# CHARACTERIZATION AND PATHOGENICITY OF ANASTOMOSIS GROUPS OF RHIZOCTONIA SOLANI ISOLATED FROM SUGARBEET IN EGYPT

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(Manuscript received 10 December 1996)

#### Abstract

Fourty-three isolates of *Rhizoctonia* were collected from sugarbeet seedlings and older plants from different fields in Egypt during 1992-1993. Of the isolates collected, only one was binucleate and the remaining 42 isolates were multinucleate with characteristics typical of *R.solani*. *R.solani* isolates belonged to three anastomosis groups: AG-2-2 (28.6%), AG-4 (64.3%) and AG-5 (7.1%). All isolates from rotted roots were AG-2-2, while 81.8% of AG-4 isolates from seedlings. Pathogenicity tests on sugarbeet seedlings in the greenhouse showed that isolates of AG- 4 were most pathogenic in causing seedling damping-off compared with AG-2-2 and AG-5, while AG-2-2 isolates were more virulent in causing root-rot to older sugar beet plants. The binucleate isolate of *Rhizoctonia* was nonpathogenic and increased growth of sugarbeet seedlings. The binucleate isolate significantly reduced damping-off caused by *R.solani*.

#### INTRODUCTION

Sugarbeet (Beta vulgaris L.) has become one of the most economically important crops in Egypt. Soil borne diseases cause problems in sugarbeet fields throughout the production areas (El-Kholi, 1979, 1984). *Rhizoctonia solani* Kuhn {Telemorph: *Thanatephorus cucumeris* (Frank) Donk} is an important soil borne pathogen with an exteremly wide host rang and world-wide distribution (Ogoshi, 1987). On sugarbeet, *R.solani* may cause damping-off, root-rot, crown rot and foliar blight depending on the pathological, physiological and morphological attrib-

utes of the particular isolates involved (Kotila, 1947; Ashour et al, 1964, El-Kholi, 1979 and 1984).

Rhizoctonia solani comprises a collection of noninterbreeding populations that are recognized through the anastomosis group concept (Ogoshi, 1987). Isolates of *R. solani* are currently grouped into 18 anastomosis groups (AG) and sub-groups on the basis of their anastomosis behaviour (Sneh *et al.*, 1991). Since virulence and host range of these groups differ, knowledge of anastomosis group affilation of an isolate involved in a particular disease has become very useful (Anderson, 1982; Sneh, *et al.*, 1991).

A preliminary characterization of anastomosis groups of *R. solani* on sugarbeet, based on eight isolates collected from fields from Kafr El-Sheikh governorate, was reported earlier (El-Kholi, 1993). These results stimulated further studies with more isolates collected in different years and from several locations.

This paper reportes the results of identification, characterization and pathogenecity of isolates of *R. solani* associated with sugarbeet plants from Kafr El-Sheikh and El-Dakahlia governorates, in Egypt. A portion of this work was reported in an abstract (Mosa and El-Kholi, 1995).

# MATERIALS AND METHODS

# Sampling and isolation:

Infected sugarbeet seedlings and older plants were collected from several fields from Kafr El-Sheikh and El-Dakahlia governorates, which represent major sugarbeet growing areas, during 1992-1993.

Small pieces (2-3 cm long) of roots and stems usually bearing distinct lesions were washed in running tap water for 1 hour. The pieces were surface sterilized with 1% sodium hypochlorite for 30 sec, and rinsed three times with sterile distilled water. Surface sterilized pieces were cut longnitudinally and placed with their cut surface down on acidified 2% water agar (WA) and incubated at 25°C for 4 days. Hyphal tips of *R. solani* emerging from the diseased tissues were transferred to potato dextrose agar (PDA) for axenic culture. For long-term preservation, *R.solani* isolates were grown in tubes (50 X 10 mm) containing sandy-loam soil with 4% (w/w) wheat bran (Butler, 1980).

#### Identification and characterization:

Characteristics of the septal pore apparatus and numbers of nuclei in vegetative cells of all cultures were determined by staining and microscopic examination. Mycelia (2 or 3 days old) were stained by two modified staining procedures with 0.5% aniline blue (Tu and Kimbrough, 1973) or with 0.5% safranin 0 and 3% KOH (Yamamoto and Uchida, 1982). Stained mycelia were examined microscopically at 400-1, 000 X. The isolates were identified as *R.solani* if they met vegetative characteristics described for the species (Parmeter and Whitney, 1970; Sneh *et al.*, 1991).

#### Anastmosis group identification :

Rhizoctonia solani isolates were tested for their ability to anastomose with known AG tester cultures (AG-1, AG-2-1, AG2-2. AG-3, AG-4, AG-5, AG-6, AG-7 and AG-BI) kindly provided by Dr. Akira Ogoshi, Hokkaido University, Japan to Cairo MIRCEN, Ain Shams University.

Pairings were made on sterile glass slides coated with 1% WA and placed on WA plates to maintain moisture. A mycelial disk, 5 mm diameter, from the edge of 3 days old colony of an unknown isolate was placed at 2 cm distance from a similar disk of known AG tester culture. After the intermingling of the hyphae, 2-3 days of growth at 24°C, slides were stained with 0.5% cotton blue in dilute lactophenol and microscopically examined for hyphal anastomosis. About 100 observations were made per isolate.

#### Growth rate:

Two representative isolates of AG 2-2, AG-4 and AG-5 were grown on PDA in 9 cm diameter Petri dishes and incubated at  $15,20,25,30^{\circ}$ C in the dark. The radial growth rate mm/day was measured at 24 hrs intervals for 4 days. Three dishes were used for each isolate.

#### Thiamine requirement :

Two representative isolates of AG-2-2, AG-4 and AG-5 were grown in 100 ml conical flasks containing 30 ml of glucose asparagin medium with or without  $10^{-5}$  M thiamine hydrochlorid (Ogoshi and Ui, 1978). A 5 mm mycelial disk from thin cultures on WA was inoculated gently to each flask. Mycelial dry weights were determined after 10 days incubations at  $24^{\circ}$ C. There were four replicates per isolate.

#### Pathogenicity determination:

Representative randomly selected isolates of AG-2-2, AG-4 and AG-5 of *R.solani* from diseased sugarbeet were used in pathogenicity tests on sugarbeet in the greenhouse. Because a culture of binucleate *Rhizoctonia* was also isolated, it was included for comparison with *R.solani*.

Pathogenicity of cultures on sugarbeet seedlings was tested as described by Windels and Nabben, (1989). The soil texture consisted of field soil; peat; sand (1:1:1) was moistened and autoclaved for 1 hr at two consecutive days. Plastic pots (20 cm diameter) were used and 600 cm<sup>3</sup> of soil was added to each pot. A 7-day-old culture growng on 2% WA in 6-cm diameter Petri dish was added and covered wth 100 cm<sup>3</sup> of soil. Sugar beet seeds were surface sterilized with 1% NaOCL for 2 min, rinsed with sterile water and dried between filter papers. Ten seeds were placed on the soil surface and covered with 100 cm<sup>3</sup> soil. Control pots contained WA only. A randomized complete block design was used with five replicates per each isolate. Numbers of seedling surviving were recorded after 4 weeks.

The same seven isolates were tested also on older plants in 20 cm pots containing autoclaved soil mix with one vigorous plant per pot. Inculation was carried out on 8-week-old sugar beet by adding a teaspon (2 gm) of barley grain inoculum of each isolate to a hole in the soil in contact with the tap root (Windels and Nabben, 1989). The control consisted of roots treated with sterilized barley grain. After four weeks of inoculation, the beets were removed from the pots, washed and rated on a 0-5 disease rating scale (0=healthy, 5 = dead) (Herr and Roberts, 1980).

#### Biocontrol of Rhizoctonia solani by binucleate Rhizoctonia:

Efficacy of the binucleate *Rhizoctonia* isolate KS 92/B to control sugar beet damping off causes by *R.solani* was tested in the greenhouse. Inoculum for experiments was produced on oat kernles medium as described by Escande and Echandi (1991). Experiments were conducted in 20 cm diameter pots contained posturized sandy loam soil. Inoculum of *R.solani* was added to soil at a rate of 2 g/kg soil, while binucleate *Rhizoctonia* was added at rates of 0.5, 1.0, 2.0, 4.0 g/Kg soil. Controls consisted of soil amended with uncolonized sterilized oat kernels. Ten sugerbeet seeds were sown in each pot and four pots were used for each treatment. Seedling survival was recorded after 4 weeks from planting. The experiment was repeated twice.

#### RESULTS

#### Anastomosis groups of R. solani:

Forty-eight hours after planting tissue sections, *Rhizoctonia* colonies were early distinguished from other colonies when present. Of 43 cultures collected from sugarbeet seedlings and older plants, only one isolate was binculeate and the remaining 42 were multinucleate and had cultural characteristics typical of *R. solani*. Only three anastomosis groups; AG-2-2, AG-4, and AG-5 were identified among the multinucleate isolates (Table 1). AG-4 isolates predominated (64.3%) and were isolated only from seedlings. AG-2-2 isolates (28.6%) were obtained from older plants, but were isolated occasionally from seedlings. AG-5 isolates were less frequent comprising 7.1% of the total isolates and were found only in Kafr El-Sheikh. AG-4 isolates were detected from both regions, while AG-2-2 was not detected in seedlings samples from El-Dakahli. (Table 1).

Table 1. Anastomosis groups of *Rhizoctonia solani* isolated from sugar beet seedlings and older plants, during 1992-1993, from Kafr El-Sheikh and El-Dakhlia governorates in Egypt.

Anastomosis group	Origin and Number of isolates			
	Seedlings		Older plants	
	KSa)	DKp)	KS	DK
AG-2-2	3	0	7	2
AG-4	19	8	0	0
AG-5	3	0	0	0
Binucleate	0	0	1	0

- a) Kafr El-Sheikh
- b) El-Dakahlia

The binucleate *Rhizoctonia* was isolated from root tissues which found to be infected by *Pythium* sp. The isolate emerged from the healthy margin of the sample. This isolate was culturally indistinguishable from *R.solani*. However, nuclear staining could differentiate it easily from multinucleate ones.

#### Growth rate:

The growth rate of representative two isolates of AG-2-2, AG-4 and AG-5

were compared at four temperature degrees (Table 2). All isolates tested grew faster on 25°C than 20°C or 15 °C . However, AG-4 isolates grew faster than AG-2-2 and AG-5 at  $30^{\circ}$ C.

Table 2. Radial growth rate (mm/day) of *Rhizoctonia solani* AG-2-2, AG-4 and AG-5 isolates from sugarbeet on PDA plates at four different temperatures degrees.

Anastomosis	Temperature <sup>O</sup> C			
group <sup>a</sup> )	15 nobs	20	25	30
AG-2-2	11.6	17.8	22.5	20.3
AG-4	12.3	19.4	29.3	30.2
AG-5	. 9.2	14.6	20.9	18.7
.SD (0.05) b)	2.4	1.9	4.2	4.8

- a) Mean of two isolates for each AG.
- b) LSD (P<0.05) values for comparison between anastomosis groups at the same temperature degree.

## Thiamine requirement:

Both AG-2-2 and AG-5 isolates of *R.solani* could not grow on glucose asparagine media without thiamine addition. In contrast, both AG-4 isolates could grow on the medium in the absence of thiamine. Variation of growth between isolates was also observed (Table 3).

Table 3. Thiamine requirement of selected isolates of *Rhizoctonia solani* of AG 2-2, AG 4 and AG-5.

Isolate Anastomosis group	Mycelial dry weight (mg/flask) <sup>a</sup> )		% growth	
	group	Thiamine		
		- (A)	+ (B)	
KS 92/2	AG-2-2	8.3	165.2	19.5
DK 93/2	AG-2-2	5.2	175-6	33.7
KS 92/4	AG-4	84.8	112.2	1.3
DK 93/4	AG-4	54.5	76.4	1.4
KS 92/5	AG-5	5.0	86.0	17.2
KS 93/5	AG-5	4.8	93.4	19.4

a) After 10 days growth on glucose-asparagine medium amended with thiamine at concentration  $10^{-5}$  M (B) or not amended (A).

#### Pathogenicity

Significant differences in seedling survival were observed among the six iso-lates of *R.solani* AG-2-2, AG-4 and AG-5. (Table 4). Both AG-4 isolates, KS 92/4 and DK 93/4, were highly pathogenic and caused 75% and 79.5% seedling damping-off 4 weeks after planting, respectively. Isolates of AG-2-2 varied in severity of reducing seedling stands 4 week after planting. Isolates KS 92/2 and DK 93/2 caused 28.4% and 44.3% seedling damping off, respectively, compared with the untreated soil control. Both AG-5 isolates, KS 92/5 and KS 93/5, were less pathogenic and caused 14.7% and 22.7% damping-off, respectively. All isolates of *R.solani* significantly reduced seedling dry weight of sugarbeet (Table 4). The relative virulence, measured as root-rot disease rating, of the *R.solani* isolates on older plants gave quite different results from those obtained with seedling test (Table 4). The results indicate that AG-2-2 isolates were significantly more virulent than either AG-4 or AG-5 isolates on older plants.

Table 4. Influence of selected *Rhizoctonia solani* isolates and a binucleate *Rhizoctonia* isolate on survival, growth and root-rot ratings of sugarbeet grown in the greenhouse (Pot experiments).

		Seedli	Root		
	Anastomosis group	Seedling survival (%)	Seedling dry weight (mg/plant)	rot index a)	
KS 92/2	AG-2-2	63 c b)	210 c	4.5 a	
DK 93/2	AG-2-2	49 d	189 d	4.4 a	
KS 92/4	AG-4	22 e	173 d	1.7 b	
DK 93/4	AG-4	,18 e	261 b	1.8 b	
KS 92/5	AG-5	75 b	232 c	1.0 c	
KS 93/5	AG-5	68 c	218 c	0.8 c	
KS 92/B	BN	86 a	318 a	0.3 d	
Control		88 a	274 b	0.0 d	

a) Based on 0-5 scale (0 = healthy, 5 = dead).

b) Means followed by the same letter are not significantly different (P = 0.05, according to Duncan multiple range test).

Surprisingly, the binuculeate *Rhizoctonia* isolate, KS 92/B, was non pathogenic and did not reduce seedling stand significantly. The binucleate isolate was also avirulent on older sugarbeet plants and increased significantly seedling dry weight compared with untreated control.

# Suppression of damping - off caused by *R. solani* by binucleate *Rhizoctonia* KS 92/B:

Results presented in Table (5) indicate that binucleate *Rhizoctonia* isolate KS 92/B reduced incidence of damping off caused by *R.solani* compared with the untreated control. Although all rates of binucleate isolate tesed were effective, the rate that gave optimum disease control was 2 g/Kg soil when damping-off was reduced from 63.6 to 3.0%. However, in non-infested soil with *R.solani*, the binucleate isolate did no affect final seedling survival significantly.

Table 5. Biocontrol of damping-off of sugarbeet by binucleate *Rhizoctonia* isolate KS 92/B, in greenhouse.

	% seedling survival (b)  Rhizoctonia solani		
Binucleate <i>Rhizoctonia</i> a)			
	(-)	(+)	
0.0	82.5 a <sup>c)</sup>	30.0 d	
0.5	87.5 a .	65.0 b	
1.0	80.0 a	65.0 b	
2.0	82.5 a	80.0 a	
4.0	80.0 a	50.0 c	

a) Rate as g/kg soil

### DISCUSSION

The results obtained in this study expands the list of anastomosis groups of *R.solani* previously reported on sugarbeet in Egypt by El-Kholi (1993) from two (AG-2-2 and 4) to three (AG-2-2, 4 and 5). This is the first report also of the isolation of binucleate *Rhizotonia* from sugar beet plants in Egypt. Distribution of AG-2-2 and AG-4 were not restricted to any particular geographical reigon while AG-5

b) After 28 days from sowing

c) Means followed by the same letter are not significantly different (P< 0.05, according to Duncan multiple range test).

isolates were isolated from Kafr El-Sheikh only. Isolates of *R.solani* from other anastomosis groups have been also reported on sugarbeet in different parts of the world. Windels and Nobben (1989), in USA, and Naito *et al.* (1975), in Japan, have reported isolating the same six anastomosis groups (AG-1, 2-1, 2-2, 3, 4, 5) from diseased sugarbeet, but AG-2-2 and AG-4 have predominated.

Temperature effect may have influenced the agroecological distribution of *R.solani* (Anguiz and Martin, 1989; Carling and Leiner, 1990). AG-2-2 and AG-5 growth is optimum between 20-25°C compared to 25-30°C for AG-4. In such condition in the field, isolates of AG-4 may develop faster in warmer season and may be able to compet with AG-2-2 and AG-5.

Thiamine requirement for *R.solani* isolates was considered as an addition criteria to characterize different AG groups and subgroups (Ogoshi and Ui, 1978). The results of this study, showing that AG-2-2 isolates were thiamine-auxotrophic while AG-4 isolates were autotrophic, are in agreement with Ogoshi and Ui (1978).

In this study, AG-4 isolates were more virulent in reducing seedling stand while AG-2-2 isolates were more virulent in causing root-rot to older plants. The ability of AG-2-2 to cause severe root-rot is supported by other studies (Naito et al. 1978; Herr and Roberts, 1980; Windels and Nabben, 1989). However, AG-2-2 was frequently isolated from seedling from Kafr El-Sheikh only. Although, AG-2-2 and AG-4 isolates have been reported to cause foliage blight of sugarbeet (Ruppel, 1972; Naito et al. 1978; Herr, 1988), the symptoms of foliage blight have not been observed in Egypt. Although AG-5 isolates were less pathogenic to sugarbeet seedling and older plants, it was reported to be isolated from 50% of diseased seedling in Japan (Naito et al. 1975).

Binculeate *Rhizoctonia* were reported on sugarbeet and other field crops and some of these isolates were plant pathogens and others were non pathogenic (Sneh, et al. 1991). Binucleate *Rhizoctonia* may have potential for the biological control of Rhizoctonia diseases including potatoes, beans and sugarbeets (Cardoso and Echandi, 1987; Herr, 1988; Escandie and Echandi, 1991). Pathogenicity tests indicate that the isolate of bincleate *Rhizoctonia* found on sugarbeet in this study was non pathogenic to seedling or older plants and greatly increased seedling dry weight. This isolate (KS 92/B), when applied to the soil in colonized oat kernels, protected sugarbeet seedling from damping-off caused by *R.solani*. The binucleate *Rhizoctonia* isolate may have potential for use as a biological control agent. Further studies on the the host range, variability and pathogenicity of bincluleate *Rhizoctonia* in Egypt should

be considered.

In conclusion, the results of this study suggest that *R.solani* AG-2-2, AG-4 and AG-5 could attack sugarbeet crops in different seasons and different locations in Egypt.

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## تحديد خصائص المجاميع الإلتحامية Anastomosis groups لفطر الريزوكتونيا سولاني وقدرتها المرضية على بنجر السكر في مصر

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يعتبر فطر الريزوكتونيا سولاني أحد ممرضات التربة الهامة لبنجر السكر في مصر – ومن المعروف حاليا ان عزلات هذا الفطر تتضمن ثمانيه عشر مجموعة وتحت مجموعه التحامية AG، وقد هدفت الدراسة لتحديد المجاميع الالتحامية RG، وقد هدفت الدراسة لتحديد المجاميع الالتحامية بكفر الشيخ لفطر الريزوكتونيا سولاني على بنجر السكر في منطقتي الزراعة الرئيسية بكفر الشيخ والدقهلية وكذلك دراسة القدرة المرضيه لتلك العزلات.

تم عزل ٤٢ عزله ريزوكتونيا من بادرات وجذور نباتات بنجر السكر وحيث أتضح ان عدد ٤٢ عزله كانت المدريزوكتونيا سولانى - بينما كانت احدى العزلات لفطر ريزوكتونيا شغزله بمصر.

- قسمت عزلات الريزكتونيا سولاني الى ثلاثة مجاميع التحاميه وهي:

AG-2-2 AG-2-2 بنسبة TA, V - AG-4 بنسبة TA, V - AG-4 بنسبة TA, V - AG-4 بنسبة TA, V - AG-2-2 العزلات المتحصل عليها من الجذور الكبيرة المصابة تنتمي للمجموعة TAG-2-2 بينما TAG-2-2 من العزلات من البادرات كانت تنتمي للمجموعة TAG-2-2 وقد عزلت TAG-2-2-2 من بادرات مصابة من محافظة كفر الشيخ.

- أوضحت دراسة القدرة المرضية أن العزلات التابعة للمجموعة AG-4كانت أكثر إمراضيه على البادرات مسببة موت بادرات بدرجة أكبر من AG-5, AG-2-2 على التوالى - بينما سببت العزلات AG-2-2A عفن لجذور النباتات الكبيرة بدرجة أكبر كثيرا من العزلات الأخرى.

- كانت عزلة الريزوكتونيا ثنانية النواه غير ممرضة للبادرات أو للجدور الكبيرة لبنجر السكر وأدت لزيادة كبيرة للوزن الجاف للبادرات المحقونة كما أدت معامله التربه بتلك العزله الى تثبيط فى موت البادرات الناشئ عن الريزوكتونيا سولانى بدرجة كبيره - وهو ما يوضح أن هذه العزله تحتاج مزيد من الدراسة لإمكانية إستخدامها فى المقاومة البيولوجية لأمراض التربة المتسببة عن فطر الريزكتونيا سولانى.