.66abc	-	-	Radial	Brown
	6	23.66abc		++	Radial	Reddish brown
Kafr El-Sheikh	7	16.33bcde	-	+++	Radial	Light brown
	8	23.00abcd	-	+	Hadial	Dark brown
	9	22.33abcde	-	++	Appressed	Brown
Qalubiya	10	13.00cde	-	+++	Appressed fluffy	Whitish gray
	11	24.33abc	+	+	Radial	Light brown
	12	10.66e	-	+	Radial	Light brown
Behaira	13	16.33bcde	-	++	Appressed	Whitish gray
	14	19.00abcde	+	+	Appressed fluffy	Whitish gray
	15	15.33cde	-	++	Appressed	Light brown
Sharkiya	16	13.00cde	-	++	Appressed	Whitish gray
	17	14.66cde	-	+	Appressed	Whitish gray
	18	23.66abc	++	+++	Radial	Light brown
Dakahliya	19	18.66abcde	++	++	Radial	Brown
	20	20.66abcde	++	++	Radial	Light brown
	21	29.66a	++	+++	Radial	Whitish gray
T (AG-4)		19.66abcde	+	+++	Radial	Reddish brown

Values followed by different letters are significantly different from another according to Duncan's Multiple Range Test (P< 0.05).

* Zonation scale
- = No zonation
+ = Week zonation
++ = Moderate zonation
+++ = High zonation

** Aerial byphae scale:
- = No aerial hyphae
+ = Rare aerial hyphae
++ = Scattered aerial hyphae
+++ = Profuse aerial hypha

As for the formation of aerial hyphae , isolates no,s 4, 7, 10, 18,21 and AG-4 formed profuse aerial hyphae, while the isolates no,s 2, 6, 9, 13, 15, 16, 19 and 20 showed scattered aerial hyphae. Rarely of aerial hyphae were formed by the isolates no,s 1, 3, 8, 11, 12, 14 and 17, however, the isolate no.5 could not form aerial hyphae. All the tested isolates were belonged to the anastamosis group 4. Similar findings were obtained by Sneh *et al.* (1991) who mentioned that isolates of *R. solani* assigned to AG-4 infect cotton plants and cause damping-off and root-rot. Also, Mosa and El-Kholi (1997) found that out of multinucleate isolates of *R. solani* belong to AG-4, while 4 iolates belonged to AG-5. These findings showed the dominance of AG-4 isolate of *R. solani* in the Egyptian soil.

Growth rate ranged from 10.66 – 29.66 for the different isolates of the causal organism, while it was 19.66 for the slandered isolates (AG-4). Therefore, it was difficult to consider this parameter for anastomosis group identification.

The different isolates showed different abilities to form zonation. Therefore, this phenomenon was seemed to be of less importance for AG diagnosis.

The type of maycelial growth was also studied which ranged from radial, apprised and fluffy. Colures of the different cultures were studied. The common colure was brown, however, it was difficult to take this character for AG diagnosis. It could be concluded that other methods for identification and diagnosis of the different AG groups are recommended.

A- Compatibility studies :

Tests for compatibility were carried out in Petri dishes on water agar. Tests were done between the standard isolate of AG – 4 and each of the obtained isolate to determine the hyphal fusion (Table, 5 and Figs 1 and 2).

Results obtained showed compatibility between the AG- 4 isolate and nine isolate i.e. 12, 8, 11, 20, 4, 2, 6, 13 and 17 which indicated that the nine isolate were of the same group (AG – 4) . On the other hand, 5 isolate were incompatible with the standard isolates of AG –4. These isolates were isolates No. 10, 18, 21, 19 and 16

C-DNA studies.

One DNA primer was used to study the genetic diversity between 15 isolates of *R. solani.* These isolates were 2, 4, 6, 8, 10, 11, 12, 13, 16, 17, 18, 19, 20, 21 and AG-4. The extracted DNA was used in PCR experiment using one ISSR primer. The results showed that 18 bands were amplified; 17 of them were polymorphic (94%) (Table, 6 and Fig 3). The extracted from *R. solani* and concentration and purity of DNA expressed as A260/A280 rations were tested. A260 ranged from 0.504 – 1.206 while A280 ranged from 0.041-0.640. Ratio of A260/ A280 ranged from 1.538- 1.968. On the other hand, DNA concentration ranged from 235.0 – 2460.0 ng/ ul (Table, 7).

Table (5): Compatibility reactions among the tester isolate AG- 4 and 14 isolates of *Rhizoctonia solani* .

	T	R2	R4	R6	R8	R10	R11	R12	R13	R16	R17	R18	R19	R20	R21
T	+	+	+	+	+		+	+	+		+			+	
R2	+	+	+	+	+		+	+	+		+			+	
R4	+	+	+	+	+		+	+	+		+			+	
R6	+	+	+	+	+		+	+	+		+			+	
R8	+	+	+	+	+		+	+	+		+			+	
R10						+									
R11	+	+	+	+	+		+	+	+		+			+	
R12	+	+	+	+	+		+	+	+		+			+	
R13	+	+	+	+	+		+	+	+		+			+	
R16										+					
R17	+	+	+	+	+		+	+	+		+			+	
R18												+			+
R19													+		
R20	+	+	+	+	+		+	+	+		+			+	
R21												+			+

+ : Copatible reaction.
[ ] : in compatible reaction
T= tester isolate (AG-4)

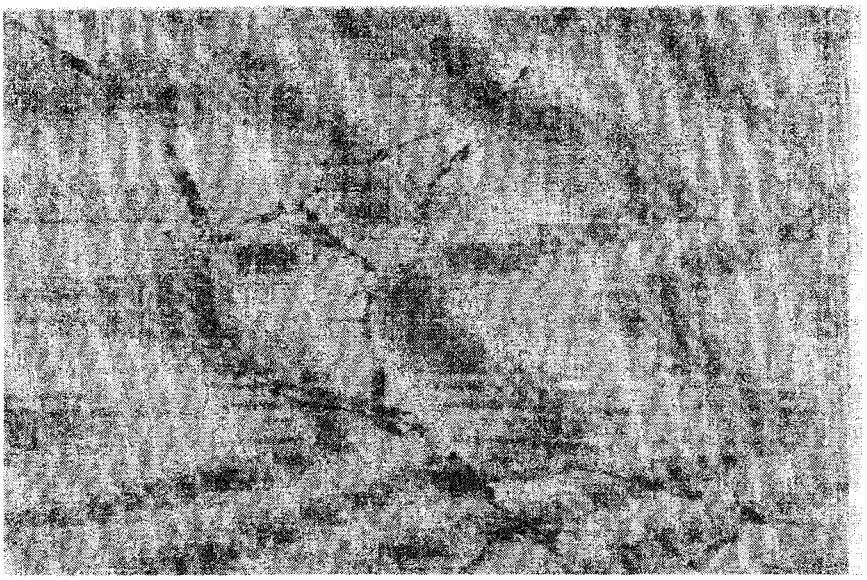

Fig.1: Perfect fusion and contact points between hyphae of *R. solani* isolate no. 2 (X = 400).

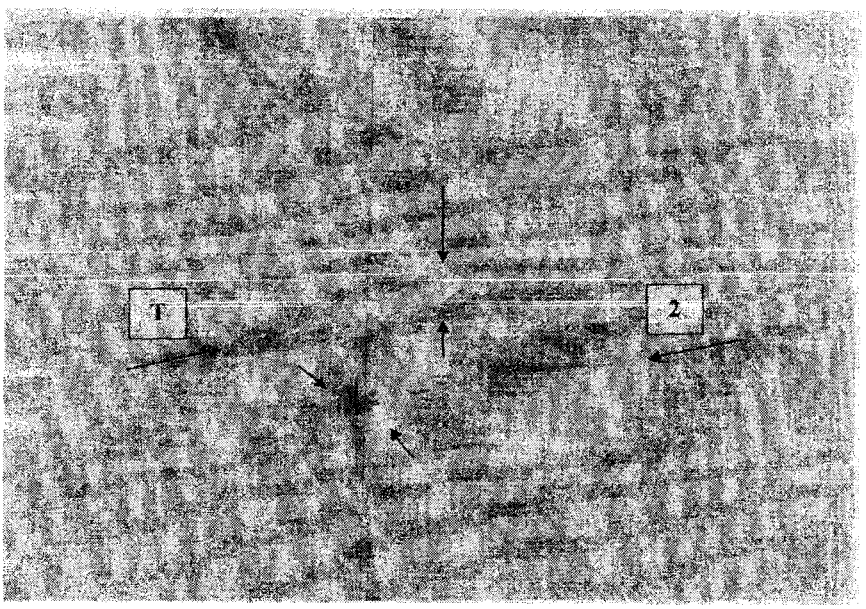

Fig.2 : Imperfect fusion and killing reaction between hyphae of *R. solani* isolate AG-4 and isolate no. 2 (X = 400).

Table (6): Inter Simple Sequence Repeat (ISSR) generated bands of 15 isolate of *Rhizoctonia solani* using primer (1).

Isolates No. /Presence of DNA bands														
T	R2	R4	R6	R8	R10	R11	R12	R13	R16	R17	R18	R19	R20	R21
-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
+	+	+	-	-	-	+	+	+	-	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
+	-	-	-	-	-	+	-	-	+	-	-	-	-	-
+	-	-	+	+	-	-	+	+	+	+	+	-	+	-
+	+	+	+	+	+	-	-	+	-	-	+	-	-	+
+	+	+	+	+	+	+	+	+	+	+	+	-	+	-
+	-	+	-	+	-	+	+	+	-	-	-	-	+	+
+	-	-	-	+	-	+	-	-	-	-	-	-	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	-	+	+	+	-	+	-	-	+	-
+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
-	-	-	+	+	+	+	-	+	-	-	-	-	-	-
-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
-	-	-	-	+	-	+	+	-	-	-	-	-	-	-
-	-	-	+	-	-	-	+	+	-	+	-	-	+	-

(T) = Tester isolate (AG4)
(R2-R21) = *Rhizoctonia solani* isolate number
(+) = Presence of the corresponding band
(-) = Absence of the corresponding band

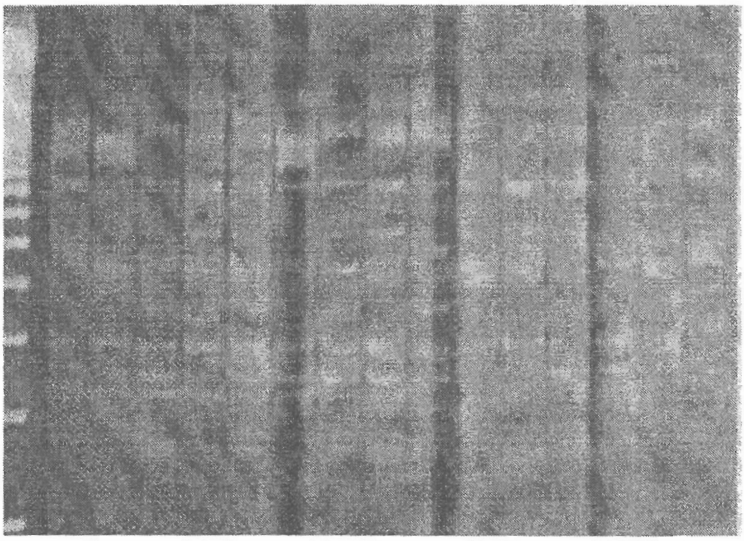

Fig 3 : ISSR patterns obtained with primer1. M, 100bp DNA ladder. Lane 1 – 15 isolates of *Rhizoctonia solani*.

Table (7): Concentration and purity * of DNA extracted from *Rhizoctonia solani* isolates.

Isolate number	Purity of DNA			Concentration ng/µl
	A_{260}	A_{280}	A_{260}/A_{280}	
T	0.754	0.435	1.730	1508.0
R2	0.504	0.351	1.660	1008.0
R4	0.625	0.391	1.598	1562.5
R6	1.03	0.621	1.658	2060.0
R8	0.841	0.532	1.581	2102.5
R10	0.271	0.155	1.740	542.0
R11	0.133	0.078	1.705	332.5
R12	0.185	0.940	1.968	370.0
R13	1.206	0.682	1.768	2412.0
R16	0.448	0.235	1.906	1120.0
R17	0.074	0.041	1.800	148.0
R18	0.155	0.093	1.667	387.5
R19	0.956	0.585	1.630	1912.0
R20	0.094	0.054	1.741	235.0
R21	0.984	0.640	1.538	2460.0

* DNA purity expressed as A260/A280 ratio.

The data of ISSR experiments were scored as (+) and (-), for the presence and absence of PCR product which presented in (Table, 6) were used to generate a matrix using the Jaccard similarity coefficient (Table 8). The Jaccard similarity coefficient ranged from 0.125 between isolates no,s 16 and 19 to 0.643 between isolates no 2 and no. 4.

Kuninaga and Yokosawa (1984) reported that the range of homology between AG4 isolates of *R. solani* using DNA-DNA resuscitation kinetics showed high homology (88%) of the same AG. While those of different AGs had low homology values (31 -48%).

Table (8): Similarity matrix of 15 isolates of *Rhizoctonia solani* based on Jaccard's coefficient.

	T	R2	R4	R6	R8	R10	R11	R12	R13	R16	R17	R18	R19	R20	R21
T	1.000														
R2	0.348	1.000													
R4	0.455	0.643	1.000												
R6	0.480	0.474	0.450	1.000											
R8	0.500	0.412	0.389	0.429	1.000										
R10	0.227	0.385	0.357	0.412	0.429	1.000									
R11	0.478	0.316	0.444	0.348	0.500	0.400	1.000								
R12	0.500	0.350	0.474	0.375	0.450	0.211	0.429	1.000							
R13	0.462	0.526	0.579	0.591	0.476	0.316	0.333	0.545	1.000						
R16	0.391	0.211	0.143	0.261	0.250	0.188	0.238	0.273	0.250	1.000					
R17	0.375	0.412	0.471	0.500	0.238	0.250	0.350	0.381	0.476	0.250	1.000				
R18	0.455	0.353	0.412	0.450	0.316	0.462	0.368	0.333	0.429	0.333	0.471	1.000			
R19	0.182	0.417	0.286	0.211	0.188	0.300	0.250	0.222	0.263	0.125	0.267	0.286	1.000		
R20	0.407	0.381	0.364	0.400	0.409	0.250	0.391	0.478	0.385	0.200	0.409	0.304	0.200	1.000	
R21	0.478	0.316	0.444	0.409	0.286	0.313	0.400	0.304	0.391	0.368	0.286	0.529	0.333	0.231	1.0000

The dendogram presented in Figure (4) reveal the genetic relationship between the tested isolates. The dendogram separated both of *R. solani* isolate no. 16 and 19 individually with similarity coefficient 0.25 and 0.27, respectively. The rest of the isolates were sub-clustered into different sub-groups with higher similarity coefficient. The isolates no,s 2 and 4 showed the highest similarity coefficient (64 %). This variation has great importance in studying pathogen populations, since it reflects its ability to evolve itself

continuously against resistance. A possible explanation may be the fusion that occurs between the isolates assigned to the same anastamosis group. This fusion causes the shifting of nuclei between fused hyphae leading to new genetic combination (Ogoshi, 1996, Carling and Kuninage,1990).

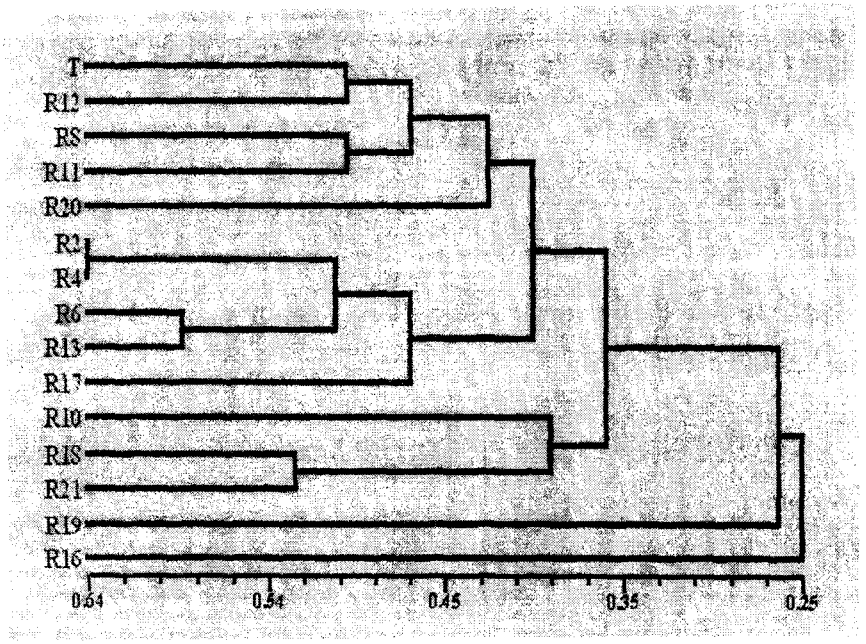

Fig.4: Dendogram generated by UPGMA cluster analysis based on Jaccard coefficient determined from 18 ISSR markers of 15 isolates of *R. solani.*

REFERENCES

Adams, G. C., J.r., and E. E. Butler (1983). Enviromental factors influencing the formation of basidia and bsidiosporses in Thanatephorus cucumeris. Phytopathology 73:152-155.

Carling, D.E. and S. Kuninaga (1990). DNA base sequence homology in *Rhizoctonia solani* Kuhn : Inter and intra-group relatedness of anastamosis group 9.Phytopathology,80: 1362 -1364.

Carling, D.E. and R. D. Leiner (1990). Effect of temperature on virulence of *Rhizoctonia solani* and other *Rhizoctonia* on potato . Phytopathology,80: 930 -934

Carling, D.E., R.E. Baird and S. Kuninaga (2002). Characterization of AG-13, a newly reported anastamosis group of *Rhizoctonia solani*. Phytopathology ,92, ISS8:893.

El-Akkad, Salwa, A. (1997). Studies on anastamosis groups of *Rhizoctonia solani*. Ph.D. Thesis, Fac. Of Agric. Cairo Univerisity.

Garrett, S. D. (1944). Root disease fungi. Chronica Botanica Company, Waltham, Mass., USA.

Herr, L. J. and DL. Roberts (1980). Characterization of *Rhizoctonia* population obtained from sugar beet fields with different soil textures. Phytopathology, 70: 476- 480.

Hoa, T. T. C. (1993). Anastamosing potential of *Rhizoctonia solani* and its pathogenic implications in crop diseases. Division of Mycology and Plant Pathology, IARI, New Delhi-110012. Roli No.7608.

Jaccard , P. (1908). Nouvelles researches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat., 44: 223 – 270.

Kuninaga, S. (1996). DNA base sequence complementary analysis.Edited by Sneh et, al., Academic Publishers,Dordrecht,Netherlands,pp,73-79

Kuninaga, S. (1980). Hyphal anastamosis behaviour of single basidiospre isolates in *Thanatephorus cucumeris* (Frank) Donk. Higashi Nippon-Gakuen J. Liberal Art and Sci., 6: 95 - 106

Kuninaga, S. and R. Yokosawa (1984). DNA base sequence homology in *Rhizoctonia solani* Kuhn. IV. Genetic relatedness within AG-4. Anals. Phytopath. Soc. Japan,50: 322 – 330.

Lee, S. B. and J. W. Taylor (1990). Isolation of DNA from fungal mycelia and single cells, pp.282- 287. In: PCR Protocols, A guide to methods and Applications. Academic Press ,San Giego.

Monga, D., Shheo and S. Raj (1994). Cultural and pathogenic variations in the isolates of *Rhizoctonia* species causing root rot of cotton. Indian. Phytopathotogy, 47:403.

Mosa, AA. and MMM. El-Kholi (1997). Characterization and pathogenicity of anastamosis groups of *Rhizoctonia solani* isolated from sugar beet in Egypt. Egypt. J. Agric. Res., 75(3):

Moubasher, A. H. (1958). Studies on the damping- off diseases of cotton in Egypt with a note on the effect of origin of *Rhizoctonia* Ph.D Thesis, Fac. of Agric. Cairo Univ., 120pp.

Ogoshi, A. (1975). Grouping of *Rhizoctonia solani* Kuhn and their perfect stages. Rev. Plant Prol. Res. Japan, 8: 98- 103.

Ogoshi, A. (1996). The genus Rhizoctonia. Edited by Sneh et al., Academic Publisher, Dordrecht, Netherlands, pp.1-9.

Parmeter, J.R., HS Whitney and WD. Platts (1967). Affinities of some *Rhizoctonia* species that resemble mycelium of *Thanatephorus cucumeris*. Phytopathology, 57: 218-223.

Parmeter, J. R., RT. Sherwood and WD Platts (1969). Anastamosis grouping among isolates of *Thanatephorus cucumeris.* Phytopathology, 59: 1270-1278.

Paulo, C. C.; H D Shew; R J Vilgalys; L. R. Galeand M. A.Cubeta (2003). Detecting migrants in populations of *Rhizoctonia solani* anastomosis group 3 from potato in north Carolina using multilocus genotype probabilities. Phytopathology, 93:610-615.

Ping QU, K. Yamashita, T. Toda, A. Priyatmoja, M. Kubota and M. Hyakumachi (2008). Heterokaryon formation in Thanatephorus cucumeris (Rhizoctonia solani) AG-1 IC . Mycological Research,112:1088-1100.

Riker, A. J. and S. Riker (1936). Introduction to research on plant diseases. Planographed by Jhon, S. Swift Co., Inc. St., Louis Chicago, New yourk, Indiana polis, 117pp.

Ritchie F., M P. McQuilken and R A. Bain (2006). Effects of water potential on mycelial growth, sclerotial production, and germination of Rhizoctonia solani from potato.Mycological Research,110:725-733.

Salem, F. (1969). Cultural, pathogenic and physiological studies on *Rhizoctonia solani* Kuhn, the causal agent of sore shin disease in the UAR., M.SC. Thesis, Plant Pathology, Fac. Of Agric., Cairo Univ.

Sherwood, R.T. (1969). Morphology and physiology in four anastamosis groups of *Thanatephorus cucumeris.* Phytopathology,59 ;1924-1929.

Sneh, B., L. Burpee and A. Ogoshi (1991). Identification of *Rhizoctonia solani* species. The American Phytopathological Society Press. Inc. Paul., Minnesota,USA.

Soleman, N. K., M. S. Mikhail, R. K. Harb and E. M. Khalil (1988). Response of broud bean plants infested with *Rhizoctonia soloni* to application of growth regulators and calcium. Egypt J. Phytophathol., zo (1) : 1- 11.

Stodart, B.J., P.R. Harvey, S. M. Neate, D. L. Melanson and E. S. Scott (2007). Genetic variation and pathogenicity of anastomosis group 2 isolates of *Rhizoctonia solani* in Australia.Mycological Research,111:891-900.

Weerasena ,O.V.D.S.J., N. V., Chandrasekharan, R.L. C. Wijesundera and E. H. Karunanayake (2004). Development of a DNA probe and a PCR based diagnostic assay for *Rhizoctonia solani* using a repetitive DNA sequence cloned from a Sri Lankan isolate. Mycological Research,108:649-653.

تعريف المجموعات المتوافقة السائدة لمسبب مرض خناق القطن في مصر

جمعة عبد العليم عامر ، محمد نظيم سيد احمد ، محمدي زكي الشنواني ، محمد علوي سليم

قسم النبات الزراعى ـ كلية الزراعة ـ جامعة المنوفية .

الملخص العربي

يعتبر مرض الخناق المتسبب عن الفطر ريزوكتونيا سولاني من الامراض الرئيسية التي تصيب نباتات القطن في مصر حيث يهاجم المسبب المرضي البذور أثناء الإنبات وفي طور البادرة مسببا أعراض سقوط البادرات وبالتالي يقلل من عدد النباتات في الحقل. تم الحصول علي عزلات من الفطر المسبب للمرض من عينات مصابة تم جمعها من جهات متفرقة من مصر . وقد بدء العمل البحثي بجمع العينات المصابة ثم تنقيتها واختبار قدرتها المرضية ضد الأصل الوراثي جيزة ٨٩ والمنزرع في الوجه البحري ثم درست الصفات المز رعية للعزلات التي تم الحصول عليها . كذلك اجري اختبار القدرة التوافقية بين تلك العزلات وكذلك ضد العزلة القياسية AG-4 وقد أوضحت النتائج سيادة المجموعة AG-4 إلي جانب عزلات تابعة آلي مجموعات أخرى وعند مقارنتها بالعزلة القياسية AG-4 أكدت نتائج دراسة التشابه الوراثي Similarity matrix بين العزلات مقارنة بالعزلة القياسية وكذلك نتائج ISSR وجود العزلة التابعة لمجموعة AG-4 إلي جانب أربعة مجموعات أخرى . لذلك فان استخدام جميع العزلات التي تم الحصول عليها يؤدي إلي تحسين كفاءة مقاومة الأصول الوراثية للقطن ضد مرض الخناق في حقول المزارعين.