Wheat Genotypes that Develop Both Tan Necrosis and Extensive Chlorosis in Response to Isolates of *Pyrenophora tritici-repentis*

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ABSTRACT

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Wheat cultivars commonly develop either tan necrosis (nec) or chlorosis (chl) in response to infection by *Pyrenophora tritici-repentis*. We report, for the first time, wheat genotypes capable of developing both symptoms. Isolates of *P. tritici-repentis* can be classified on the basis of their ability to induce tan necrosis and/or extensive chlorosis. A selection of winter wheat cultivar Norstar and spring wheat line 6B699, tested to isolates from pathotypes 1 (nec+ chl+), 2 (nec+ chl−), 3 (nec− chl+), and 4 (nec− chl−), developed tan necrosis in response to nec+ isolates as well as extensive chlorosis in response to chl+ isolates. Both lines were sensitive to the Ptr-necrosis toxin. This finding suggests that tan necrosis and extensive chlorosis are controlled by different genes.

Pyrenophora tritici-repentis (Died.) Drechs. (anamorph *Drechslera tritici-repentis* (Died.) Shoem.) is an important leaf spot pathogen of wheat (*Triticum aestivum* L.) worldwide (5). Hosford (4) described the symptoms caused by this fungus as "light-brown lesions with distinct yellow halos." Recently, Lamari and Bernier (6) distinguished two symptoms within the tan spot syndrome: tan necrosis (nec) and chlorosis (chl). About 6% of the 695 wheat cultivars and accessions tested developed extensive chlorosis that covered most of the leaf.

Lamari and Bernier (8) also showed that individual isolates of *P. tritici-repentis* can be characterized by their ability to induce tan necrosis and/or chlorosis. They identified three pathotypes in the fungus, designated (nec+ chl+), (nec+ chl−), and (nec− chl+) for the symptoms they induce in appropriate genotypes. The necrosis-chlorosis model was later validated by the identification of a fourth pathotype, (nec− chl−), lacking the ability to induce either symptom (L. Lamari, *unpublished*).

In culture, *P. tritici-repentis* produces a cultivar-specific toxin (7,13), designated Ptr-necrosis toxin (1,7), which has been associated with the development of tan necrosis in susceptible wheats. Furthermore, the Ptr-necrosis toxin was produced by both (nec+ chl+) and (nec+ chl−) isolates but not by (nec− chl+) (7) or (nec− chl−) isolates (L. Lamari, *unpublished*).

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Host resistance to tan spot has been reported to be quantitatively (10) and qualitatively (7,9) inherited. Lamari and Bernier (7) found that tan necrosis and toxin sensitivity are controlled by the same dominant gene. To date, there are no reports on the genetics of chlorosis and extensive chlorosis in tan spot.

No previous studies have demonstrated the existence of wheat lines or cultivars capable of developing both tan necrosis and extensive chlorosis. This study reports on two such genotypes: spring wheat line 6B699 and a selection from winter wheat cultivar Norstar.

MATERIALS AND METHODS

Inoculum. Conidia of *P. tritici-repentis* were produced on V-8 PDA (150 ml of V-8 juice, 10 g of Difco potato-dextrose agar [PDA], 3 g of $CaCO_3$, 10 g of Bacto agar, and 850 ml of distilled water) as described previously (6). Cultures were incubated in the dark at 20 C until colonies reached about 4 cm in diameter. They were then flooded with sterile distilled water, the mycelium was flattened, and the excess water was decanted. The cultures were incubated for 18–24 hr at room temperature (20–24 C) under light (approximately 90 $\mu E \cdot m^{-2} \cdot s^{-1}$), followed by 18–24 hr in the dark at 15 C. Inoculum concentration was adjusted to 3,000–3,500 spores per milliliter with a cell counter (Hausser Scientific, Blue Bell, PA).

Seeds of line 6B699 (*T. aestivum* var. *ferrugineum* 'Akakomugi') (obtained from All Union Inst., Soviet Union, in 1961) and a selection of cultivar Norstar (pedigree: Winalta/Alabaskaja) (2) were planted in pots containing a commercial potting medium (Metro-Mix 220, W.R. Grace & Co., Ajax, Ontario) at the rate of four seeds per pot. Isolates ASC1 (nec+ chl+), 86-124 (nec+ chl−), D308 (nec− chl+), and 88-1 (nec− chl−) were used to test for the development of tan necrosis and extensive chlorosis. Each treatment (isolate) consisted of four pots each of 6B699 and Norstar.

Two pots of Glenlea (tan necrosis) and two of 6B365 (chlorosis) were used as controls. The reactions of these lines were previously characterized (7,8). Glenlea develops tan necrosis in response to nec+ isolates but no extensive chlorosis in response to isolates from any pathotype. Line 6B365 develops extensive chlorosis in response to chl+ isolates but no tan necrosis in response to any known pathotype. Glenlea is sensitive to the Ptr-necrosis toxin, whereas 6B365 is insensitive.

Seedlings at the two-leaf stage were sprayed to runoff with a spore suspension to which 10 drops of Tween 20 (polyoxyethylene sorbitan monolaurate) per liter had been added to reduce surface tension. Seedlings were incubated for 24 hr at 22 C with a 16-hr photoperiod. Continuous leaf wetness was provided by two computer-controlled ultrasonic humidifiers (6) filled with distilled water. The pots were then transferred to a growth room bench and kept at day-night temperatures of 22 and 18 C with a 16-hr photoperiod (181 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 6–8 days, when they were evaluated for the presence of tan necrosis and extensive chlorosis. Symptoms were rated on 16 seedlings from each combination of line and isolate. Symptoms were usually assessed on the youngest fully expanded leaf at inoculation. In addition, four seedlings from each line were infiltrated with approximately 50 μl of the Ptr-necrosis toxin (1,7) with a Hagborg device (3) and checked for reaction 48 hr later. The experiment was repeated three times.

RESULTS AND DISCUSSION

Reactions of Norstar and 6B699 are shown in Table 1. Both lines developed extensive chlorosis in response to isolates ASC1 (nec+ chl+) and D308 (nec− chl+) (Fig. 1B,C) and only tan necrosis in response to isolate 86-124 (nec+ chl−) (Fig. 1A). Tan necrosis was visible on seedlings of both lines 3–4 days after inoculation with isolate ASC1 but was later masked by extensive chlorosis.

Neither tan necrosis nor chlorosis developed following infection with isolate 88-1 (nec− chl−). This isolate

Table 1. Reactions[a] of four wheat lines and cultivars to four pathotypes of *Pyrenophora tritici-repentis*

	Genotype							
	Norstar		6B699		Glenlea		6B365	
Isolate	Nec	Chl	Nec	Chl	Nec	Chl	Nec	Chl
ASC1 (nec+ chl+)	+	+	+	+	+	−	−	+
86-124 (nec+ chl−)	+	−	+	−	+	−	−	−
D308 (nec− chl+)	−	+	−	+	−	−	−	+
88-1 (nec− chl−)	−	−	−	−	−	−	−	−

[a]Nec = tan necrosis; Chl = extensive chlorosis; + and − indicate the presence or absence of a symptom, respectively.

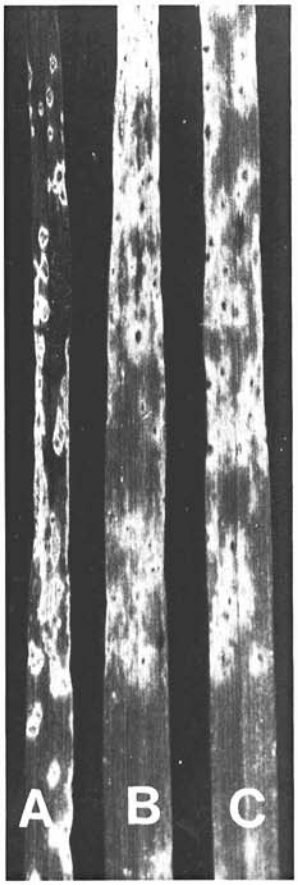

Fig. 1. Leaves of wheat cultivar Norstar plants inoculated with isolates 86-124 (nec+ chl−) **(A),** D308 (nec− chl+) **(B),** and ASC1 (nec+ chl+) **(C)** of *Pyrenophora tritici-repentis.* Note the well-defined edges of necrotic lesions and the absence of extensive chlorosis in leaf A and the extensive chlorosis and lack of well-defined lesion edges in leaves B and C.

induced small dark brown to black spots at the sites of penetration. Although avirulent, isolate 88-1 was previously found to penetrate and colonize the epidermal layer and to move to the mesophyll, where its growth was halted (L. Lamari, *unpublished*).

Line 6B699 and cultivars Glenlea and Norstar were sensitive to the Ptr-necrosis toxin, whereas line 6B365 was insensitive. The reactions of Glenlea and 6B365 to all isolates (Table 1) were as described previously (8).

The development of either tan necrosis or extensive chlorosis in wheat genotypes in response to the pathotypes of *P. tritici-repentis* has been recognized (8). However, it was believed that wheat lines could express only a single symptom (7,8). This is the first report of wheat genotypes capable of expressing tan necrosis in response to nec+ isolates as well as extensive chlorosis in response to chl+ isolates.

Net blotch of barley resembles tan spot of wheat in that barley genotypes can develop either net or spot blotch in response to infection by *P. teres* Drechs. f. *teres* and f. *maculata* Smedeg., respectively (11). No individual isolates of *P. teres* capable of causing both the net- and the spot-type lesions have been reported, although genetic analyses of crosses between isolates that induce spot blotch and net blotch indicated that these phenotypes are controlled by two different loci (12).

The differential development of tan necrosis and extensive chlorosis by the same wheat genotype in response to isolates of *P. tritici-repentis* indicates that these symptoms are controlled by different gene loci. Genetic analyses are needed to determine the relationship between these loci. Our results suggest that rating extensive chlorosis and tan necrosis as one symptom (i.e., one interaction) may lead to unusual genetic ratios. Also, the data have implications for breeders seeking to incorporate resistance to tan spot in wheat and point to the importance of testing segregating populations with both nec+ and chl+ isolates.

The effect of the existence of wheat genotypes capable of developing both tan spot symptoms on the epidemiology of the disease is not known. Further studies are needed to assess the behavior of such lines under field conditions.

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