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Resistance of *Hordeum chilense* against loose smuts of wheat (*Ustilago tritici*) and barley (*U. nuda*), and its expression in amphiploids with wheat

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Key words: disease resistance, *Hordeum*, *Triticum*, tritordeum, *Ustilago tritici*, *Ustilago nuda*

Abstract

Hordeum chilense is wild barley with high potential for cereal breeding purposes given its high crossability with other members of the Triticeae tribe. It is resistant to loose smuts of wheat (*Ustilago tritici*). The resistance is expressed in x*Tritordeum* amphiploids, offering perspectives for its utilization both in tritordeum breeding and for its transfer to wheat. *H. chilense* and tritordeums are also resistant to barley loose smut (*U. nuda*).

Introduction

Loose smuts of wheat (*Ustilago tritici* (Pers.) Rostr.) and of barley (*U. nuda* (Jens.) Rostr.) are important seed-borne diseases of worldwide distribution. They are seldom devastating, but cause low to moderate annual losses (Nielsen and Thomas, 1996). Loose smuts can be controlled by seed treatment with systemic fungicides such as carboxin. However, currently there is no seed treatment available under organic farming conditions. Also, resistance to the fungicide has already been developed by both *U. tritici* and *U. nuda* (Dhitaphichit and Jones, 2007; Menzies, 2008).

Resistance remains the most economical and environmentally sound way to control smuts, with a number of single resistance genes available in both wheat and barley (Metcalf and Johnston, 1963; Nielsen and Thomas, 1996). However both *U. tritici* and *U. nuda* populations are capable of evolving new races (Thomas, 1984; Nielsen 1987; Randhawa et al., 2009) that are easily disseminated in seed by man. New race expansion requires continuous breeding efforts. Broadening the genetic base of cultivated wheat by the introgression of resistance genes from related species or genera may provide additional valuable sources of resistance to diseases.

Hordeum chilense Roem. et Schult is an extremely polymorphic, diploid wild barley that occurs exclusively in Chile and Argentina. After *H. vulgare/spontaneum* and *H. bulbosum*, *H. chilense* is the *Hordeum* species with the highest potential for cereal breeding purposes, given its high crossability with other members of the Triticeae tribe (*Triticum*, *Hordeum*, *Secale* and *Agropyron*) and its agronomically interesting characteristics (Martín et al. 2000). Plant geneticists have been interested in hybridising barley with wheat for more than 100 years but have had little success (Kruse 1973). No fertile wheat x *H. vulgare* amphiploids have been produced even after many attempts (Fedak 1992). In contrast, fertile amphiploids with wheat were easily produced using *H. chilense*. Among several approaches for its use in cereal breeding are the development of hybrids and amphiploids with wheat to be used as a possible new crop, or as bridges to transfer useful genes to cultivated cereals (Martín et al. 2000; Rubiales et al. 2001). The purpose of the present experiments was to determine the level of resistance of *H. chilense* accessions to loose smuts, and its expression in amphiploids with wheat.

Materials and Methods

H. chilense accessions, together with wheat accessions with whom they were crossed and their resulting tritordeum amphiploids (*H. chilense* x wheat) (Martín et al. 2000) (table 1) were grown in the greenhouse in 2 litter pots and inoculated with *U. tritici* and *U. nuda*. Two pots of each entry

were sown at weekly intervals to allow availability of plants of similar growth stage at the time of inoculation.

Inoculums were collected from diseased spikes collected at experimental fields at Córdoba and stored in paper envelopes in a refrigerator at 4°C till use. For each of the *Ustilago* species, five spikes of each of the test lines were inoculated at early to mid-anthesis growth stage. A suspension of spores (1 g/L) in distilled water was injected by a 10 mL syringe with gauge 22 hypodermic needle. Each single floret received 20-30 µL of inoculum. The central florets in the spikelets and the uppermost spikelets in the spike were removed. Inoculated spikes were covered with small paper bags and labelled. The suspension of spores was used for inoculations performed during 3-4 days. After this, new suspensions were made.

Inoculated spikes were collected at maturity, and stored. Resistance was studied following season in the field at Córdoba. Sowing took place in middle November following standard agronomic practises in the area. Each accession was represented by a 2 m rows with 10 plants each, with 4 replications in a completely randomized block design. Observations were made at heading, counting the number of smutted spikes per plant. The scores were angular transformed ($\arcsin(\sqrt{1/x})$) prior to an analysis of variance using SPSS version 11.0.

Results and discussion

We found *H. chilense* to be highly resistant to both *U. tritici* and *U. nuda* (Table 1). This is in agreement with Nielsen (1987b). *U. tritici* is known to infect both durum and bread wheats, however, there is a degree of host specialization. Most races are virulent only on either bread wheat or durum wheat, except race T39 that can infect both species (Randhawa et al., 2009). We used a *U. tritici* population specialized on bread wheat, thus we included only one accession each of *T. tauschii* and of *T. turgidum* that were resistant. Their resulting tritordeums (HT105 and HT251 (4x) and HT73 (6x)) were also resistant. On the contrary, all hexaploid wheat accessions

(both *T. aestivum* and *T. sphaerococcum*) studied were very susceptible. Some *U. tritici* infection was observed in their resulting octoploid tritordeums, but markedly reduced compared to the wheat parent.

Similar results were previously obtained with common bunt (*Tilletia caries*) to which *H. chilense* is resistant. All hexaploid tritordeums were immune, whereas octoploid ones and addition lines in bread wheat showed incomplete resistance (Rubiales et al. 1996; Rubiales and Martín, 1999). *H. chilense* was also found resistant to Karnal bunt (*Neovossia indica*). This resistance was transmitted to some secondary tritordeums but not to others (Chauhan and Singh 1997). Expression of resistance in other amphiploids to bunts has also been reported. Resistance to Karnal bunt was expressed in synthetic hexaploid wheats (Villareal et al. 1994). Triticale is considered resistant to common bunt (Gaudet and Puchalski 1989) and possesses some resistance to Karnal bunt (Warham 1988).

U. nuda infected only the barley check (Table 1). All *H. chilense* accessions tested were immune to *U. nuda* as were all *Triticum* accessions and the resulting tritordeums. We can not conclude if the resistance of tritordeum was conferred by the *H. chilense* or by the wheat parent or by both of them. However, the information that *H. chilense* is resistant to *U. nuda* is useful to breeders as this discards any concern that breeders might have of introducing susceptibility to barley smut when using *H. chilense* in their introgression programs. For instance, triticale can be infected by any race of loose smuts that attacks the parental wheat or rye (Nielsen, 1973). Results presented here indicate that tritordeums are highly resistant to loose smut of wheat and immune to loose smut of barley.

H. chilense appears to offer a valuable reservoir of genes for disease resistance that potentially can be exploited in cereal breeding. *H. chilense* was also found to be resistant to the barley, wheat and rye brown rusts, the powdery mildews of wheat, barley, rye and oat and to Septoria leaf blotch suggesting that *H. chilense* is a non-host of these pathogens (Rubiales et al.

1991, 1992, 1993a). There are also lines resistant to wheat and barley yellow rust, stem rust and to *Agropyron* leaf rust, as well as lines giving moderate levels of resistance to *Septoria* glume blotch, tan spot and *Fusarium* head blight (Rubiales et al. 1991, 2001). *H. chilense* chromosome addition and substitution lines in durum and bread wheat and wheat - *H. chilense* translocations have been
5 obtained and the feasibility of transferring traits from *H. chilense* to cereals previously discussed (Martín et al. 1998, 2000). Therefore, *H. chilense* resistance to *U. tritici* appears accessible for wheat breeding purposes.

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Table 1: Percentages of smutted spikes in *H. chilense*, wheat and tritordeum amphiploids inoculated with *U. tritici* or with *U. nuda*

Accession	Species	Pedigree	Ploidy level	Genomic constitution	<i>U. tritici</i> % smutted spikes	<i>U. nuda</i> % smutted spikes
H26	<i>H. chilense</i>		4x	H ^{ch} H ^{ch} H ^{ch} H ^{ch}	0	0
H108	<i>H. chilense</i>		4x	H ^{ch} H ^{ch} H ^{ch} H ^{ch}	0	0
T6	<i>T. tauschii</i>		4x	DDDD	0	0
HT105	<i>xTritordeum</i>	H26xT6	4x	DDH ^{ch} H ^{ch}	0	0
HT251	<i>xTritordeum</i>	H108xT6	4x	DDH ^{ch} H ^{ch}	0	0
H8	<i>H. chilense</i>		2x	H ^{ch} H ^{ch}	0	0
T31	<i>T. turgidum</i>		4x	AABB	0	0
HT47	<i>xTritordeum</i>	H8xT31	6x	AABBH ^{ch} H ^{ch}	0	0
H55	<i>H. chilense</i>		2x	H ^{ch} H ^{ch}	0	0
T79	<i>T. aestivum</i>		6x	AABBDD	50	0
HT77	<i>xTritordeum</i>	H55xT79	8x	AABBDDH ^{ch} H ^{ch}	1 ***	0
H46	<i>H. chilense</i>		2x	H ^{ch} H ^{ch}	0	0
T90	<i>T. aestivum</i>		4x	AABBDD	60	0
HT81	<i>xTritordeum</i>	H46xT90	6x	AABBDDH ^{ch} H ^{ch}	10 **	0
H7	<i>H. chilense</i>		2x	H ^{ch} H ^{ch}	0	0
T59	<i>T. sphaerococcum</i>		6x	AABBDD	40	0
HT18	<i>xTritordeum</i>	H7xT59	8x	AABBDDH ^{ch} H ^{ch}	6 **	0
Vada	<i>H. vulgare</i>		2x	HH	0	85

, * differences with its parental wheat are statistically significant at p<0.01 or p<0.001, respectively.