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Molecular mapping of resistance to *Pyrenophora tritici-repentis* in wheat

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Introduction

Tan spot, caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis*) (Died.) Shoem., is a major disease of wheat (*Triticum aestivum* L.) worldwide (Hosford 1982; Wiese 1987). Grain yield losses of 2–50% (Hosford 1971; Hosford and Busch 1974; Rees *et al*. 1982; Rees and Platz 1983) and as high as 75% (Rees *et al*. 1981) have been attributed to this disease. Tan spot is the fastest growing disease in the Southern Cone region of South America including Argentina, Brazil, Chile, Paraguay and Uruguay (Kohli *et al*. 1992). In Argentina, the disease was found for the first time affecting wheat crops in the north-central region of Buenos Aires province in the early 1980s (Annone 1985). Subsequently, the disease has gained predominance among the wheat diseases in most of the wheat growing areas in the country (Annone 1997; Kohli *et al*. 1992; Carmona *et al*. 1999). The increase in the severity of tan spot has been linked with the change in the varietal composition, and the expansion of the area under conservation tillage practices with continuous wheat cultivation, which allows the buildup inoculum on the wheat stubble over time.

Based on the ability to induce necrosis or chlorosis on a set of differential cultivars, isolates of *P. tritici-repentis* are classified into eight races. Race 1 isolates can induce necrosis on the susceptible cultivar 'Glenlea' and chlorosis on the differential line '6B365'. Isolates that produce necrosis on 'Glenlea' only and chlorosis on '6B365' only were designed as race 2 and 3 respectively. Race 4 isolates do not induce either symptoms in susceptible differential genotypes and are considered avirulent. Race 5 isolates produce chlorotic symptoms similar to race 3 but in cultivar 'Katepwa'. Race 6 combines the virulence of races 3 and 5. Race 7 combines the virulence of races 2 and 5, and race 8 combines the virulences of races 2, 3 and 5 (Lamari et al. 2003).

Three host-specific toxins produced by *Pyrenophora tritici-repentis* have been identified (Ptr Tox A, Ptr Tox B, and Ptr Tox C) and sensitivity and susceptibility to their producer races are each controlled by the same gene (Lamari and Bernier 1989; Orolaza *et al*. 1995; Gamba *et al*. 1998; Effertz *et al*. 2002). One well-characterized host selective proteinaceous toxin, Ptr ToxA (Ciuffeti and Tuori 1999; Lamari and Bernier 1989), produced by races 1, 2, 7, and 8 (Lamari *et al*. 2003), is the main factor causing the necrotic symptom in susceptible wheat cultivars. Two chlorosis-inducing host-specific toxins, designated as Ptr ToxB (Orolaza *et al*. 1995), isolated from race 5, and Ptr ToxC (Effertz *et al*. 2002), isolated from race 1, have been identified and well characterized.

Reports on the mode of resistance to tan spot in *T. aestivum* have ranged from qualitative (1 or 2 genes) (Lee and Gough 1984; Lamari and Bernier 1991, Sykes and Bernier 1991) to quantitative (Nagle *et al*. 1982, Elias *et al*. 1989). Number and location of genes for resistance have been determined in recent years in different germplasm. Genetic studies indicated that a single recessive gene, *Tsr1 (formely tsn1)* controls resistance to necrosis caused by races 1 and 2 in both durum and bread wheat and is located on the long arm of chromosome 5B (Anderson *et al.* 1999; Faris *et al.* 1996). In addition *Tsr2* (*tsn2*) on 3BL conferring resistance to race 3 (Singh *et al*. 2006), *Tsr3* (*Tsn3*) on 3D *(*Tadesse *et al.* 2006a, 2007*)* and *Tsr4 (tsn4)* on 3A *(Tadesse et al. 2006b)* have been identified. Effertz *et al.* 1999 mapped the gene, *tsc1,* controlling insensitivity to toxin Ptr ToxC (race 3) in hexaploid wheat on the short arm of chromosome 1A. Furthermore, Singh *et al*. (2007) based on the reactions of *T. diccocoides* substitution lines determined that genes for resistance to races 1 and 2, and 3 and 5 were determined to be in chromosomes 5B and 3B, respectively. Resistance to toxin Ptr Tox A was located on chromosome 5B. Gurung *et al.* 2011 using 567 landraces detected resistance to race 1 on chromosomes 1D, 2A, 2B, 2D, 4A, 5B and 7D explaining 1.3 to 3.1% of the phenotypic variance, while markers associated with resistance to race 5 were distributed on 2D, 6A and 7D and explained 2.2 to 5.9% of the phenotypic variance.

Faris *et al.* (1997) in a population derived from crossing the synthetic hexaploid wheat 'W7984' and the CIMMYT (International Maize and Wheat Improvement Center) bred hard red spring wheat 'Opata 85' observed that resistance to extensive chlorosis by race 1 in seedlings was quantitative with a major proportion of the variation controlled by a quantitative trait locus on chromosome 1AS, a gene with a minor effect on 4AL, and an interaction between the 1AS gene and a gene in 2DL. Effertz *et al*. (2001) found that the major QTL located on 1AS also conferred resistance in adult plants. In addition minor QTLs associated with resistance were found on chromosomes 1AL, 7DS, 5AL and 3BL of this population. The same major QTL conferred resistance to race 1 isolates, and the QTL in chromosome 4AL to a race 3 isolate in seedlings of the 'W7984' x 'Trenton' population (Effertz et al. 2001). Friesen and Faris (2004) using seedlings of the same population located the toxin-insensitivity gene to Prt Tox B produced by race 5 on the short arm of chromosome 2B. Additional minor QTLs were identified on the short arm of chromosome 2A, the long arm of chromosome 4A and on the long arm of chromosome 2B.

Objective

The aim of this work was to determine the chromosomal location for resistance (expressed as necrosis and chlorosis) in the population of W 7984 x Opata 85 using two Argentinean isolates of *Pyrenophora tritici-repentis*.

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Materials and methods

Plant materials

A selection of 49 RILs from the ITMI (International Triticeae Mapping Initiative) mapping population created by crossing the synthetic hexaploid wheat `W7984´ with the spring wheat cultivar `Opata 85´ was examined. `W7984´ was generated via a cross of the *Triticum tauschii* accession `CIGM86.940´ (DD) with the tetraploid wheat `Altar 84´ (AABB)

Seedling stage examination

The experiments were performed in three environments: at the Facultad de Ciencias Agrarias y Forestales, La Plata (FCAyFLP) Argentina during 2003 (enviroment1) and 2004 (environment 2) and at the Experimental Station of Los Hornos, Argentina in 2004 (environment 3). For each environment, seeds were germinated in petri dishes and vernalised for 4 weeks at 4-8ºC. For the first two environments, germinated seeds were planted in 10 L pots in a randomized design with 2 replications for each isolate (H0019 and H0120, FCAyF). Isolates were selected from a preliminary screening of the parents using 8 isolates, because of the highest differences in resistance among them, being 'W7984' moderately resistant and 'Opata 85' susceptible. Eight to ten seeds were grown in each pot. Pots were fertilized with 50 kg ha^{-1} N as urea and 50 kg ha^{-1} P as ammonium diphosphate at sowing and with 50 kg ha^{-1} N as urea at tillering. For the third environment germinated seeds were planted with the same design in rows in the field and fertilized in the same way.

Plants were inoculated with the H0019 and H0120 isolates from the FCAyFLP, La Plata, Argentina. Mycelial plugs from both isolates were taken from the advancing margin of a 6 days culture of *Pyrenophora tritici-repentis* on potato dextrose agar (PDA).These plugs were then placed on petri dishes with V8 medium (150 ml of V8 juices, 3g of CaCO3, 15 g of agar and 850 ml distilled water) and incubated for 5 days at 20ºC with light alternating (3500 lux dark cycles of 12 h plus the addition of near UV light (365 nm). At the 6th day of incubation, the aerial mycelium was knocked down with a sterile, bent glass rod. The dishes were then incubated in continuous light for 24 h. Finally, the dishes were incubated in darkness for another 24 h. The light cycle simulated conidiophores formation and reduced mycelial fragments while the dark cycle induced completion of the sporulation process (Platt *et al*. 1977; Odvody and Boosalis 1982).The conidial harvest was made by flooding the plate with 5ml of sterile distilled water and dislodging the conidia with a bent glass rod. The resulted suspension was filtered through cheesecloth and the concentration of inoculum suspension was adjusted to 2×10^6 and with 0.05% Tween 20 added as a surfactant.

Spores suspension was applied with a hand sprayer until run-off. Plants were inoculated at the two leaf stage. After inoculation, pots were covered with transparent plastic to maintain wet conditions for 48 hs. Plants were regularly irrigated. Field experiment was maintained wet by spraying with water for 15 minutes every two hours for three days. Ratings of genotypes for reaction to tan spot were made 28 days after inoculation as percentage of the leaf area affected by the disease (necrosis and chlorosis) on a visual basis.

Adult stage examination

Similar experiments as for seedling stage examination were planted in the same way and the same three environments. The plant material was inoculated with the same isolates and in the same way as for the seedling experiment at the flag leaf stage (GS 37-39, Zadoks *et al*. 1974). After inoculation the conditions were similar to the seedling stage experiment for the first 48 h. As for the seedling stage, plants were fertilized and the pots experiment was regularly irrigated. Disease reaction was visually estimated as a percentage of the leaf affected by the disease (necrosis and chlorosis) at GS 82

QTL mapping

An extensive RFLP map exists for the W-7984/Opata 85 RI population (http://wheat.pw.usda.gov/ggpages/map_summary.html). Markers were used to indentify associations with percentages of disease severity. The computer program QGENE was used to perform QTL analysis (Nelson 1997). Single marker regression map was applied

Results and discussion

At seedling stage 'W7984' was moderately resistant to resistant and 'Opata 85' moderately susceptible to susceptible. At the adult stage 'W7984' was moderately resistant and 'Opata 85' moderately susceptible to susceptible. The RILs population segregated for reaction to both isolates at both growth stages. Most of the lines have little necrosis but varying in chlorosis percentage according to its level of resistance.

Analysing the disease scores, no clear cut deviations allowing a qualitative approach were detected either at seedling or adult stage for the three environments and the two isolates used.

Investigating the RILs at the seedling stage, a locus coming from 'W7984' was discovered on the short arm of chromosome 6A linked to *Xksuh4c* with LOD scores between 1.90 and 3.76 when considering isolate H0019 for two of the environments (environment 1 and 3) and on the same chromosome linked to the same marker for isolate H0120 with LOD scores between 2.25 and 4.29. When considering the average of the three environments for each isolate the same locus was detected on chromosome 6A with LOD scores of 3.76 and 5.87 for isolates H0019 and H0120 respectively (Fig.1). No other consistent loci were detected at seedling stage. In addition no consistent loci through the three environments were detected on adult stage either. No consistent QTLs were determined in adult stage

Several genes have been identified conferring resistance to the pathogen in *Triticum aestivum* populations. It is known that isolates of *Pyrenophora tritici-repentis* are classified into at least 8 races, however new races can risen. Genes or QTLs effective against races 1; 2; 3 and 5 have been identified earlier. No genes have been identified at present effective against races 4 (avirulent on differential lines), 7 and 8.

Using the same material as in the present work Faris *et al.* (1997) observed that resistance to extensive chlorosis by race 1 in seedlings was quantitative with a major proportion of the variation controlled by a quantitative trait loci on chromosome 1AS, a gene with a minor effect in 4AL, and an interaction between the 1AS gene and a gene in 2DL. Effertz *et al*. (2001) also found that the major QTL located on 1AS conferred resistance in adult plants. In addition minor QTLs associated with resistance were found on chromosomes 1AL, 7DS, 5AL and 3BL of this population. The same major QTL conferred resistance to race 1 isolates, and the QTL on chromosome 4AL to a race 3 isolate in seedlings of the W 7984 x Trenton population (Effertz *et al*. 2001). Friesen and Faris (2004) using seedlings of the same population located the toxin-insensitivity gene to Prt Tox B produced by race 5 on the short arm of chromosome 2B. Additional minor QTLs were identified on the short arm of chromosome 2A, the long arm of chromosome 4A and on the long arm of chromosome 2B.

One of the isolates used in this work was race 1 (H0019), the other is not virulent on the set of diferential lines (that would indicate that is race 4) (Moreno et al unpublished data). However did not amplify for *Tox* b gene, according with suggested primer by Andrie *et al.* (2007). The H0019 isolate was assigned as race 1, it produced necrosis on both 'Glenlea' and 'Katepwa', and chlorosis on '6B365'. Chlorosis on '6B365' can be attributed to the production of Ptr Tox C (Effertz *et al*. 2002), however the gene(s) has not been characterized and it is not possible to confirm this result by PCR at the moment (Moreno *et al.,* unpublished data).

The isolate H0120 considered as race 4, based in the absence of symptoms on the cultivars and lines selected, did not amplified for the Ptr-tox b, however amplified for the Tox A gene, however phenotypically was considered as non-pathogenic on the differential set of cultivars. If we consider that we need the Ptr-tox b amplification to assign an isolate to race 4, we can consider isolate H0120 as a potential new race.

Although isolate H0019 was considered as race 1, as it was said it is not possible confirm this result by PCR, and as chromosomal location for this race in this population has been established mainly on chromosome 1AS, we speculated that it could belong to a new race. In addition it is known that new races can appear adding new lines to the differential set.

P. tritici-repentis-wheat interaction exhibits a complex race structure. Many researches showed discrepancy between the phenotypic findings and the PCR determination for *P.tritici-repentis Tox A* and *Tox B* genes (Ali *et al*. 2010; Andrie *et al*. 2007; Lepoint *et al*. 2010; Moreno *et al*. unpublished data). Races determination of the pathogen in Argentina is recent (Moreno *et al*. unpublished data). According to the results of this work, we suggest the possibility that both isolates belong to new races of the pathogen

Fig.1 QTL for resistance to *Pyrenophora tritici-repentis* in the synthetic wheat W7984