ANASTOMOSIS GROUPS OF *RHIZOCTONIA SOLANI* ISOLATED FROM SUGARBEET AND THEIR VIRULENCE IN RELATION TO CROP SEQUENCE

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Abstract

Eight isolates of *Thanatephorus cucumeris* Frank (Donk) (*Rhizoctonia solani* Kuhn) isolated from sugarbeet seedlings and roots were multinuclate. Considering anastomosis, the isolates fell into two groups corresponding to AG-2 and AG-4.

A greenhouse test was conducted to determine the effect of cotton, soybean, sugarbeet, beams and corn as crops preceding sugarbeet on Rhizoctonia seedling disease of the latter.

All isolates of AG-2 and AG-4 showed different degrees in infection percentage and disease severity on seedling stage (15 and 30 days) on all crops.

Cotton, soybean and sugarbeet significantly increased pre- and post-emergence damping-off, while beans and corn were less conducive. Therefore, lower disease incidence in pre- and post-emergence stages could be obtained by not following cotton, soybean, or sugarbeet.

Advantage of reduced damping-off in sugarbeet following corn in rotation is apparent.

INTRODUCTION

Rhizoctonia solani Kuhn (*Thanatephorus cucumeris* Frank (Donk) is a common soil borne plant pathogen of commercially grown crops in Egypt.

The fungus causes damping-off, root rot, crown rot and foliar blight of sugarbeet (Kotila, 1947; Ashour *et al.*, 1965; Rupple, 1972, El-Kholi, 1979 and 1984 and

Witney and Duffus, 1986).

Rhizoctonia solani is currently classified into several anastomosis groups (AG). A review by Ogoshi (1987) stated that there are at least nine anastomosis groups of *Rhizoctonia solani* (Telemorph: *Thanatephorus cucumeris* (Frank) Donk, and one of these groups is further divided into two subgroups (AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-5, AG6, AG-7, AG-8 and B1). Corling *et al.* (1987) has described a new anastomosis group, AG-9 in Alaska.

Crop rotation has been recommended as a control of sugarbeet (*Beta vulgaris* L.) root rot caused by *Rhizoctonia solani* (Maxson, 1948; Schaster and Harris, 1960 and El-Kholi, 1984).

Greenhouse and field inoculation by Schuster and Harris (1960), with seven isolate of *Rhizoctonia solani*, indicated that corn and beans were nonhosts for sugarbeet, isolates which were pathogenic to potato. In 4 or 6-year rotation, however, it made little difference whether potato or bean preceded sugarbeet. Also, *Rhizoctonia solani* is a ubiquitous pathogen of many crops and weeds species, including alfalfa, barley, bean corn, potato, sorghum and wheat. These crops are usually planted in rotation with sugarbeets in Colorado and adjacent states. Little damage attributed to *Rhizoctonia solani* infection has been observed, however, in various crops planted immediately after a sugarbeet crop heavily infected with the pathogen.

The objectives of this study were to characterize anastomosis groups of *Rhizoctonia solani* isolated from disease sugarbeet seedlings and roots and their relative virulence to other crops included in the crop rotation.

MATERIALS AND METHODS

Sampling and isolation:

Rhizoctonia - infected sugarbeet seedlings, older plants and plant debris in sugarbeet fields were collected from the Governorate of Kafr El-Sheikh and adjacent areas in Egypt. The decayed or lesioned portions of the root were selected and cut into pieces (1x0.5 cm). Root pieces were surface-disinfected in 0.5% sodium hypo-

chlorite for 15 sec., rinsed twice in sterile distilled water and incubated at 20-25°C for 2-5 days. Root pieces were examined microscopically, then dried on filter paper placed on potato dextrose agar (PDA) or on Ko and Hora's medium (1971) and incubated at 20-25°C for 5-7 days. Vultures of Rhizoctonia were transferred to water agar (WA) containing streptomycin sulfate (30 mg l). After 1-2 days, each culture was hyphal tipped to PDA and incubated at 20-25°C.

Identification and Characterization:

Characteristics of the septal pore apparatus and numbers nuclei in vegetative cells were recorded for each culture. Mycelia (2 or 3 days' old) were stained with 0.5% safranin 0 and 3% KOH. Stained mycelia were examined microscopically at 400x according to Bandoni (1979).

Anastomosis Grouping:

Rhizoctonia isolates were subjected to hyphal anastomosis pairing by using the tester isolates (ATCC) AG-1, AG-2, AG-2-1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8 and B1, supplied by W.M. Bugbee, USDA, ARS, Northern Crop Science Laboratory, North Dakota State University, Fargo, ND, 58105 USA. Hyphae were paired on sterile microscopic slide coated with 2% WA and incubated at 20°C for 24-48 hr., until the hyphae stored to overlap and intermingle. Mycelial growth was then stained with lactophenol-cotton blue and microscopically examined by hyphal anastomosis (Parmeter *et al.* and Herr and Roberts, 1980). At least two slides were examined to verify each pairing before identification.

Pathogenicity test (crop rotation):

Test A:

Eight isolates of *Rhizoctonia solani* were tested for their ability to cause seedling damping-off in cotton (*Gossypium hirsutum* L.) variety Stardel, soybean (*Glycine max* L.) variety Maple Amber; Red kidney bean (*Vigna sinensis* Endl.) variety Charlaovix, sugarbeet (*Beta vulgaris* L.) variety Ultramono and corn (*Zea mays* L.) variety A66X M105. These crops are usually planted in rotation with sugarbeet in Kafr El-Sheikh Governorate and adjacent areas.

Test B:

Sugarbeet (variety Ultramono) seeds were planted following all crops in the same pots, after being harvested in (Test A). Surviving plants were recorded at 15

and 30 days and disease severity was recorded twice. Five replicates were used in a randomized complete block design.

Inoculum preparation:

Inoculum of *Rhizoctonia solani* was prepared according to Pierson and Gaskill (1961).

Statistical analysis:

Statistical analysis of variance and mean separation were performed. A significance level of p=0.05 was used in all statistical tests.

Disease severity in five replicates, except when mentioned, was rates on a 0-4 scale according to Bolakan and Ribeiro (1985) as follows:

0 : Symptomless plants (healthy).

1 : 1 -25%.

2 : 26-30%.

3 : 51-75%.

4 : Seedling killed.

RESULTS

Characterization of Rhizoctonia isolates:

Eight isolates of Rhizoctonia, originating from diseased sugarbeet seedlings and roots were multinucleate and showed typical characteristics of *Rhizoctonia solani* as described by Parmeter *et al.*, 1969; Sherwood, 1969 and Ogoshi, 1987. These isolates were identified to the anastomosis groups AG-2 and AG-4.

In AG-4, mycelium light to dark camel brown, appressed to moderately aerial, uniform texture and color over the colony, Sclerotia rare, usually small and rounded. This was represented by isolates R3, R4 and R6.

In AG-4, mycelium whitish to light brown, compact, aerial, sclerotia few,

variable in size, usually small light brown, flat to round as in isolates R1, 2, 5, 7 and 8.

Pathogenicity test on seedlings the greenhouse (crop rotation):

Test A:

Cotton, sugarbeet, beans, soybean and corn were planted in a crop rotation in the North Delta to determine their reaction to *Rhizoctonia solani* sugarbeet isolate, Pre- and post-emergence damping-off was recorded under greenhouse conditions 15, 30 days after planting.

Date in Table (1) show that both cotton and sugarbeets were highly susceptible fifteen days after planting, during the pre-emergence damping-off, with 27.5 and 23% reduction in stand, respectively. Beans and soybean were less affected during the same period and corn was the least affected during the pre-emergence damping-off, with reduction of only 7.0%. Thirty days after planting, during the post-emergence damping-off, sugarbeet, beans, soybean and cotton, were the most affected in stand, showing 58, 51, 49.5 and 47% infection, respectively. Corn showed the least infection in this stage, with 13% reduction, Table (1). The disease was most severe on sugarbeet, beans, soybean, as it was, 46.625, 44.000 and 33.37%, respectively, while corn was less affected at 6.25%.

Isolates of *Rhizoctonia solani* uses in these studies differed in their virulence on hosts included in crop rotation i.e., cotton, sugarbeet, beans, soybean and corn. Results in Table (1) show that during the pre- and post-emergence damping-off stages 13, 30 days after planting and severity percentage, at isolates No. 8, 7, 5 and 1 caused highest infection; isolates 4, 2 and 3 were less virulent and isolate No. 6 showed the least virulence to all crops tested in the experiment.

Test B:

Rhizoctonia damping-off disease increased after 15 days, when cotton, soybean and sugarbeet, preceded sugarbeet and decreased in sugarbeet planted after corn at 15 and 30 days intervals.

When beans preceded sugarbeet the percentage of pre- and post-emergence damping-off, 15 and 30 days from planting, were intermediate. On the other hand, infection with *Rhizoctonia solani* was more severe when sugarbeet came after soybean or cotton, less in beet after beet and beans and least in beets following corn,

Table 1. Effect of pathogenic *R. solani* isolates on pre- and post emergence damping-off and severity of disease on crops (Test A).

Crops	Infection %	Grouping	*R. solani* isolates	Infection %	Grouping
	Planted after 15 days				
Cotton	27.5	A	8	30.4	A
Sugarbeet	23.0	AB	7	22.4	AB
Beans	18.0	B	5	21.6	AB
Soybean	15.5	B	1	20.8	AB
Corn	7.0	C	4	20.0	CBD
			2	13.6	CBD
			3	10.4	D
			6	6.4	D
L.S.D. at 0.05 level 7.6738				9.7666	
	Planted after 30 days				
Sugarbeet	58.0	A	8	65.6	A
Beans	51.0	AB	7	57.6	AB
Soybean	49.5	AB	5	48.8	CB
Cotton	47.0	B	1	43.2	CD
Corn	13.0	C	2	38.4	CED
			3	35.2	ED
			4	32.8	ED
			6	28.0	E
L.S.D. at 0.05 level 7.6738				12.635	
	Severity % after 30 days				
Sugarbeet	46.625	A	8	56.6	A
Cotton	44.000	AE	5	48.6	A
Beans	35.5000	CE	1	47.0	A
Soybean	33.375	C	7	45.6	A
Corn	6.250	D	4	22.6	B
			2	19.6	BC
			6	16.2	BC
			3	9.6	C
L.S.D. at 0.05 level 7.6738				12.454	

* Means with the same letter are not significant different at P=0.05 by Duncan's multiple range test. Means based on 5 replications per Cultivar (1955).

Table 2. Effect of proceding crops on pre- and post emergence damping-off and severity of disease caused by *R. solani* isolates on sugarbeet plants in the same pots (Test B).

Crops	Infection %	Grouping	*R. solani* isolates	Infection %	Grouping
	Planted after 15 days				
Cotton	57.0	A	8	67.2	A
Sugarbeet	55.5	A	1	57.0	B
Beans	40.5	B	2	46.4	BC
Soybean	34.0	BC	7	43.2	BC
Corn	22.5	C	5	41.6	BC
			4	39.2	BC
			3	34.4	C
			6	16.0	D
L.S.D. at 0.05 level 11.314				14.387	
	Planted after 30 days				
Cotton	71.0	A	8	92.0	A
Soybean	70.5	A	7	77.6	B
Sugarbeet	58.5	B	1	68.8	BC
Beans	52.0	B	5	56.8	DC
Corn	41.5	C	2	54.4	D
			4	46.4	D
			3	45.6	D
			6	28.0	E
L.S.D. at 0.05 level 10.249				12.963	
	Severity % after 30 days				
Soybean	63.625	A	8	91.2	A
Cotton	63.500	A	7	62.8	B
Sugarbeet	42.125	B	1	59.0	BC
Beans	37.125	B	5	47.0	DC
Corn	23.500	C	2	43.2	DE
			4	33.0	FE
			3	21.0	FG
			6	10.6	G
L.S.D. at 0.05 level				13.002	

(Table 2). When sugarbeet was planted after cotton, soybean, sugarbeet or corn, *Rhizoctonia isolate* No. 8 showed the highest Pathogenicity followed by isolate No. 1, 2, 7 and 5, while isolates 4 and 3 were intermediate in their effect. On the other hand isolate 6 caued the lowest damping-off after 15 days.

After 30 days, post-emergence damping-off was clear with isolate No. 8 causing 92% damaging sugarbeet following all crops under the test, followed by isolates No. 7, 1 and 5, while isolates No. 2, 4 and 3 were intermediate. Isolate no. 6 was the least virulent on all crops, causing 28% post-emergence damping-off for all crops (Table 2).

Severity data were in the same order as for the 30 days data. Rhizoctonia isolates were most effective during pre- and post-emergence damping-off and severity, at 15 and 30 days when sugarbeet was planted after cotton or soybean. Isolates were least effective when planting after corn. Also, Rhizoctonia isolate No. 8 was the most virulent during the pre- and post-emergence damping-off and caused more severe infection on all crops. *Rhizoctonia solani* isolates 7, 1 and 5 were intermediate in their effect, isolates 2, 4 and 3 were less effective and isolate No. 6 showed very little incidence on all crops under test.

DISCUSSION

Anastomosis among 8 isolates of (*Thanatephorus cucumeris* (Frank) Donk.) (*Rhizoctonia solani* Kuhn) isolated from sugarbeet seedlings and roots showed that these isolates fell into two anastomosis groups, AG-2 (isolates 3, 4 and 6) and AG-4 (isolates 1, 2, 5, 7 and 8) as described by Parmeter *et al.*, 1969; Sherwood, 1969 and Ogoshi, 1987. Rupple (1985) tested 10 Colorado sugarbeet isolates of *Rhizoctonia solani* isolated from root rot of sugarbeet and belonging to anastomosis group 2 (AG-2). Also, Windels and Nabben (1989) found that from 361 cultures of Rhizoctonia isolates from sugarbeet seedlings, 325 cultures were multinuclate with characteristics typical of *Rhizoctonia solani* and were identified to six anastomosis groups, with AG-4 predominating followed by AG-5 and AG-2-2.

Crop rotation is a common practice for improving crop production and reduc-

ing disease incidence. Many authors included crops such as cotton (Rogers, 1942), wheat (Rovira, 1986), corn (Sumner *et al.*, 1981) and potato (Goss and Afanasiev, 1938). The latter indicated its general effectiveness in controlling a wide variety of foliar and soil born plant pathogens. Numerous studies have been conducted to determine the appropriate crops to rotate with sugarbeet. Many of these have been agronomic in nature and made no mention of disease incidence, Grimes (1959), Stockinger *et al.* (1963).

Relatively few studies involving crop rotation and disease intensity in sugarbeets has been conducted. Most have evaluated how specific crops and crop sequences affected Rhizoctonia root rot development El-Kholi (1984), Rupple (1985) and Schuster and Harris (1960).

Rhizoctonia solani can survive saprophytically and colonize crop residues. In the present study, the high levels of residues incorporated into the soil of cotton, soybean and sugarbeet-planted pots may have resulted in elevated pathogen populations. This could readily explain the increased disease incidence in treatments other than beans and corn. Increased residue levels of certain plants may have affected disease incidence by creating a favorable environment for disease development. Sugarbeet following bean, or corn showed low level of disease, whereas beets after cotton, soybean and sugarbeet suffered high levels of infection. Results of the host range study incidence that, of the crops tested, corn is the only nonhost crop to precede sugarbeet where Rhizoctonia seedling is endemic.

Greenhouse studies and field observations by Coons and Kotila (1935) showed that corn reduced and alfalfa increased sugarbeet damping-off when these crops preceded sugarbeets. Also, Schneider and Robertson (1975) found that black root severity was higher after alfalfa than after corn or soybeans. Crown rot incidence was higher where beets followed alfalfa than where beets followed corn, soybean or navy beans.

It is probable that the fungus *Rhizoctonia solani* when living saprophytically during a season would be less virulent than in the presence of a host. This may show why *Rhizoctonia solani* was more virulent after cotton (host) than after corn (nonhost). Other soil microflora could be involved and their behavior in response to different plant species may affect the behavior of the pathogen.

Generally, rotation with non hosts can be practised to reduce disease incidence in subsequent host crops; however, lack of definite conclusions concerning the

reasons for the observed results indicates a need of additional studies.

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علاقة عزلات الفطر ريزوكتونيا سولانى المعزولة من بنجر السكر بتعاقب المحاصيل ودراسة خصائص تلك العزلات لظاهرة الـ ANASTOMOSIS فى مصر

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قسم بحوث الآفات (الامراض والحشرات) معهد بحوث المحاصيل السكرية - مركز البحوث الزراعية - الجيزة - مصر

عزلت ثمانى عزلات من الفطر ريزوكتونيا سولانى (وطوره الكامل: ثاناتيفورس كيوكيموريس) من بادرات وجذور بنجر السكر لها صفة تعدد الانوية وتقع العزلات تحت مجموعتين هما AG-4 و AG-2 من مجاميع التوافق الخضرى Anastomosis.

أجريت الاختبارات فى الصوبة لدراسة تأثير محاصيل القطن - فول الصويا - بنجر السكر - الفاصوليا - والأذرة على مرض موت البادرات فى بنجر السكر والمتسبب عن عزلات الفطر ريزوكتونيا وذلك فى تعاقب (دورة زراعية) مع محصول بنجر السكر فى تواجد نفس العزلات للفطر الممرض.

أظهرت العزلات المختلفة الثمانية والتى تقع تحت مجموعتين AG-2 و AG-4 درجات مختلفة فى قدرتها على احداث الاصابة وشدة الاصابة للأعمار ٢٥ و ٣٠ يوم لكل من المحاصيل المختبرة.

ازدادت نسبة موت البادرات قبل وبعد الانبات معنويا فى الأصص المنزرعة بالقطن وفول الصويا وبنجر السكر على التوالى بينما انخفضت فى حالة الفاصوليا والأذرة.

أوضحت النتائج أن زراعة بنجر السكر بعد قطن أو فول الصويا أن بنجر السكر يزيد من نسبة حدوث موت البادرات قبل وبعد الإنبات بينما قلت نسبة الضرر عند زراعة بنجر السكر عقب ذرة.