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A DIFFERENTIAL MEDIUM FOR DISCRIMINATING BETWEEN *DRECHSLERA BROMI* (DIED.) SHOEMAKER AND *D. TRITICI-REPENTIS* (DIED.) SHOEMAKER

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A B S T R A C T

Drecheslera tritici-repentis (*Dtr*) causes tan spot, one of the important leaf diseases of wheat worldwide. The fungus has a broad host range and causes leaf spots on many members of family Poaceae including smooth bromegrass. *Drechslera bromi* (*Db*), causes brown leaf spotonsmoothgrass. *Dtr* isolates have been grouped into eight races based on their ability to induce two distinct symptoms, necrosis and chlorosis, on appropriate wheat differential genotypes. Of these eight races, race 4 and *Db* induce either symptom except small pinhead size black spots on tan spot differential lines. *Dtr* and *Db* are morphologically very similar and are difficult to discriminate between the two species. *Db* isolates can be characterized as race 4 while *Dtr* isolates recovered from bromegrass are race characterized because both are avirulent on the tan spot wheat differential genotypes. It is imperative to develop a culture medium that could distinguish these two species and avoid the chances of mischaracterization of *Db* isolates as *Dtr* race 4. In this study, we evaluated V8PDA (V-8 juice potato dextrose agar) medium, a routinely medium used to grow *Dtr* and *Db* for spore production, as a differential medium. Forty-single-spore isolates each of *Db* and *Dtr* were grown on V8PDA plates and slants. Colony growth diameter and presence or absence of protothecia data of each isolate were recorded after 6 and 13 days for cultures grown in plates and after 10 and 13 days for cultures grown in slants. All *Db* isolates identity was confirmed by inoculating them individually on seven brown spot susceptible smooth bromegrass cultivars. All *Db* isolates grew slower with average colony diameter 2.5 cm than *Dtr* with average colony diameter 5 cm. Also, all *Db* but not *Dtr* isolates developed protothecia in cultures both in plates and in slants. Based on our results, we recommend V8PDA medium as a differential medium for discriminating between *Dtr*and *D. bromi.*

Keywords: Tan spot, *Pyrenophora tritici-repentis*, Bromegrass, and wheat.

INTRODUCTION

Drechslera tritici-repentis (Dtr), telomorph *Pyrenophora tritici-repentis*, is an important foliar pathogen of wheat (*Triticum aestivum*) causing tan spot worldwide (Hosford, 1982). The fungus has also been reported to be pathogenic on many members of the family Poaceae including smooth bromegrass (*Bromus inermus*) (Ali and Francl, 2003; Hosford, 1971; Krupinsky, 1982; 1986; 1992; Drechsler, 1923; Sprauge, 1950; Howard and Morrall, 1975). The fungus produces lens shaped, tan necrotic lesions with a chlorotic halo on susceptible wheat cultivars. Yield losses due to tan spot ranging from 3-50% have been reported, depending on the cultivar, amount of inoculum, plant growth stage when infected, and pathogen virulence (Shabeer and Bockus, 1988; Rees *et al*., 1982).

Smooth bromegrass, a species from Siberia, is utilized in pasture and wind breaks around wheat fields in the northern Great Plains (Newell, 1973; Berg and Sherwood, 1994). In addition, naturalized stands exist along railroads, state parks, and in grasslands of the northern Great Plains. It serves as a primary host of *Drechslera bromi* (*Db*), the cause of brown leaf spot, and also is an alternate host of *D. tritici-repentis* (Noviello, 1963; Chamberlain and Allison, 1945; Zieders and Sherwood, 1986; Hosford, 1971; Krupinsky, 1992). *Db* and *Dtr* are morphologically very similar and difficult to separate (Shoemaker, 1962). They also look similar on field-collected samples (Krupinsky, 1986) and *Dtr*

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isolated from bromegrass produced symptoms indistinguishable from those caused by *Db* (Shoemaker and Berkenkamp, 1970).

Dtr isolates have been grouped into eight races based on their ability to induce necrosis and chlorosis (nec+chl+), necrosis only (nec+chl-), extensive chlorosis (nec-chl+), and lack of either necrosis or chlorosis (nec-chl-) on appropriate wheat genotypes (Lamari *et al*., 2003) and races 1-5 have been observed in the US (Ali and Francl, 2003). Of eight races described to date, race 4 isolates produce neither symptom, except a small black spot, on susceptible or resistant wheat genotypes. When studying *Dtr* population prevalent on smooth bromegrass, *Db* isolates can be characterized as race 4 as they are not pathogenic on wheat. However, addition of smooth bromegrass to the wheat differential lines or development of a differential medium, which could discriminate between *Dtr* and *Db*, can overcome this limitation. The addition of a smooth bromegrass genotype into the differentials is difficult because: 1) it takes more time to have the seedlings ready as compared to the wheat seedlings; 2) a universally susceptible genotype to *Db* is needed but hasnot yet been identified; and, 3) a loss of time and resources in screening unwanted isolates.

A differential medium looks more promising than a bromegrass differential as it would allow isolates of both species to be distinguished in the laboratory and would save time and greenhouse resources. Shoemaker (1962) used sucrose proline agar medium (SPA) for distinguishing between the two species based on their growth rate, development of protothecia (immature pseudothecia), and pigmentation. In an independent study, Lima bean agar (LBA) and SPA differentiated these two species based on spore production and growth rate, respectively (Krupinsky, 1986). Sucrose proline agar medium requires special chemicals for its preparation and extra time in transferring and culturing every isolate in question. Also, spore production and their counting on LBA is time consuming and labor intensive, especially when a large number of isolates are under investigation. In this paper, V8PDA is evaluated as a differential medium by comparing growth of a large number of isolates from both species. If the two species can be differentiated on V8PDA medium, it will save time and resources by eliminating growing cultures on SPA for identification and spore production steps on LBA, respectively.

MATERIALSAND METHODS

***Drechslera bromi* isolates:** Forty single-spore isolates were obtained from 20 diseased leaf samples of smooth bromegrass. The samples were collected from state parks, national grasslands, and around cultivated wheat fields from Minnesota and North Dakota in July 1997 and from South Dakota in August 1998. Each sample was comprised of 10 to 12 leaves. All leaves of each sample were cut into pieces about 2 cm long. Fifty leaf pieces were chosen randomly and placed in plastic Petri plates (9 cm diameter) with three layers of dampened Whatman #1 filter paper. Ten leaf pieces were placed in each dish. The dishes were incubated for 96 h with alternate cycles of 24 h light at 22 ^{0}C and dark at 16 ^{0}C, respectively. The incubated leaf pieces were examined under a stereoscope for *Db* conidia. Single conidia were picked if present with a flamed sterilized needle, and transferred onto plates of V8PDA (10 g Difco potato dextrose agar, 10 g agar, 3 g $CaCO_3$, 150 ml V8 juice, and 850 ml of distilled sterilized water). One conidium was transferred on each plate. The plates were incubated in darkness at 22 ^{0}C for 12 days or until the colony growth reached about 8 cm in diameter and then the cultures were stored at -20 ^{0}C following the procedure described by Jordahl and Francl (1992). The culture grown in each dish was considered as a single-spore isolate.

Seedling production and pathogenicity: Seedlings of seven smooth bromegrass cultivars Barton, Badger, Carton, Lincoln, Radisson, Rebound, and Saratoga were raised in 9 x 2.5 cm plastic cones containing Fison Sunshine Mix #1 (Fison Horticulture, Vancouver, B.C.) in a greenhouse. Two seedlings were maintained in each cone until they were used.

To obtain spore suspensions, 10 plugs of each isolate, stored at -20 ^{0}C, were plated on V8PDA plates. Two plugs were placed in the center with about 2 cm apart on each dish. The dishes were incubated at 22 ^{0}C under darkness until colony diameter reached about 6 cm. After incubation, the dishes were flooded with sterilized distilled water and the mycelia growth was flattened with the bottom of a flamed test tube. The dishes were incubated with an alternate cycle of 24 h light and dark at 22 ^{0}C and 16 ^{0}C, respectively, for inducing conidiophores and conidia. The conidia were dislodged by adding 30 ml distilled sterilized water to each dish and rubbing with a looped inoculating needle. Spore suspension was adjusted to 3000 spores/ml as described by Jordahl and Francl (1992).

Fourteen seedlings of each cultivar were inoculated with spore suspensions of 25 *Db* isolates individually. The seedlings were placed in a humidity chamber at 100% RH for 24 h. Thereafter, the seedlings were moved to a growth chamber at 22 ^{0}C with 16 h photoperiod and rated for disease reaction 8 days post-inoculation. Seedlings of wheat line ND495 were also inoculated with one isolate of *Dtr* and two isolates of *Db.* Isolations were made from the inoculated seedlings as described earlier except the leaf pieces were incubated for 48 h. Twenty percent of the isolates were repeated once to verify consistency of response.

***P. tritici-repentis and P. bromi growth on sucrose proline agar (SPA) and V8PDA*:** Forty-single-spore isolates each of *Dtr* and *Db* were grown both on slants and Petri dishes containing SPA and V8PDA. Both V8PDA and SPA were prepared as described by Lamari and Bernier (1989) and Dhingra and Sinclair (1985), respectively. *Dtr* isolates were chosen in a way that they should represent all five described races. In addition, the isolates were obtained from durum, hard red spring wheat, winter wheat, and alternate host samples collected from different locations. Each isolate tested was grown on V8PDA to obtain a fresh culture. Twelve plugs, 0.5 cm diameter, of each isolate weremade using a brass cork borer. Three plugs, one per slant, of each isolate were placed on SPA and V8PDA slants. Similarly, the plugs were placed on three SPA plates and three V8PDA plates. Both the slants and dishes were incubated in the dark at 22 ^{0}C for 13 days. The colony diameter of each isolate was measured after 6 and 13 days of growth on SPA and V8PDA plates; and after 10 days and 13 days of growth on slants.

RESULTS AND DISCUSSION

A t-test (two samples assuming equal variance) was conducted to compare the growth rate of *Db* and *Dtr* on V8PDA.All 40 isolates of *Db* grew more slowly than *Dtr* isolates on both sucrose proline agar (SPA) and V8PDA media. The average 6-day old culture colony growth diameter of *Db* was significantly smaller (1.65 and3.19 cm on SPA and V8PDA, respectively) as compare to *Dtr* 5.35 and6.3 cm on SPA and V8PDA [t (26) = 36.23, p =0.001], respectively (Fig. 1 and Table 1).

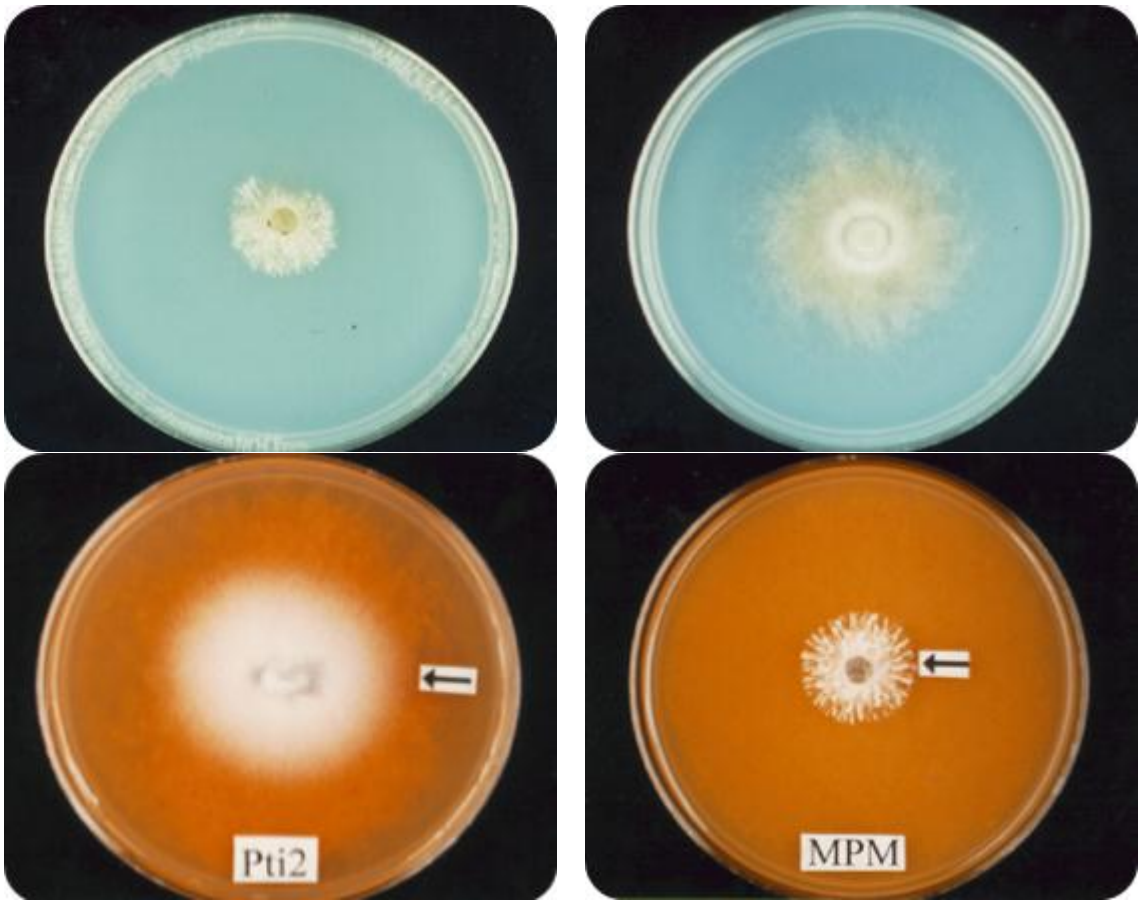

Fig. 1. Six-day old *D. bromi* isolate MPM (top -L) and *D. tritici*-repentis isolate Pti2 (top-R) cultures grown on SPA and on V8PDA (Bottom-L) and (bottom-R). Arrow indicates edge of the hyphal growth.

Table 1.Growth characteristics of *D. tritici-repentis*(*Dtr*) and *D. bromi*(*Db*) on V8PDAand SPA media.

Fungal Species	Colony diameter (cm)				Pigmentation		Psueodthecia		Colony color
	SPA		V8PDA		SPA	V8PDA	SPA	V8PDA	V8PDA
	Average	Range	Average	Range					
Dtr	5.35[a]	4.5 - 6.0	6.3[a]	5 - 7.5	Present	Absent	Absent	Absent	White to grey, dense
Db	1.65[b]	1.4 - 2.1	3.19[b]	2.5 - 4.5	Absent	Present	few	many	White, submerged

The letters a and b in the same column indicate the treatments are significantly different at $p = 0.001$.

Only*Db*7-day old cultures produced pseudothecia on V8PDA (Fig. 2). Similar growth trends of *Db* and *Dtr* were observed when they were cultured on SPA and V8PDA slants. The average colony diameter of 11-day old cultures of *Db* and *Dtr* was 1.04 and 4 cm, respectively. All *Dtr*isolates, except one, 13-day old cultures produced brownish pigmentation in SPA. *Db* produced many pseudothecia on V8PDA (Fig. 2). These results are in agreement with previous studies that showed sucrose proline agar could discriminate between the two species based on their growth rate and pigmentation (Shoemaker, 1962; Krupinsky (1986). The differences in growth rate and presence or absence of pigmentation in SPA verified the identity of *Db* and *Dtr*. The results from this study also confirmed previous studies that *Db* grows very slowly regardless of the medium used (Chamberlain and Allison, 1945; Krupinsky, 1986; Shoemaker, 1962; 1995; Carter and Dickson, 1961; Kaufmann et al., 1961; Tiffany, 1955). Some of the isolates grew fast in this study, but they also produced pseudothecia in six-day old cultures and incited brown spots on the smooth bromegrass cultivars. This result is in agreement with Tiffany (1955), who also found some quick growing *Db* isolates. Shoemaker (1962) stated that quick growing isolates described by Tiffany (1955) might be of *Dtr*. The pathogenicity results of fast growing isolates in this study confirmed the identity as *Db*.

Krupinsky (1986) suggested Lima bean agar as a differential medium for *Db* and *Dtr* based on spore production. Similar results were observed in this study (data not shown). Spore counting is time consuming, especially when a large number of isolates are under investigation. The fungus produced slightly more spores on Lima bean agar than on V8PDA (data not shown). However, this limitation can be overcome by using one extra plate of V8PDA for an equivalent amount of inoculum production.

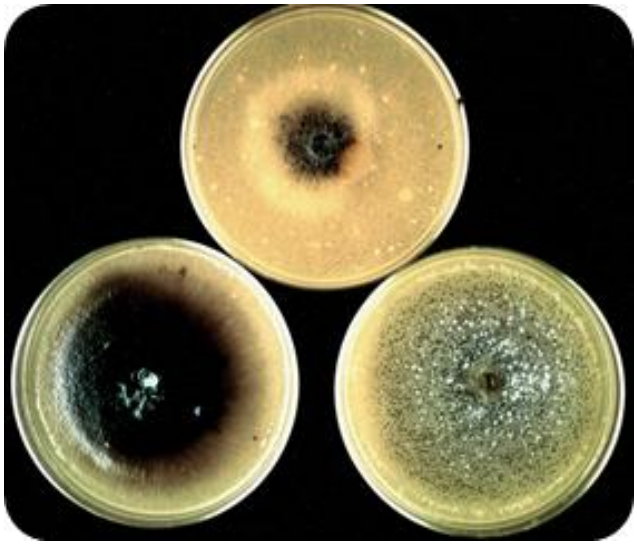

Fig. 2. Seven-day old *D. bromi* culture (top) with protothecia (black dots in black area), seven-day old culture of *D. tritici-repentis* without protothecia (bottom left), and 13-day old *P. bromi* culture with pseudothecia (black dots) (bottom right). All were grown on V8PDA.

Though both V8PDA and SPA works well in differentiating the two closely morphologically related species, V8PDA has several advantages such as 1) no transferring of each isolate needed as they are already on V8PDA, 2) V8PDA requires very few ingredients, 3) less time consuming as the test requires 6 days to complete as compared to 11 days on SPA as suggested by Shoemaker (1962), 4) it is a standard medium for inoculum production for both species (Lamari and Bernier 1989; Ali and Francl, 2003). The main drawback appears to be the scarcity of V8 Juice in some countries; however, substitution of raw ingredients may suffice (Postnikova, 1996).

All 25 *P. bromi* isolates produced brown leaf spots as described by Chamberlain (1945) on the smooth bromegrass cultivars, and the wheat line ND495 developed a few very small black pin head size spots to all *Db* isolates (Fig. 3).

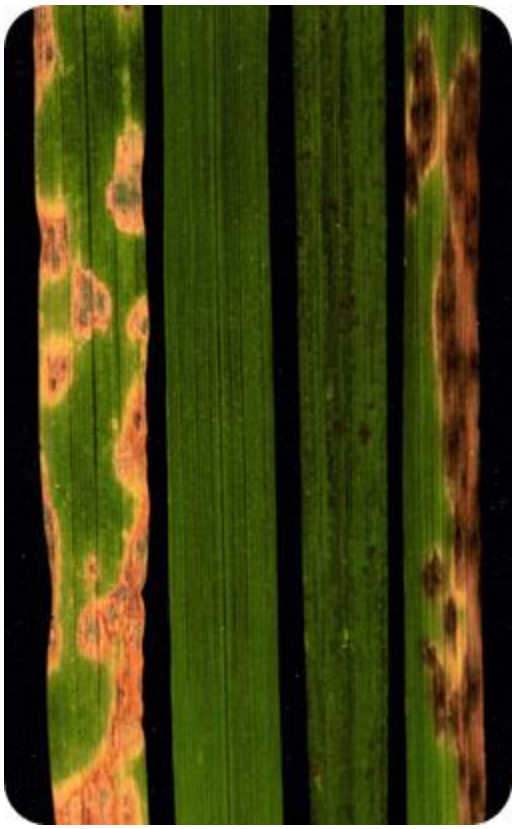

Fig. 3. (L-R). Wheat line ND495 inoculated with *D. tritici-repentis*, inoculated with *D. bromi*, smooth bromegrass cultivar Badger inoculated with *D. tritici-repentis*, and inoculate with *D. bromi.*

In contrast to *Db*, *Dtr* incited small black spots on smooth brome cultivars; whereas, it produced typical tan spot symptoms on ND495 (Fig. 3). These inoculations results verify the identity of isolates of *Db* and *Dtr*. During this experimentation, a little variation was observed among the *P. bromi* isolates based on lesion size and time required for onset of symptoms (data not shown).

Based on this study, V8PDA medium is recommended to separate two closely morphologically related *Drechslera* species as it is easy to prepare, requires few ingredients, is less time consuming and inexpensive, and is good for inoculum production. The main drawback of using V8PDA as a differential medium could be the scarcity of V8 juice in some countries; substitution of raw ingredients may suffice.

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