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Some characteristic of *Rhizoctonia* spp. in sharp eyespot of wheat

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Isolates of *Rhizoctonia* sp. with multinucleate and binucleate cells were obtained from sharp eyespot lesions on wheat culms in Olsztyn region. (NE Poland). These isolates were compared to isolates of AG-4 and GAG-1 testers with reference to cultural morphology of colony, growth rate, hyphal anastomosis and pathogenicity to wheat seedlings. The wheat binucleate isolates were similar in morphology of colonies and anastomosed with the *Ceratobasidium* anastomosis group GAG-1 tester isolates of *R. cerealis*. Growth rates on PDA ranged from 9 to 11 mm/24h for wheat isolates and from 1 to 11 mm/24 h for tester isolates GAG-1 of *R. cerealis*. The wheat multinucleate isolates were similar in morphology of colonies and anastomosed with *Rhizoctonia solani* Kühn group AG-4 tester isolate. *R. solani* AG-4 isolates were morphologically distinct from the *R. cerealis* isolates. These isolates on PDA were dark and grow rapidly (20-30 mm diam./24 h/20°C) and significantly contrasted with slowly growing white-creamy isolates of *R. cerealis* (GAG-1). Isolates of *R. solani* (AG-4) and *R. cerealis* (GAG-1) developed sharp eyespot lesions on culms and white head symptoms typical of the disease. None of the wheat isolates of *R. cerealis* (GAG-1) caused root-rot on wheat seedlings. In the present work the classification system of vegetative groups of *Rhizoctonia* spp. in present work is also discussed.

Key words: *Triticum aestivum, Rhizoctonia solani, R. cerealis*, anastomosis group, sharp eyespot disease, cultural characteristes, pathogenicity.

INTRODUCTION

Sharp eyespot is a common disease of *Triticum aestivum* in the temperate regions of the world. The disease generally and frequently occurs in association with more damaging rot-stem diseases (Bruehl, 1951; Pitt, 1964; Reinecke, Fehrmann, 1979; Lipps, Herr, 1981, 1982; Carling, Summer, 1982; Clarkson, Cook, 1983; Hollins, Jellis, Scott, 1983; Hollins, Scott, 1983; Deacon, Scott, 1985; Moen, Harris, 1985; Carling, Leiner, Kebler, 1986; Ogoshi, Cook, Bassett, 1990).

The first report of sharp eyespot on wheat was presented by Sprague (1934). Later Sprague (1937) attributed the disease to the presence of *Rhizoctonia* sp. Based on morphological characteristics Blair (1942) identified the fungus isolated from sharp eyespot lesions on wheat stems from Canada and England as *Rhizoctonia solani* Kühn. Further works (Glynne, 1950; Pitt, 1964; Carling, Summer, 1992; Clarkson, Cook, 1983) supported Blair's identification of *R. solani* as the casual agent of sharp eyespot. Recently, Stern and Jones (1978) indicated *R. solani* as the cause of sharp eyespot and assigned it to the hyphal anastomosis group AG-4 as erected by Parameter, Whitney, Platt (1967). Stern and Jones (1978) reported that only AG-4 isolates of *R. solani* caused damping-off and sharp eyespot lesions on wheat. In 1977, Van der Hoeven described a new species, *R. cerealis*, as the cause of sharp eyespot lesions on wheat in the Netherlands. *R. cerealis* differs from *R. solani* in having predominantly binucleate hyphal cells and a relatively slow growth rate. Thus, two different fungi resembling one another in morphological feature cause similar symptoms on small grains (Parameter, Whitney, Platt, 1967; Sherwood, 1969; Parameter, 1970; Ogoshi, 1972, 1975, 1985; Ogoshi, Omiki, Sakai, 1979; Ogoshi, Omiki, Araki, 1983; Burpee, 1980; Burpree, Sanders, Cole, 1980; Martin, Lucas, 1984; Murray, Burpree, 1984; Carling, Leiner, Kebler, 1986; Oniki, Ogoshi, Araki, 1986; Kataria, Hoffman, 1988; Mordue, Carrah, Bridge, 1989; Sneh, Burpree, Ogoshi, 1991; Carling, Summer, 1992).

Grouping based on hyphal anastomosis among isolates having common biological affinities has greatly facilitated the identification of *R. solani*. Burpree, Sanders, Cole (1980) observed hyphal anastomosis among isolates of *Ceratobasidium cornigerum* (Bourd) Rogers and related fungi having *Rhizoctonia* imperfect states. Based on hyphal pairing, seven *Ceratobasidium* anastomosis groups (GAG) were established. Within each GAG, little homogenity occurred among isolates with respect to host, except for GAG-1 isolates, which were associated with members of the *Gramineae* (Murray, Burpree, 1984; Oniki, Araki, 1986; Kataria, Hofman, 1988; Sneh, Burpree, Ogoshi, 1991; Burpree, Martin, 1992). Burpree (1980) later found that isolates assigned to CAG-1 anastomosed with the type culture of *R. cerealis*, demonstrating that isolates of *R. cerealis* comprise a common anastomosis group CAG-1. He reported that isolates of *R. cerealis* from the United States and elsewhere were the cause of yellow patch of turfgrass. *R. cerealis* has been reported as the cause of sharp eyespot in the Netherlands, Germany, and South Africa (Scott, Visser, Ruzenacht, 1979; Lipps, Herr, 1982; Hollins, Scott, 1983; Moen, Harris, 1985; Deacon, Scott, 1988; Kataria, Hoffman, 1988; Ogoshi, Cook, Bassett, 1990).

Plants with white heads and sharp eyespot lesions on the base of culms were found in several locations during a survey of wheat fields in Poland. Fungi resembling *R. solani* were isolated from these lesions. However, these isolates grew more slowly and had narrower hyphae than typical isolates of *R. solani*.

The purpose of this study was to identify the isolates obtained from sharp eyespot lesions and to determine their pathogenicity to wheat.

MATERIALS AND METHODS

I s o l a t i o n a n d s o u r c e s. Wheat culms with sharp eyespot lesions were collected from commercial fields in Olsztyn region and plots in Experimental Station Bałcyny in the years 1994-1996. Plants samples were obtained from the experimental fields under the crop rotation: sugar beet-spring wheat-winter barley amended with different organic manures (biological compost, straw and stable manure) and four levels of mineral nitrogen (0, 30, 60, 90, 120 N/hectare). Spring wheat cultivar 'Inga' was cropping as follows: 1994 – wheat, 1995 – sugar beet following wheat, 1996 – spring barley following wheat.

The crop rotation was established in 1994 year. *Rhizoctonia cerealis* and *R. solani* were isolated from diseased plant using standard procedure.

S e l e c t i v e m e d i a. 1) Ko-Hora medium amended: benomyl – 500 mg/l, prochloraz – 500 mg/l (K o, H o r a, 1971).
2) 2 % water agar (WA) amended with ethanol – 20 ml/l, $NaNO_3$ – 5 g/l, KH_2PO_4 – 0.5 g/l (T r u j i l l o et al., 1987).
3) 2 % WA amended with fungicides: benomyl (Benlate 50 WP), penycurone (Monceren 25 WP), cyproconazole (Alto 25 WP), toclosfosmethyle (Rizolex 10 WP) each 500 mg/l (S u m m e r, 1987; K a t a r i a, H o f f m a n, 1988; K a t a r i a, G i s i, 1989).

In comparative tests (V i n c e l l i, B e a u p r e, 1989) these media were rated equally effective in the selective recovery of *Rhizoctonia* spp. from soil and plant samples. Both media were dispensed at approximately 12 ml per Petri dish (PD) (9 cm in diam.). The dishes with three selective media were used for plant samples assay. Tissue sections 1-1.5 cm long were cut from the margins of lesions, washed under tap water, rinsed with an antibiotic solution (10 mg chloramphenicol and 100 mg of neomycin per 1l of distilled water) and transferred aseptically to Ko-Hora medium and to two other selective media. After the tissues had been grown for3 days at 21-22°C in the dark, cultures were examined at 400 x for mycelia of *R. solani.*

N u c l e a r s t a i n i n g. Cultures on PDA were stained by a rapid technique with 0.5 % aniline blue and by a HCL-Giemsa nuclear staining procedure, which to allowed count of nuclei in vegetative cells (H e r r, 1979).

H y p h a l a n a s t o m o s i s. Hyphal anastomosis was observed on agar-coated slides. All the wheat culm isolates were paired with GAG-1 and AG-4 tester isolates to determine their affinities. Isolates of *R. cerealis, R. solani* were paired in all possible combinations with representative isolate from deseased culms. GAG-1 (*R. cerealis*) tester isolate was provided by Prof. Burpree, Canada and AG-4 by Prof. Carling, USA.

G r o w t h r a t e a n d c h a r a c t e r i s t i c s o f c o l o n i e s. Agar discs (5-7 mm in diam.) cut from margin of actively growing colonies on PDA were transferred to PD containing 15 ml of PDA freshly prepared from potatoes. Three dishes of each isolate were incubated at 22-23°C in the dark. Two measurements at right angles were taken and the increase in colony diameter between 24 and 48 hour of growth was recorded. The morphology and colour of colonies were compared during the first week of growth and after 24 days.

P a t h o g e n i c i t y t e s t s. Oat kernel inoculum was prepared by autoclaving 100 ml of whole oat kernels and 50 ml of distilled water in a 250 ml Erlenmeyer flask for 1 hour on two consecutive days. Colonised agar discs of isolate tested were transferred to flasks and incubated at 23-26°C for 14-21 days prior to use. Seeds of spring wheat cv. 'Inga' were surface sterilized in a 95 % ethyl alcohol for 30 seconds, then rinsed with sterile water and planted wet on sterile sand in a plastic pot 15 cm diam. Five replicate pots were used per isolate. Seedlings were inoculated by placing one infected oat kernel 1 cm below the sand in contact with the coleoptile. After 21 days, plants were washed free of adhering sand and rated for development of lesions.

The ability of isolates of *Rhizoctonia* sp. obtained from culms to cause root-rot was tested using inoculum layer technique (L i p p s, H e r r, 1982). Plastic pots with sterile sand as described above were used. A completely colonized 2 % WA layer inoculum from a PD culture was placed to cover the top of the sand. A noncolonized agar layer was used for the control. Seven surface-sterilized wheat seeds were arranged on top of the agar inoculum and then covered with 50 ml of the sand. Five replicate pots were randomized and plants were maintained at 10-25°C. Seedlings were washed free of adhering sand and both root length and fresh weight of tops were recorded.

A g a r - p l a t e v i r u l e n c e a s s a y. Seeds were washed in a 0.3 % sodium hypochlorite solution in deionized water for 5 minutes, rinsed in deionized water and air-dried before use. The seeds were placed in a circle 1 cm from the edge of a 15 cm diam. sterile, disposable PD 15 cm in diam. containing 20 ml of 1.5 % WA. About 1 mm diam. mycelial disc from the edge of a 2-3 day old 1.5 % WA culture of each of the isolates was transferred aseptically to the centre of each disc (one/disc). Control plates have a noncolonized agar dishes.

Two days after inoculation, 3-5 drops of sterile distilled water were dispensed aseptically onto each seed. Dishes were sealed at two points with clear adhesive tape and incubated in continous darkness at room temperature for 5 days. Dishes were then placed in a laboratory and opposed to light for 12 hours (one day). The percentage of seedlings with infected roots and/or coleoptile and disease severity on individual seedlings was recorded 9 days after inoculation. Disease severity was rated based on a 1-5 scale: 1 = no symptoms, normal root development; 2 = localized tissue discoloration without necrosis, near-normal root development; 3 = localized lesions with extensive tissue discoloration, near-normal root development; 4 = nearly

complete root necrosis, partially restricted root legth; and 5 = complete root-rot, length severely restricted. A dishe containing 10 seedlings represented one replication. The experiment was conducted four times, with each repetition in time representing one block of randomized complete design.

RESULTS

Altogether 110 isolates belonging to *Rhizoctonia* spp. were isolate from wheat culms with sharp eyespot lesions. Among these isolates 61 % were assigned to *R. cerealis* (GAG-1), 22 % to AG-4 while 17 % were unassignable to any of the anastomosis group. These isolates did not anastomosed neither with tester GAG-1, or with the tester of AG-4 and all had binucleate hyphal cells. All the isolates from wheat which anastomosed with tester GAG-1 had binucleate cells. All the isolates of *Rizoctonia solani* Kühn anastomosing with testers AG-4 had 5-7 nuclei per cell. Using hyphal anastomosis tests, hyphae of wheat isolates of *R. cerealis* and of the GAG-1 tester isolate fused with one another. Wheat isolates of *R. solani* belonging to AG-4 group fused with AG-4 tester isolates, confirming common anastomosis affinities among isolates tested. Other wheat isolates of binucleate *Rhizoctonia* spp. failed to anastomose with the GAG-1 tester and one another.

C h a r a c t e r i s t i c s o f c o l o n i e s. All the binucleate isolates of *Rhizoctonia* obtained from wheat culms formed yellow-white to light-tan coloured mycelium on PDA during the first week of growth. In some cultures, mycelial pigmentation increased with age, resulting in light-tan coloration after 23 days of growth. Sclerotial development varied greatly among isolates. Some produced or no very few sclerotia and others formed many darkly pigmented sclerotia covering the agar surface. Some isolates which failed to anastomose with CAG-1 produced no sclerotia covering hyphal remained yellow-white during 24 days of the study. Hyphae of all the wheat isolates studied ranged from 3.0-7.5 µm in diameter. Most of the isolates of *R. cerealis* had white-cream mycelium which remained creamy even after 3 weeks.

G r o w t h r a t e s. The growth rate of the CAG-1 tester isolate (11 mm/24 h) was within the range of growth raters of the wheat isolates that were assigned to CAG-1 (10.7-11.5 mm/24 h). Some isolates of *R. cerealis* grew slowly than the tester CAG-1 (7.4 mm/24 h). The binucleate isolates which failed to anastomose with tester isolates GAG-1 grew slowly than the GAG-1 isolates (14.9 mm/24 h).

The multinucleate isolates of *R. solani* Kühn belonging to anastomose group AG-4 grew about three times as fast as the wheat isolates assigned to GAG-1 (*R. cerealis*) and other binucleate isolates of *Rhizoctonia* spp. (24.2-28.8 mm/24 h). The growth rate of the AG-4 tester isolate was 29.8-30 mm/24 h. The isolates of AG-4 had width hyphae 5.2-8 µm in diam. on average. The width of the hyphae of

R. cerealis varied considerably and an average hyphal width of *R. cerealis* was significantly lower than that of the all isolates of *R. solani* anastomose group AG-4 [hyphae < 5 μm in diam. (mean 4-5 μm)].

On PDA colonies of AG-4 isolates were white to cream coloured after 4-6 days of growth. Initial colony growth was dense and spidery. Small sclerotia (< 1.0 mm in diam.) were dispersed over the surface of cultures. After 14 days of growth colonies of AG-4 isolates were tan to chocolate brown. Nonrinded chocolate brown sclerotia (2-5.2 mm in diam.) were dispersed over the surface of cultures. Some isolates of AG-4 (ca 2-3 %) had a fluffy appearance on PDA, with abundant aerial tan or chocolate brown mycelium and tufts of monilioid cells adhering to the PD lids.

Pathogenicity tests. All the binucleate isolates of *Rhizoctonia cerealis* (GAG-1) which caused sharp eyespot disease of wheat in the field were pathogenic to wheat seedlings indicating that they were *R. cerealis* (GAG-1). Wheat stems sharply definied with dark-brown margins lesions with creamy central areas were observed in the field. Lesions frequently coalesced into larger multiple patches, extending up stems for several centimeters or girdling the stems. One or more tillers per plant were usually killed prematurely due to the development of sharp eyespot lesions. Death of tillers caused the heads to lose colour resulting in the white-head symptoms.

Variation was found in the virulence of binucleate isolates of *Rhizoctonia* sp. using the wheat seedling assay. Wheat isolates anastomosing with the GAG-1 tester isolate produced sharp eyespot lesions on wheat seedlings but significant differences in the severity of disease occurred. Other of the binucleate isolates of *Rhizoctonia* spp. which failed to anastomose with the GAG-1 tester were non-pathogenic and caused only slight browning on some seedlings. None of the wheat isolates of *R. cerealis* (GAG-1) caused visible root-rot, root stunting or reduction of the top growth o seedlings when roots were allowed to grow through an agar layer colonized by the fungi-tested. Isolates of AG-4 were less pathogenic to wheat compared with *R. cerealis* isolates. Wheat isolates that anastomosed with the AG-4 tester isolate produced sharp eyespot lesions on wheat seedlings but several of AG-4 did not produce symptoms. Some tested isolates of AG-4 caused root-rot on wheat seedlings.

DISCUSSION

The results of this study indicate that the pathogenic binucleate isolates of *Rhizoctonia* spp. obtained from wheat culms with sharp eyespot lesions identified as *R. cerealis* as described by Van der Hoeven and Bollen (1980). The morphology of the wheat isolate cultures paralleled was similar to that of *R. cerealis* reported previously but more variation in colour was detected. Considerable variation in sclerotial production occurred among different isolates of *R. cerealis* from wheat. Burpree (1980) also found sclerotia production to be of limited taxonomic

value. The growth rate of the CAG-1 tester isolate was similar to that of the isolates from wheat. Binucleate isolates which failed to anastomose with the CAG-1 tester isolate grew somewhat faster than the *R. cerealis* isolates. These results indicate that growth rate may facilitate separation of *R. cerealis* from other binucleate isolates. Burpree (1980), Murray, Burpree (1984), Oniki, Ogoshi, Araki (1986), Kataria and Hoffman (1989), Mordue, Currah, Bridge (1989) and Burpree, Martin (1992) also suggested that hyphal anastomosis and host specificity would be valuable characters for identifying *R. cerealis*. In an anastomosis test, hyphal fusion occurred between isolates of *R. cerealis* paired in all possible combinations regardless of their origin. When tested for pathogenicity, only isolates which anastomosed with CAG-1 were pathogenic to wheat seedlings. These findings also support the results of Burpree (1980)), Murray, Burpree (1984), Oniki, Ogoshi, Araki (1986), Kataria, Hoffman (1989), Burpree and Martin (1992) indicating that isolates of *R. cerealis* comprise a common anastomosis group CAG-1.

Other binucleate isolates of *Rhizoctonia* sp. obtained from wheat culms produced no symptoms on inoculated seedlings.

These binucleate isolates of *Rhizoctonia* spp. may have been present on the diseased culms together with *R. cerealis* (GAG-1) and *R. solani* anastomose group AG-4. Because none of these isolates anastomosed with any of the CAG tester isolates, further investigations should be conducted to identify them.

Stern and Jones (1987) reported that most of the isolates of *Rhizoctonia* sp. obtained from diseased wheat culms anastomosed with the AG-4 tester isolate of *R. solani* and that pathogenicity trials proved that the *R. solani* AG-4 isolates caused sharp eyespot lesions on wheat plants grown in fields and in greenhouse. These and other results of studies with *R. solani* and *R. cerealis* indicate that two different fungi species may cause sharp eyespot lesions on cereals. Taking under consideration the above results nuclear staining and observations of hyphal anastomosis is recomended to be used for differentiating isolates of *Rhizoctonia* spp. from wheat and other cereals. Parameter, Whitney, Platt, 1967; Parameter, Sherwood, Platt, 1969; Parameter, 1970; Ogoshi, 1975, 1985; Herr, 1979; Burpree, 1984; Oniki, Ogoshi, Araki, 1986; Mordue, Currah, Bridge, 1989).

Considerable variation in the ability of isolates to parasite roots and culms of cereals has been reported. It has been observed that isolates identified as *R. solani* produced two distinct types of injuries on wheat plants. Isolates from England caused severe root-rot whereas those from Canada, the United States and Australia attacked only the lower culms of wheat plants. The results of the present study and the findings of Bruehl (1951), Pitt (1964), Stern, Jones (1978) and Lipps, Herr (1981) indicate thak isolates from sharp eyespot lesions do not attack roots. The authors reported a severe root-rot of wheat caused by multinucleate isolates

of *R. solani* anastomose groups AG-2-2, AG-4 and AG-8. They also indicated that although roots were severely rotted, culms were never affected by these anastomosis groups of *R. solani*. In view of reports of *R. solani* attacking roots and culms of cereals and the relatively recent use of nuclear staining and hyphal anastomosis as an aid in differentiating isolates and species of *Rhizoctonia* sp. A comparative study should be conducted to determine which isolates or species *R. cerealis* and *R. solani* attack roots and/or culms of plants in Poland.

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Grupy zgodności wegetatywnej grzybów z rodzaju *Rizoctonia* spp. wywołujących łamliwość źdźbła pszenicy

Streszczenie

Z porażonych roślin pszenicy z objawami łamliwości źdźbła wyosobniono wielojądrowe i dwujądrowe izolaty *Rizoctonia* spp. Przynależność izolatów do grup zgodności wegetatywnej oznaczono na podstawie ich zdolności do tworzenia heterokarionów z testerami. Stwierdzono, że łamliwość była wywołana głównie przez dwujądrowe izolaty *R. cerealis* Van den Hoeven [st. dosk. *Ceratobasidium cereale* Murray et Burgee] należące do grupy zgodności wegetatywnej GAG-1. Łamliwość źdźbła pszenicy była również wywołana przez wielojądrowe izolaty *Rizoctonia solani* Kühn [st. dosk. *Thanatheporus cucumeris* (Frank) Donk] należące do grupy zgodności wegetatywnej AG-4. W pracy podano charakterystykę obu izolatów.