Genomic interactions in the resistance to mildew and rust fungi in hybrids and amphiploids involving the genera *Triticum*, *Hordeum* and *Secale*.

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Abstract

Hordeum chilense is highly resistant to mildew and brown rust fungi. The *H. chilense* resistance to powdery mildew conferred a substantial reduction of the infection frequency of *Erysiphe graminis* f.sp. *tritici* in wheat - *H. chilense* amphiploids (tritordeum) and of *E. graminis* f.sp *secalis* in rye - *H. chilense* genome combinations. The reaction of *H. chilense* to rusts was overruled by the wheat reaction in tritordeum. Resistance of *H. chilense* to rye brown rust was expressed in rye - *H. chilense* combinations. In tetraploid triticale (DDRR) wheat contributed to *E. graminis* f.sp. *secalis* and to *P. recondita* f.sp. *recondita* resistance, and rye contributed to *E. graminis* f.sp. *tritici*, *P. recondita* f.sp *tritici* and *P. striiformis* f.sp. *tritici* resistance.

Key words: disease resistance, *Hordeum chilense*, intergeneric hybridization, mildews, rusts

Introduction

Wide hybridization provides a means to exploit the wide source of genetic variation and to study phylogenetic relationships. Crossing *Triticum* and *Secale* has produced the triticale, the first manmade cultivated alloploid. The interest in crossing *Triticum* and *Hordeum* is old. Hybrids have been produced, but their respective amphiploids were only obtained when wild species of barley were used. From crosses between *H. chilense* and *Triticum* spp. a wide range of "tritordeum" amphiploids have been produced that are proposed as a new crop (MARTÍN 1988). Also "hordecale" (*Hordeum* x *Secale*) has been suggested as a potential crop (FEDAK 1986a). These hybrids and amphiploids may be used as intermediates to transfer desirable traits to the cultivated cereals.

Disease resistance is the most readily exploited character in wide crosses. Resistance has been successfully transferred to cultivated cereals from related species and genera (FEDAK 1986b; GALE and MILLER 1987). Cases have been reported, however, in which the resistance is not expressed in the new background due to genomic interactions or dilution at the higher ploidy levels (QUINONES et al. 1972; THE and BAKER 1975; KERBER and GREEN 1980; CHEVRE et al. 1989; RUBIALES et al. 1991; BAI and KNOTT 1992; VILLAREAL et al. 1992).

The present experiment was performed to study the *Triticum*, *Hordeum* and *Secale* genome interactions in the expression of resistance to rust and mildew fungi.

Material and Methods

Plant material. The hybrids and amphiploids and their parental lines are listed in table 1. As some genomic combinations ($H^{ch}R$, $H^{ch}H^{ch}RR$, $H^{ch}DR$, $H^{v}H^{ch}AB$ and $H^{v}H^{ch}D$) were sterile, they had to be vegetatively multiplied.

Inoculum. The fungi applied were:
- *E. graminis* f.sp. *tritici*, (*Egt*), isolates WC11 and WC28, from Dr. J.K.M. Brown, Cambridge Laboratory, Norwich, United Kingdom.
- *E. graminis* f. sp. *hordei*, (*Egh*), isolate CC52, from Dr. J.K.M. Brown.
- *E. graminis* f.sp. *secalis*, (*Egs*), an isolate from Dr. H.G. Welz, University of Hohenheim, Germany.
- *P. recondita* f.sp. *tritici*, (*Prt*), isolate 'Flamingo', from Plant Breeding Department (PBD), Wageningen, The Netherlands.
- *P. recondita* f.sp. *recondita*, (*Prr*), an isolate collected at Wageningen.
- Putative *P. recondita* f. sp. *agropyri*, ("*Pra*"), collected at Wageningen on *H. jubatum*.
- *P. hordei*, (*Ph*), isolate 1-2-1, from PBD, Wageningen.
- *P. striiformis* f.sp. *tritici*, (*Pst*), race 39E134, from Dr. R.W. Stubbs, IPO, Wageningen.

Inoculation. The mildew inoculation was performed at Cambridge Laboratory, UK. A middle 30 mm segment of leaf of each plant was excised and placed, adaxial surface up, on 0.4% agar containing 125 ppm benzimidazole in transparent boxes. Young conidia were applied into a settling tower. The boxes were then transferred to a growth cabinet at 15 °C.

The rust spores were applied in a settling tower at PBD, NL. After inoculation the plants were incubated overnight in darkness in a mist chamber at 16 °C (the plants inoculated with *Pst* were incubated for 24 hours at 10 °C) and then transferred to a greenhouse at 20-16 °C (day-night range).

Observations. The Infection Type (IT) of the segments inoculated with mildew was determined seven days after inoculation according to a 0-4 scale (MOSEMAN et al. 1965). The number of mildew colonies developed per square centimetre (IF) was recorded. The IT of the plants inoculated with rusts was determined about 17 days after inoculation according to the 0-9 scale of McNEAL et al. (1971).

Results

Table 1 shows the reaction of the genomic combinations to the *Egt*, *Egs*, *Prt*, *Ph*, *Prr* and "*Pra*". The *H. chilense*, and rye lines were highly resistant (IT 0) to the two isolates of *Egt* (Table 1). The reaction of the wheats was of resistance (IT 0) or susceptibility (IT 4). The IT of the tritordeums were that of the wheat parent although with a considerable reduction in the number of colonies. The rye lines studied were susceptible to the *Egs* isolate. The *H. chilense*, wheat, tritordeum, triticale and the trigeneric hybrid $H^{ch}DR$ studied were highly resistant. The hybrid $H^{ch}R$ and the

Table 1. Infection Type (IT) of the lines inoculated with *E. graminis* f.sp *tritici* (*Egt*), *E. graminis* f.sp. *secalis* (*Egs*), *P. recondita* f.sp. *tritici* (*Prt*), *P. recondita* f.sp. *recondita* (*Prr*), *P. recondita* f.sp *agropiry* (*Pra*), *P. hordei* (*Ph*) and *P. striiformis* f.sp. *tritici* (*Pst*).

Species	Line	Genome composition	Fungus *Egt* WC11	*Egt* WC28	*Egs*	*Prt*	*Prr*	"*Pra*"	*Ph*	*Pst*
H. chilense	H1	$H^{ch}H^{ch}$	0	0	0	1	0	1	1	7
	H7	$H^{ch}H^{ch}$	0	0	0	0	0	1	0	0
	H26	$H^{ch}H^{ch}H^{ch}H^{ch}$	0	0	0	0	0	0	0	7
T. tauschii 4x	T6	DDDD	0	4	0	8	4/5	6	0	7
T. turgidum	[a]	AABB	0/4	0/4	0	0/9	0	0	0	0/9
T. aestivum	[a]	AABBDD	0/4	0/4	0	0/9	0	0	0	0/9
S. cereale	Petkus	RR	0	0	4	1-3	9	1-2	0	0
	C4x	RRRR	0	0	4	0	9	0	0	0
H. vulgare	Betzes	$H^{v}H^{v}$	-	-	-	0	0	0	9	-
Tritordeum	HT(4x)[b]	$H^{ch}H^{ch}DD$	0	4↓↓	0	6-7	0	6-	0	7+
	HT(6x)[c]	$H^{ch}H^{ch}AABB$	0/4↓	0/4↓	0	0/9	0	0	0	0/9
	HT(8x)[d]	$H^{ch}H^{ch}AABBDD$	0/4↓	0/4↓	0	0/9	0	0	0	0/9
	[e]	$H^{v}H^{ch}AB$	-	-	-	S[f]	0	-	0	-
	[g]	$H^{v}H^{ch}D$	-	-	-	1	-	-	1	-
Triticale	[h]	DDRR	0	0	0	5	7	6+	0	4
Hordecale	[i]	$H^{ch}R$	0	0	4↓↓	1	2-4	-	0	-
	[j]	$H^{ch}H^{ch}RR$	0	0	4↓↓	0-1	2	-	0	-
Hordetricale	[k]	$H^{ch}DR$	0	0	0	1	3	-	-	2

Footnotes:
[a] five lines.
[b] H26 x T6 (CABRERA and MARTÍN 1991)
[c] seven lines. H^{ch} x W4x (MARTÍN and SÁNCHEZ-MONGE 1982)
[d] seven lines. H^{ch} x W6x (MARTÍN et al. 1987).
[e] 'Betzes' x HT108 (MARTÍN 1991)
[f] The only leaf of $H^{v}H^{ch}AB$ was processed for microscopy so the IT could not be recorded. It showed a high percentage of established colonies without necrosis. This suggests susceptibility.
[g] 'Betzes' x HT105 (MARTÍN unpublished)
[h] T6 x 'C4x' (CABRERA and MARTÍN unpublished)
[i] H7 x 'Petkus' (MARTÍN et al. 1988)
[j] H26 x 'C4x' (MARTÍN et al. 1988)
[k] $H^{ch}H^{ch}DD$ x R.'Petkus' (CABRERA and MARTÍN 1992)
- non determined.

amphiploid $H^{ch}H^{ch}RR$ showed a very low number of colonies of the susceptible type (IT 4↓↓).

The *H. chilense* lines were resistant to *Prt* as previously reported (Table 1) (RUBIALES and NIKS 1992). As previously found (RUBIALES et al., 1991) the reaction of the hexaploid and octoploid tritordeums ranged from resistance to susceptibility, being that of their respective wheat parent. The tetraploid *T. tauschii* showed a susceptible reaction. The tetraploid tritordeum $H^{ch}H^{ch}DD$ showed an intermediate reaction (IT 6-7) similar to that of the parental T6. Rye 'Petkus' and 'C4X' were resistant. The reaction of Petkus (IT 1-3) is not unexpected, as rye may be rather susceptible to *Prt* (BÓCSA and KISS 1966; NIKS and DEKENS 1987). The tetraploid triticale DDRR showed an intermediate reaction (IT 5). $H^vH^{ch}AB$ was susceptible. However, the hybrid 'Betzes' x tetraploid tritordeum ($H^vH^{ch}D$) was resistant (IT 1).

Susceptibility of barley 'Betzes' (IT 9) to *Ph* was not expressed in its hybrids $H^vH^{ch}D$ and $H^vH^{ch}AB$. The amphiploids $H^{ch}H^{ch}DD$, $H^{ch}H^{ch}RR$ and DDRR and the hybrid $H^{ch}R$ were resistant as were their respective parents.

Although some *H. chilense* lines have been previously found to be susceptible to "*Pra*" (RUBIALES and NIKS 1992), the lines used here, H1, H7 and H26, were very resistant. Also the rye lines were very resistant. The reaction of the amphiploids $H^{ch}H^{ch}DD$ and DDRR was that of their wheat parental line T6.

The *H. chilense*, barley and wheat lines were very resistant to *Prr*. The tetraploid *T. tauschii* showed IT 4-5. This IT is higher than usual in *Triticum*, but commonly found in *T. tauschii* accessions (RUBIALES, unpublished results). The rye lines were very susceptible. The $H^{ch}H^{ch}DD$ showed IT 0. The amphiploid DDRR showed IT 7. The susceptibility of rye was not expressed in the amphiploid $H^{ch}H^{ch}RR$ and the hybrids $H^{ch}DR$ and $H^{ch}R$.

Discussion.

As in previous studies the reaction of the *H. chilense* x *Triticum* spp. amphiploids to rust fungi was that of the wheat parent with little influence of the resistance of the *H. chilense* genome (RUBIALES et al. 1991). In the hexaploid, and octoploid tritordeums a possible explanation could be a 'genome dose effect'. The behaviour of the tetraploid tritordeum ($H^{ch}H^{ch}DD$) to *Prt* and to "*Pra*", and of the hybrid $H^vH^{ch}AB$ to *Prt* support the hypothesis of epistasis of the AB(D) or D genomes over the H^{ch} genome with suppression of the expression of the resistance carried on H^{ch}. The hybrid $H^vH^{ch}D$ was, however, resistant to *Prt* indicating expression of H^v or genome dose effect of the *Hordeum* genomes over D genome. Suppression of rust resistance due to intergenomic interactions has previously been reported in cereals (see introduction). Such suppressors may possibly be removed through mutation as has been reported for the 7DL suppressor locus of wheat 'Canthatch' (KERBER 1991) that inhibited resistance to stem rust.

The resistance of *H. chilense* to rust fungi is suppressed by the wheat genomes, but not by the rye genome. The suppression is not found for other diseases. The *H. chilense* resistance to *Septoria tritici* was found to be fully expressed in hexaploid tritordeum, with some dilution of the resistance at the octoploid level (RUBIALES et al. 1992). Tritordeum is also more resistant than wheat to septoria glume blotch, fusarium head blight, smut (RUBIALES, unpublished data) and to the root-knot nematode (PERSON-DEDRYVER, et al. 1989). Although the IT of tritordeum to *Egt* is that of the wheat parent, there is considerable reduction in the infection frequency of tritordeum with respect to that of the wheat. The resistance to powdery mildew of *H. chilense* is not completely inhibited by the wheat genome resulting in partial resistance (i.e. quantitative resistance with a compatible infection type) of tritordeum.

The reaction of the amphiploid DDRR to *Prt* and *Pst* was intermediate between its parental lines T6 and C4X showing some contribution of the resistance carried by the rye. Genes for hypersensitive resistance to *Prt* in triticale may be contributed by either the wheat or the rye parental line (NIKS and DEKENS 1987; SINGH and McINTOSH 1990). The IT of DDRR to "*Pra*" is, however, that of its wheat parent (T6). More DDRR combinations should be studied to verify this observation.

Table 2. Summary of the observed genome interactions in the expression of resistance to rust and mildew fungi.

Fungus	Genome dominance			
	Tritordeum	Triticale	Hordecale	Hordetricale
Egt	H^{ch} > ABD, AB, D	R > D		$H^{ch}R$ > D
Egs		D > R	H^{ch} > R	$H^{ch}D$ > R
Prt	ABD, AB, D > H^{ch} AB > $H^{ch}H^{v}$ $H^{v}H^{ch}$ > D	R ≈ D		$H^{ch}R$ > D
Ph	$H^{ch}AB$ > H^{v} $H^{ch}D$ > H^{v}			
Pra	ABD, AB, D > H^{ch}	D > R		
Prr		D ≈ R	H^{ch} > R	$H^{ch}D$ > R
Pst	ABD, AB, D? > H^{ch}	D ≈ R	$H^{ch}R$ > D	

The DDRR was susceptible to *Prr* (IT 7). Despite this susceptibility, there was some reduction on the IT with respect to its rye parent. The susceptibility of this tetraploid triticale contrasts with previously reported high resistance of hexa- and octoploid triticales to *Prr* (QUINONES et al. 1972; STUCHLÍKOVÁ and BARTOŠ 1976; NIKS and DEKENS 1987) although Quinones et al. (1972) present data on triticale susceptible to *Prr*. Niks and Dekens (1987) concluded that the rye genome does not make the triticale a host to *Prr*. This seems to be true for hexa- and octoploid triticale, but the host status of this tetraploid triticale (DDRR) suggests a 'genome dose effect' although the unexpected intermediate IT of T6 to *Prr* makes it difficult to compare this tetraploid triticale with the hexaploid and octoploid ones.

Tetraploid triticale was immune to *Egt* and *Egs*. Both the rye resistance to *Egt*, and

the wheat resistance to *Egs* were fully expressed in DDRR. This means that the contribution of R genome in DDRR to resistance to *Egt* is stronger than that of H^{ch} in $H^{ch}H^{ch}DD$ where some colonies were recorded. Linde-Laursen (1977) reported triticale to be resistant to *Egs* and to *Egt*, although some octoploid lines could be susceptible to *Egt*.

The hybrid $H^{ch}R$ and amphiploid $H^{ch}H^{ch}RR$ showed a reduction in the IT to *Prr* and in the IF to *Egs*. The nonhost *H. chilense* genome conferred in the hybrid hypersensitive resistance to *Prr* and partial resistance to *Egs*.

In $H^{ch}R$ and $H^{ch}H^{ch}RR$ the *Hordeum* resistance to *Egs* was expressed. This is in agreement with Wojciechowska (1978) that reported (without further data on IT, severity or formae speciales) the *H. jubatum* ($H^{j}H^{j}H^{j}H^{j}$) x *S. cereale* (RR) hybrid ($H^{j}H^{j}R$) to be as susceptible to *P. graminis* as the rye parent, although other traits such as tillering capacity, perennial habit and resistance to *E. graminis* were inherited from the wild barley parent. The susceptibility of the $H^{j}H^{j}R$ hybrid to stem rusts does not support the 'genome dose effect' hypothesis. In the $H^{ch}R$ and $H^{ch}H^{ch}RR$ combinations studied here there was, however, a contribution of the H^{ch} genome to the resistance to *Prr*.

The resistance to *Prt* carried by the *Hordeum* genomes was not expressed in the hybrid $H^{v}H^{ch}AB$, nor in hexa- and octoploid tritordeums, but was fully expressed in the hybrid $H^{v}H^{ch}D$. Both hybrids $H^{v}H^{ch}AB$ and $H^{v}H^{ch}D$ were highly resistant to *Ph*. The susceptibility of 'Bętzes' to *Ph* was fully overruled by the AB(H^{ch}?) and D(H^{ch}?) resistance respectively. The observed $H^{v}H^{ch}$ homoeologous chromosome pairing (MARTÍN, 1991; unpublished) in this material is promising to achieve introduction of *H. chilense* resistance genes into cultivated barley.

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