Prehaustorial and posthaustorial resistance to wheat leaf rust in diploid wheat

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Corine C. Anker

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Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
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in het openbaar te verdedigen
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des namiddags te vier uur in de Aula.

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Stellingen

1. *Triticum monococcum* kan worden beschouwd als een niet-waard voor tarwe bruine roest, *Puccinia triticina*. Dit proefschrift

2. De afwezigheid van celwandafzettingen bij de helft van de prehaustoriaal geaborteerde infectie eenheden in *T. monococcum* accessie Einkorn, wijst er op dat celwandafzetting bij prehaustoriale abortie in diploïde tarwe in het algemeen meer een reactie is op beschadiging dan de oorzaak van resistentie. Dit proefschrift

3. Het tarwe genus (*Triticum* L.) was en is nog immer onderworpen aan een verwarrende veelheid van taxonomische indelingen waarvan de –waarschijnlijk enige- overeenkomst is dat geen enkele indeling afdoende fylogenetische realiteit met gebruiksgemak combineert. Nesbitt en Samuel, Hulled Wheats

4. De duurzaamheid van ziekteresistentie in planten wordt minstens evenveel beïnvloed door het evolutionaire potentieel van de pathogeenpopulatie als door het resistentiemechanisme in de plant.

5a. Het is eenvoudiger een ethische discussie te voeren over euthanasie dan over genetische modificatie. n.a.v. Prophyta 55, jan/feb 2001

6. Dat 'de wetenschap in Nederland moeilijk jonge wetenschappers kan interesseren voor het metier omdat een doctorstitel niet sexy is', valt moeilijk te verifiëren.
emeritus hoogleraar J.M. Winter, UU (de Volkskrant februari 2001)

7. Voor het opkrikken van het imago van groene studies is veel meer nodig dan alleen promotiecampagnes die aansluiten bij de belevingswereld van potentiële studenten. Promotiecampagne Wageningen Universiteit
Promotiecampagne AOC onderwijs

8. Everything is a matter of degree.

Stellingen behorende bij het proefschrift "Prehaustorial and posthaustorial resistance to wheat leaf rust in diploid wheat" van Corine Anker. In het openbaar te verdedigen op 20 juni 2001 in de Aula van Wageningen Universiteit.

'Poems and Hums are not things that you get, they are things that get You!
And the only thing you can do is to go where they can find you.'
Pooh Bear

Contents

Chapter 1

General Introduction

plant disease

Plant diseases are a major constraint in agriculture. Yield reduction due to plant pathogens is estimated at 12% world wide, despite extensive control measures (Agrios, 1997). Of the two most widely applied control measures, the use of resistant varieties gains importance as increasing environmental and health awareness impedes the continued application of pesticides. However, adaptation of pathogens causing the loss of resistance in plants is a serious problem that breeders of all major crops have to consider. Knowledge on mechanisms and genetics of resistance facilitates breeding for durable forms of resistance. This thesis presents a study into the occurrence and the genetics of a potentially durable type of resistance to wheat leaf rust in diploid wheat.

plant defence

Plants are continuously exposed to a wide variety of pathogens. All plant species, however, are nonhosts for the majority of the potential plant pathogens. Either the plant perceives the invading pathogen and a general defence reaction follows or the plant does not fulfil the growth requirements of the potential pathogen (Heath 1981a, 1991).

If the pathogen circumvents or tolerates the more general defence reactions of a plant and the requirements for pathogen growth are met, the plant species is a host to the pathogen that may establish and reproduce itself in a compatible interaction with the plant (Heath 1981a). Still, in such a host-pathogen interaction, the invading pathogen may be recognised by the host, and an activation of a host defence mechanism that confines the infection may ensue (Heath 1991). The incompatibility between host and pathogen can result from a hypersensitivity resistance mechanism or a non-hypersensitivity resistance mechanism in the plant.

hypersensitivity resistance

Defence responses in incompatible interactions are frequently associated with host cell death at the infection site. This so-called hypersensitivity response has been proposed to play a causal role in resistance, as the host cell death would prevent the uptake of nutrients by the pathogen, inhibiting its growth (Heath 1980). Also, plant cell collapse is associated with the production of new and/or the release of stored anti-microbial components that inhibit fungal growth (Hammond-Kosack and Jones 1996). The hypersensitivity response can be phenotypically diverse. The timing and extent range from very early in the infection process with only one or a few host cells involved not resulting in macroscopic symptoms, to late in the infection process involving a large number of host cells around the colonies resulting in macroscopically visible necrotic flecks on the host.

Pathogen recognition and the ensuing hypersensitivity reaction are presumed to be determined by the interaction of a plant resistance gene product and a corresponding pathogen avirulence gene product. Flor (1942) first assessed this gene-for-gene relationship in flax and flax rust. It holds true for many plant-pathogen interactions as studies with isolated resistance genes and some of the corresponding avirulence genes have shown in the last few years (e.g. Hammond-Kosack and Jones 1997, Laugé and De Wit 1998). The gene-for-gene model can explain the lack of durability found for many monogenic hypersensitivity resistance genes as a single mutation in the avirulence gene can result in loss of recognition of its product in the host and thus in a compatible interaction.

In the interaction between plants and biotrophic rust fungi and powdery mildews, the hypersensitivity is initiated in plant cells in which the pathogen is forming a haustorium to absorb nutrients. The hypersensitivity resistance in these interactions is therefore posthaustorial. In wheat, almost all of the leaf rust resistance genes used in wheat breeding cause a hypersensitivity reaction (McIntosh et al. 1998), as are most of the powdery mildew resistance genes in wheat and in barley (Jørgensen 1992b, Chen and Chelkowski 1999).

non-hypersensitivity resistance

A few cases of plant-pathogen interactions are known in which the mechanism of resistance is non-hypersensitive. The *Rx* gene of potato to potato virus X mediates a rapid arrest of the potato virus X accumulation in the infected host cell, most likely through suppression of virus replication or the promotion of virus degradation (Bendahmane et al. 1999). The *Arabidopsis thaliana* mutant *dnd1* has an effective resistance to *Pseudomonas syringae* based upon the constitutive induction of systemic acquired resistance (Yu et al. 1998). Resistance to *Alternaria alternata* f.sp. *lycopersici* in tomato and *Cochliobolus carbonum* in maize depends on the detoxification of fungal toxins by the *Asc1* and *Hm1* gene respectively (Johal and Briggs 1992, Van der Biezen et al. 1996).

The non-hypersensitivity resistance to the biotrophic rusts and powdery mildews acts prehaustorially, before the fungus enters the host cell to form a haustorium. The *mlo* gene in barley confers nearly complete monogenic non-hypersensitivity resistance to powdery mildew (*Blumeria graminis* f. sp. *hordei*; syn. *Erisyphe graminis* f. sp. *hordei* Jørgensen 1992a). Failure of haustorium formation by the fungus is accompanied by a cell wall apposition in the plant cell directly beneath the site of attempted penetration (Jørgensen and Mortensen 1977).

Incomplete non-hypersensitive resistance, or partial resistance, can be conferred by single genes, such as *Lr34* and *Lr46*, in wheat to wheat leaf rust. In plants possessing one of these genes the macroscopic reaction is susceptible, but fewer infections per surface area are established, and the disease develops at a slower rate than in a compatible interaction without partial resistance (Rubiales and Niks 1995, Singh et al 1998). Partial resistance can also be conferred by several genes, as was demonstrated in barley and hexaploid wheat genotypes with their respective leaf rusts, that show a similar macroscopic reaction as caused by *Lr34* and *Lr46* (Jacobs and Broers 1989, Qi et al. 1998). Histologically, partial resistance arrests infection units early in the infection process without plant cell necrosis, although some infection units continue to grow and form pustules (Niks 1983, Rubiales and Niks 1995).

In nonhost interactions of plants to inappropriate rust fungi, the failure to infect is also often primarily due to the failure of haustorium formation (Heath 1980, Heath 1981b, Niks and Dekens 1991). Up to 100% the infection units of the inappropriate rust initially develop normally, including the penetration of the stoma, but are prehaustorially aborted subsequently (e.g. rye leaf rust, *P. recondita*, on barley and on wheat and *Uromyces dactylidis* on rye; Niks and Dekens 1991). In some nonhost interactions the reactions are, however, mainly posthaustorial (e.g. barley leaf rust on rye; Niks and Dekens 1991).
Although some non-hypersensitivity resistances are governed by a gene-for-gene relationship, such as the *Rx* mediated resistance (Harrison 2000), it is assumed that non-hypersensitivity resistance is in general more durable than hypersensitivity resistance. Indeed, the widespread application of *Lr34* in wheat and *mlo* and partial resistance genes in barley have thus far not led to the erosion of the resistances governed by these genes (Niks et al. 2000). Also, nonhost resistance mechanisms appear to be durable: the move of a pathogen to a new host species is very uncommon (Heath 1980). Thus it would seem worthwhile to incorporate resistance based on non-hypersensitivity in breeding programs.

plant disease – plant defence in this thesis: wheat leaf rust versus diploid wheat

wheat leaf rust

Wheat leaf rust, *Puccinia triticina* Erikss. (syn. *Puccinia recondita*, Rob. ex Desm., f.sp. *tritici*) is a biotrophic pathogen of wheat that occurs practically everywhere where wheat is produced. Yield losses due to the disease may be more than 10% (Roelfs et al. 1992). Typical signs of wheat leaf rust infection are small round to oval pustules, or uredia, that can measure up to 0.2 cm in diameter, and appear mainly on the leaf blades. The uredia produce large numbers of orange-red urediospores for up to several weeks and each spore can initiate a new infection in the asexual cycle of wheat leaf rust.

The sexual cycle of wheat leaf rust consists of four different developmental stages, two of which infect the alternate host *Thalictrum* spp. (Ranunculaceae). In most wheat growing areas, the alternate host, if present, is thought to provide little direct inoculum at the beginning of the growing season. In general, volunteer wheat, winter wheat and/or wind-dispersed spores transported from region to region are the prime source of inoculum each year (Roelfs et al. 1992). The alternate host is, however, thought to play a role in the evolution of new races (Samborski 1985) as is somatic hybridisation (Park et al. 1999) although the main source of variation in the pathogen is most likely mutations (Groth 1984).

For the research described in this thesis, only urediospores were used. When inoculated on a susceptible wheat leaf, an urediospore forms a germ tube that in its turn forms an appressorium over a stoma. The fungus enters the plant via the stoma with an infection peg and develops a substomatal vesicle just below the stoma. From the substomatal vesicle an infection hypha is formed that grows toward a host mesophyll cell, and, upon touching the mesophyll cell, initiates a haustorium mother cell. From the haustorium mother cell a penetration peg is developed to penetrate the host cell wall (Figure 1).

Once inside the host cell, the tip of the penetration peg expands to a haustorium. The haustorium only invaginates the plant cell membrane leaving the membrane intact. A tight association between the host and fungal membranes allows the transport of nutrients from the host to the fungus (Littlefield and Heath 1979, Mendgen 1981, Baka et al. 1995). The haustorium might also modify the metabolism of the host cell to the pathogen's own requirements (Harder and Chong 1984). A group of *Pig* (*in plant induced*) genes, single-copy genes that code for proteins involved in amino acid transport and other physiological functions, is expressed in haustoria in host plant leaves (Hahn and Mendgen 1997).

The formation of haustoria is the keystone in the infection process. If no haustoria are formed or, at any moment in the infection process, the number of functional haustoria is not sufficient to support the infection unit's nutritional requirements, the infection unit will not form a spore-producing pustule to complete its growth.

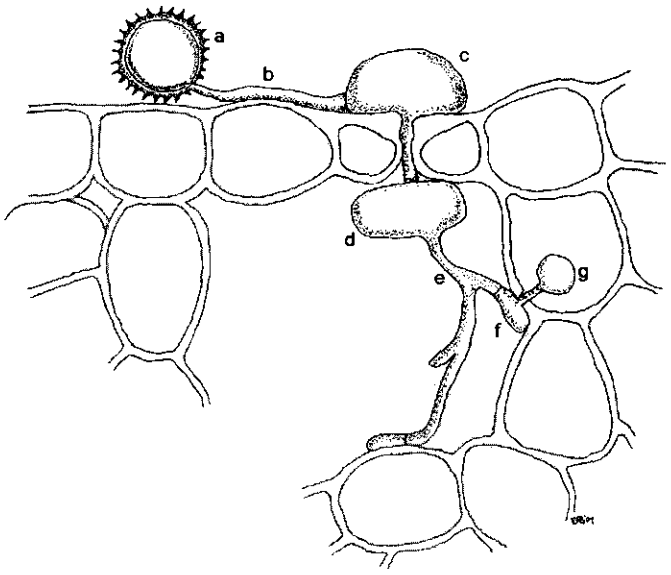

Figure 1. Infection unit of wheat leaf rust on a susceptible wheat leaf. a: urediospore, b: germtube, c: appressorium, d: substomatal vesicle, e: infection hypha, f: haustorium mother cell, g: haustorium. (drawing: Dorothée Becu)

diploid wheat

The name 'diploid wheat' is in general used for diploid *Triticum* species with the A-genome. This A-genome is very similar to one of the genomes of tetraploid (macaroni-) and hexaploid (bread-) wheat. Diploid wheat consists of two wild species: *T. urartu*, the most likely donor of the A-genome of the polyploid wheats, and *T. boeoticum*. The third diploid species, *T. monococcum*, is considered the cultivated 'form' of *T. boeoticum*. *T. monococcum* has a tough rachis that prevents shattering of the seeds making it suitable for cultivation, whereas *T. boeoticum* and *T. urartu* have a brittle rachis.

T. monococcum was one of the staple crops in ancient times grown from the British Isles to central Asia. Archeobotanists have found rachis and chaff remains of *T. monococcum* dating back to the 8th millennium BC at a Neolithic site in Syria (Nesbitt and Samuel 1996). It is still grown commercially

in a small area near Mt Ventoux and Sault in France for the production of specialty whole-grain foods and in marginal areas in Turkey for domestic and local use (Vallega 1996).

T. monococcum and the other two diploid species are of interest for cereal research and breeding because of their diploid nature and agronomically interesting characters, amongst which resistances to many pathogens of wheat, that can be transferred to polyploid wheat. Resistance to powdery mildew, stem rust, leaf rust and the Russian wheat aphid have recently been transferred to polyploid wheat from diploid wheat (Potgieter et al. 1991, Valkoun en Mamluk 1993, Hussien et al. 1997, Shi et al. 1998).

In 1991 Niks and Dekens found a non-hypersensitivity resistance mechanism to wheat leaf rust in diploid wheat. Two *T. monococcum* genotypes in a set of eleven resistant genotypes showed a high level of prehaustorial resistance: over 50% of the infection units was early aborted without plant cell necrosis. Infection units that were not prehaustorially aborted were either aborted early with necrosis or were aborted in a later developmental stage, also associated with necrosis, resulting in complete resistance (Niks and Dekens 1991). These findings formed the basis for the research described in this thesis.

scope of the thesis

The research described in this thesis had two major objectives:

- To assess the frequency of pre- and posthaustorial resistance to the wheat leaf rust fungus in diploid wheat.
- To determine the inheritance of the prehaustorial resistance to leaf rust in diploid wheat.

To address these objectives, about 600 diploid wheat accessions were tested for resistance to wheat leaf rust. The resistant accessions were studied microscopically for the frequency of prehaustorial and posthaustorial resistance (chapter 2). A subset of the accessions was studied using morphological characters and AFLP markers to determine whether the three diploid wheat species are distinct taxa (chapter 3). An F_2 of a susceptible *T. boeoticum* and a resistant *T. monococcum* with a high level of prehaustorial

resistance was studied macroscopically for the inheritance of leaf rust resistance in a series of greenhouse experiments. The influence of temperature on the prehaustorial and posthaustorial resistance was determined in a series of temperature controlled experiments in climate chambers (chapter 4). The indications for a quantitative inheritance from this study led to a quantitative approach in which the first and fifth leaf of a set of recombinant inbred lines (RILs) from a cross between a resistant *T. monococcum* and a susceptible *T. boeoticum* were inoculated. Macroscopic and microscopic observations on these RILs were used to locate quantitative trait loci (QTLs) on an RFLP linkage map. To verify the findings in the RIL population, an AFLP map was produced from the F_2 population and QTLs for the same microscopically and macroscopically observed traits were mapped (chapter 5). The final chapter summarises the results and places them in a wider perspective.

Chapter 2

Prehaustorial resistance to the wheat leaf rust fungus, *Puccinia triticina*, in *Triticum monococcum* (s.s.)

Corine C. Anker and Rients E. Niks

abstract

Diploid wheat, *Triticum monococcum* s.l., is a host for the wheat leaf rust fungus, *Puccinia triticina*. Some accessions have been reported to show a high degree of prehaustorial resistance. This is non-hypersensitivity resistance, which acts before the formation of haustoria by the pathogen. To assess the frequency of prehaustorial resistance 598 accessions of diploid wheat were inoculated with the wheat leaf rust isolate Felix. Most *T. monococcum* s.s. accessions (84%) were resistant whereas all *T. urartu* and all but three *T. boeoticum* accessions were susceptible. Histological components analysis revealed that a high percentage of prehaustorial resistance to *P. triticina* was found in only three *T. monococcum* accessions. No haustoria were observed in such infection units confirming the prehaustorial nature of the resistance. Prehaustorial abortion of certain infection units in an accession always coincided with posthaustorial abortion of the other infection units.

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introduction

Rapid adaptation of biotrophic fungal pathogens in response to the introduction of varieties with hypersensitivity resistance is a common phenomenon. Other resistance mechanisms, not based on hypersensitivity, are presumed to be more durable. In cereals, examples of such mechanisms include partial resistance to *Puccinia hordei* in barley (Niks 1986), *mlo* resistance to *Erysiphe graminis* in barley (Jørgensen 1992a), *Lr34* resistance to *P. triticina* in wheat (Rubiales and Niks 1995) and avoidance of leaf rust in *Hordeum chilense* (Rubiales and Niks 1996). To breed wheat varieties with non-hypersensitivity resistance, it is essential to determine the mechanism of the resistance found in the available germplasm.

Diploid wheat, *T. monococcum* s.l. (also often referred to as a species complex of *T. monococcum* s.s. L., *T. boeoticum* Boiss. and *T. urartu* Tum.; this classification will be used in this publication), is the donor of the A-genome in the cultivated wheat species (Kerby et al. 1988). High levels of resistance to the wheat leaf rust fungus, *Puccinia triticina* (syn. *P. recondita* f. sp. *tritici*), have been reported in this species complex (The 1976, Zitelli 1980, Dhaliwal and Gill 1982).

Based on histological observations two types of resistance can be described in diploid wheat: prehaustorial and posthaustorial resistance (Niks and Dekens 1991). Prehaustorial resistance prevents the formation of haustoria by the fungus. Normal haustorium mother cells are formed but haustoria do not develop and a papilla is often induced at the site of attempted cell wall penetration (Heath 1981a, Jacobs 1989, Niks and Dekens 1991). Prehaustorial resistance is very common in nonhost interactions (Heath 1977, 1985). In genotypes with posthaustorial resistance the plant cells die after the formation of a haustorium by the fungus (Littlefield and Aronson 1969, Heath 1981a, Niks 1983, Southerton and Deverall 1989). In general, race-specific hypersensitivity resistance is posthaustorial.

The purpose of this study was to determine the frequency of resistance to *P. triticina* in *T. monococcum* s.l. and the incidence of the prehaustorial resistance mechanism.

material and methods

plant material. Twohundred-and-eleven accessions of *T. boeoticum* (of which 29 were classified as *T. boeoticum*, 179 as *T.b.* ssp. *boeoticum* and 3 as *T.b.* ssp. *thaoudar*), 182 accessions of *T. monococcum* s.s. and 205 accessions of *T. urartu,* were provided by the National Small Grains Collection, Idaho, USA. These accessions were inoculated in 8 sets, each containing approximately 80 accessions. In a separate inoculation experiment four of the resistant accessions (PI 272560, PI 418158, PI 518452 and PI 119435) were compared in two series with the reference lines Tm2126/5 and TmEinkorn with prehaustorial resistance, and Tm2-1500 and Tb1-1082, with posthaustorial resistance (Niks and Dekens 1991). The seeds were sown in plant boxes (39x37 cm) and plants were grown in a greenhouse compartment with a minimum day temperature of 19°C, a maximum of 26°C and a minimum day length of 16 hours. *T. aestivum* cv. Little Club was used in both experiments in each box as the susceptible control.

inoculation. Twelve to 13 days after sowing, first leaves of at least five seedlings per accession were fixed horizontally and inoculated in a settling tower. Five mg of urediospores of leaf rust isolate Felix mixed with about ten times the volume of *Lycopodium* spores was used for each plant box resulting in an urediospore density of circa 300 cm^{-2}. The virulence/avirulence pattern of isolate Felix on seedlings is *Lr 2b, 2c, 3ka, 10, 11, 14a, 14b, 15, 16, 18, 20, 21, T, Ech, E, Egl 1, 2a, 3, 3bg, 17, 23, 24, 25, B* (Broers 1989b).

sampling. Four-cm-long segments of the middle of three first leaves per accession were collected six days post inoculation in the first experiment. In the first series of the second experiment, three to four cm long central segments of three first leaves per accession were collected after 42 h and after six days, in the second series after six days only.

macroscopic observations. Ten to 12 days post inoculation the infection type (IT) was determined according to the scale for seedlings of McNeal et al. (1971), with some modifications (Table 1). Since the first leaves were usually longer than 15 cm, the proximal eight cm of sampled leaves was used to evaluate infection type. Between six days post inoculation and the day of

scoring, accessions were observed twice a day for substantial differences in latency period.

Table 1. Adapted scale of McNeal et al. (1971) for wheat leaf rust infection types (IT) in diploid wheat seedlings

IT	description	interpretation
0	no visible symptoms	resistant
1	very small, pinpoint necrotic flecks	,,
2	large necrotic flecks	,,
3	very small pustules surrounded by necrosis	,,
5	small and large pustules, necrotic and chlorotic flecks	intermediate
7	large pustules with extensive chlorosis	susceptible
8	large pustules with some chlorosis	,,
9	large pustules, little or no chlorosis	,,

microscopic observations. Leaf segments harvested 42 hours post inoculation were fixed for 30 min in acetic acid/ethanol (1:3 v/v), boiled for 10 min in 0.005 % trypan blue in lactophenol/ethanol (1:2 v/v) and cleared for at least 24 hours in an almost saturated solution of chloral hydrate (5:2 w/v; Niks 1986). The number of haustorium mother cells and haustoria per infection unit were observed for 15 infection units per leaf segment. Results were averaged over the three leaf segments per accession. One segment per accession was then washed in a series of 80, 90 and 100% ethanol, stained for five min in a saturated solution of picric acid in methyl salicylate and then cleared in methyl salicylate (Niks 1986) to count the number of papillae per infection unit. Fifteen infection units per leaf segment were observed using phase contrast light microscopy at 1000x.

Leaf segments harvested six days post inoculation were stained following the procedure by Rohringer et al. (1977) using Uvitex 2B (Ciba-Geigy) instead of calcofluor. Fifty infection units, i.e. mycelial structures originating from one urediospore that had formed at least one haustorium mother cell, were scored per leaf segment. The number of haustorium mother cells (one to five or more than five) and the presence of necrosis, indicated by plant cell autofluorescence at the infection site, were scored. Fluorescence microscopy at 100x (Zeiss axiophot, exciter filter BP 395-440, chromatic beam splitter FT 460 nm and barrier filter LP 420) was used for the observations. All infection units with at least one but no more than five haustorium mother cells were

considered early aborted (Niks and Kuiper 1983). The results were averaged over the three leaf segments per accession.

results

The macroscopic evaluation of the 598 accessions indicated clear differences between the diploid wheat species (Table 2). All *T. urartu* accessions and all but three *T. boeoticum* accessions were susceptible (IT 7-9, see Table 1). Most of the *T. monococcum* accessions were resistant (84%; IT 1-3), 1.5% did not have a uniform infection type (susceptible and resistant plants in an accession), 1.5% had an intermediate infection type (IT 5) and 13% was susceptible (IT 7-9). The susceptible control Little Club invariably scored infection type 9. No substantial differences in infection frequency or latency period were observed between the susceptible accessions. Resistance to *P. triticina* was therefore confined to *T. monococcum* s.s. and rare or absent in the other two species.

Table 2. Number of accessions per diploid wheat species resistant and susceptible to wheat leaf rust isolate 'Felix' in the seedling stage, based on macroscopic observations

Species	Total number of accessions	Resistant	Susceptible	Intermediate IT[a] or heterogeneous accession
T. monococcum s.s	182	152	24	6
T. urartu	205	0	205	0
T. boeoticum	29	0	29	0
ssp. *boeoticum*	179	2	177	0
ssp. *thaoudar*	3	1	2	0

[a] infection type

The infection units of the pathogen could be classified unambiguously as early aborted (in general two to three haustorium mother cells) and established (in general more than eight haustorium mother cells). The percentage of early aborted infection units in resistant accessions varied from 0% to 100% (Figure 1). In the susceptible control Little Club all fungal colonies were well developed, between 1 and 1.7 mm long, and the frequency of early aborted

infection units was negligible. Four of the resistant accessions only had non-sporulating established infection units and two accessions only had early aborted infection units with necrosis. Early abortion without necrosis was always found in combination with infection units with early abortion with necrosis (Figure 1).

The accessions PI 272560, PI 428158 and PI 518452 had the highest percentage of early abortion without necrosis in combination with a high level of early abortion with necrosis in the first experiment. The second experiment confirmed their relatively high level of prehaustorial resistance (Table 3).

Table 3. Development of infection units of *Puccinia triticina* isolate Felix and infection type (IT) on eight diploid wheat accessions and breadwheat cultivar Little Club at 42 hours, 6 days and 10-12 days post inoculation

	42 hours post inoculation (p.i.)			6 days p.i.				10-12 days p.i.
Accession	Percentage of early aborted infection units[a] without a visible haustorium	Total percentage of early aborted infection units[a]	Number of infection units[b] with at least one papilla per infection unit	Percentage of early aborted infection units[c] without necrosis		Percentage of early aborted infection units[c]		IT[d]
				I[e]	II	I	II	
TmEinkorn	91	100	7/15	67*	35	97*	99	0
Tm2126/5	91	93	11/16	58*	56	100*	99	0
PI 272560	89	100	13/14	78	81	99	99	0
PI 428158	62	96	5/15	65	54	86	76	1
PI 518452	76	89	12/16	49	60	94	97	0
Tm2-1500	4	73	0/15	0*	19	91*	86	0
Tb1-1082	8	91	0/15	13*	16	89*	100	0
PI 119435	44	96	3/15	4	27	96	95	0
Little Club	0	33	0/30	0	0	0	0	9

[a] based on at least 44 infection units per accession
[b] based on at least 14 infection units per accession
[c] based on at least 150 infection units per accession. An infection unit was considered early aborted if at least one and no more than five haustorium mother cells were formed
[d] infection type (see Table 1) based on at least 5 primary seedling leaves per accessions.
[e] I and II are first and second series
*data from Niks & Dekens (1991).

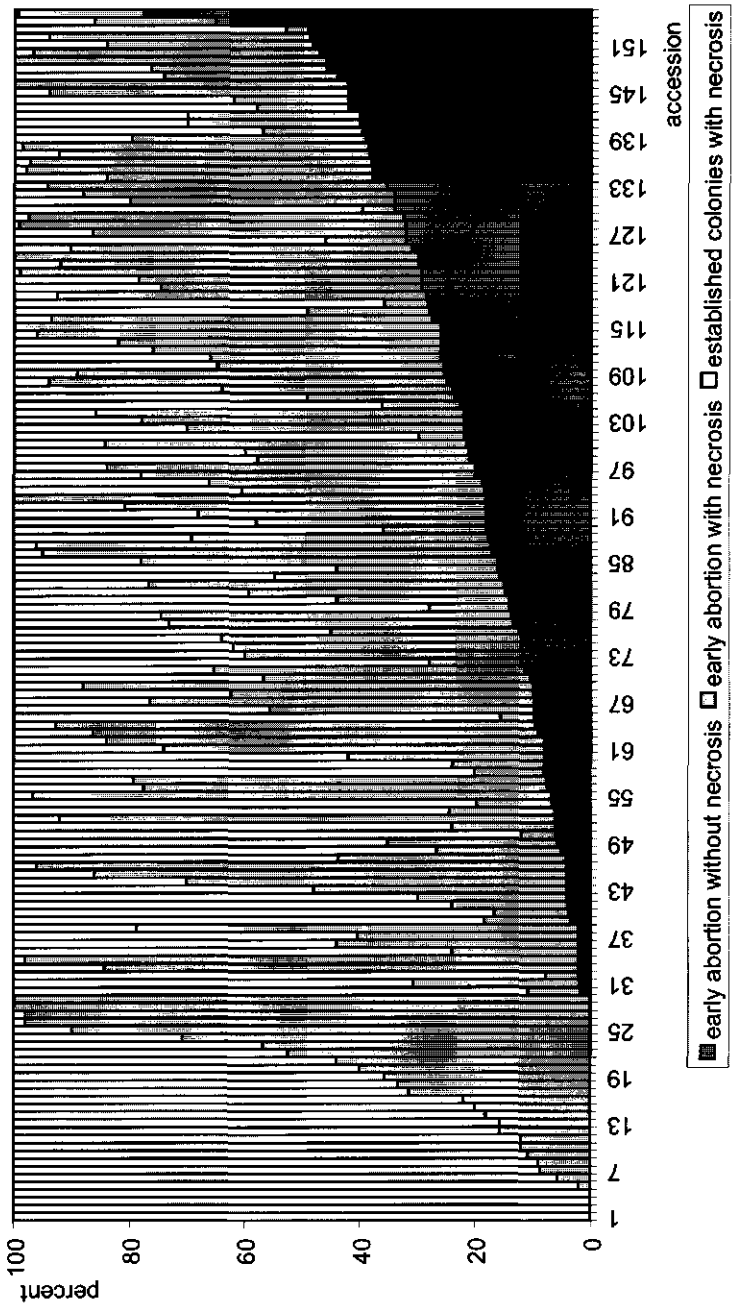

Figure 1. Percentage of early aborted infection units without necrosis and of early aborted and established infection units with necrosis in 155 resistant diploid wheat accessions.

Averaged over both experiments the percentage of early aborted infection units without necrosis was more than 50% for each accession. This is about the same level of prehaustorial resistance as in the prehaustorially resistant reference lines TmEinkorn and Tm2126/5 (Niks and Dekens 1991). Thus, accessions of *T. monococcum* s.s. with a high frequency of prehaustorial resistance are rare. TmEinkorn showed a lower percentage of early abortion without necrosis in the present study than reported by Niks and Dekens (1991; Table 3).

Further histological observations were performed to verify the absence of haustoria in early aborted infection units without necrosis and the possible presence of papillae therein. PI 272560, PI 428158 and PI 518452, with a high percentage of early abortion without necrosis, had a high percentage of infection units without any haustorium similar to the reference lines with prehaustorial resistance (Table 3).

Plant cells surrounding the infection units in accessions with a high level of prehaustorial resistance were stained darker by the trypan blue than other leaf cells, but did not collapse. This dark staining could indicate a physiological change in the cell membrane or the cell contents or both. In the reference lines with posthaustorial resistance (Tm2-1500 and Tb1-1082) dark staining combined with granulation and collapse of the plant cells at the infection site was clearly visible in many cases. In those accessions, on average two or more haustoria per infection unit were observed. In PI 119435, a much lower number of haustoria per infection unit was observed. Very dark staining of plant cells, combined with granulation and plant cell collapse around the infection units, may have obscured haustoria in this accession.

The reference lines with prehaustorial resistance often formed papillae as did the three accessions selected for a high level of early abortion without necrosis (Table 3).

Papillae were not observed in the reference lines with posthaustorial resistance and in the susceptible control Little Club. Although PI 119435 displayed posthaustorial resistance, sometimes a papilla was found at an infection site. Where a papilla was present at one of the contact sites between a haustorium mother cell and a plant cell in any accession, a papilla was also visible near most other haustorium mother cells of the same infection unit.

The pathogen never penetrated a papilla to form a haustorium. Half of the haustorium mother cells that failed to form a haustorium in TmEinkorn were not associated with a papilla (Table 3). This indicates that a papilla may be the consequence rather than the cause of the pathogen's failure to penetrate the cell wall. Of the other lines with a high percentage of early abortion without necrosis PI 428158 had a percentage of papillae similar to TmEinkorn (Table 3). The absence of a papilla in this accession mainly occurred in infection units that had induced necrosis.

These results confirm the prehaustorial nature of resistance in accessions with a high percentage of early abortion without necrosis. This prehaustorial resistance is usually, but not necessarily, associated with the formation of a papilla at the site of attempted cell wall penetration.

discussion

The diploid wheat species examined in this study differed clearly from each other in resistance to *P. triticina*. Almost all *T. monococcum* (s.s.) accessions were resistant to isolate Felix. All *T. urartu* accessions and almost all *T. boeoticum* accessions were susceptible to this isolate. Our results confirm the report by The (1976) who found 110 of 121 accessions of *T. monococcum* resistant to two isolates of *P. triticina* and 11 with intermediate resistance. Zitelli (1980) tested 122 accessions of *T. monococcum*, all of which were resistant. However, both authors do not mention *T. urartu* or *T. boeoticum*, although The (1976) does mention the existence of a wild and a cultivated form of *T. monococcum*. Dhaliwal and Gill (1982) reported resistance to the wheat leaf rust fungus in 47 *T. monococcum* accessions, but in contrast to the present study also reported resistance in ten out of 19 *T. urartu* accessions and in 29 out of 39 *T. boeoticum* accessions. Niks and Dekens (1991) reported posthaustorial resistance in a *T. boeoticum* accession. Also, Hussien et al. (1997) tested two leaf rust resistance genes transferred to common wheat from 'var. *boeoticum*'. The reported resistance in *T. urartu* and *T. boeoticum* that contrast with our findings could be ascribed to at least four causes: different isolates used in the tests, different accessions tested,

incorrect assignment of accessions to a species and criterion differences to consider an accession resistant. The identity of the resistant *T. boeoticum* and susceptible *T. monococcum* accessions in this study should be verified.

Partial resistance, which is characterised by a reduced rate of epidemic build-up in spite of a susceptible infection type, was not found in the present study as all susceptible accessions were equally susceptible. Substantial variation in latency period or infection frequency did not occur as reported for partial resistance in barley to *P. hordei* (Parlevliet 1975, Parlevliet and Kuiper 1977) and for partial resistance to *P. triticina* in spring wheat (Broers 1989a). In those plant-pathogen systems a large number of minor genes occur that cause variation in partial resistance (Broers and Jacobs 1989, Qi et al. 2000). Diploid wheat is clearly not a good source for such resistance genes.

The fact that *P. triticina* isolate Felix has species specific pathogenicity to the three diploid wheat species suggests that *T. boeoticum* and *T. urartu* harbour few or no *Lr*-genes or that the *Lr*-genes present have been overcome by the pathogen. The almost uniform resistance of *T. monococcum* does not necessarily imply that there is a large diversity of resistance genes present in that species. It is also conceivable that *T. monococcum* has a high allele frequency of one or few *Lr*-genes that correspond with avirulence-factors in the pathogen. It has been pointed out (Niks 1988, Heath 1991, Tosa 1996) that nonhost resistance to inappropriate formae speciales may be based on few major genes for resistance that correspond in a gene-for-gene manner with avirulence genes in the pathogen. If those genes occur in high allele frequency in host and pathogen, respectively, this would result in (almost) nonhost status of the plant species to that forma specialis.

A large variation in the amount of prehaustorial and posthaustorial resistance was observed within the resistant *T. monococcum* accessions. However, a high percentage of prehaustorial resistance to *P. triticina* (i.e. more than 50% of the infection units early aborted without necrosis) was found in only three out of 182 *T. monococcum* accessions (<2%). The previously reported two out of 13 diploid wheat accessions with a high level of prehaustorial resistance (Niks and Dekens 1991) apparently did not reflect the real frequency of this phenomenon in diploid wheat.

Phase contrast microscopy observations confirmed that early abortion without necrosis is indeed prehaustorial (Niks and Dekens 1991). Few or no haustoria were formed and papillae were in general present where a haustorium mother cell was in contact with the plant cell. In TmEinkorn papillae were not found in about half of the infection units that failed to form haustoria. This indicates that papillae are not a prerequisite for resistance and probably are the consequence rather than the cause of the failed cell wall penetration.

Prehaustorial abortion always coincided with posthaustorial hypersensitivity resistance in the other infection units. It seems therefore likely that the regulation of these two mechanisms is dependent. Segregation in the progeny of a cross between a resistant and a susceptible diploid wheat should allow more definite conclusions on the (in)dependence of the pre- and posthaustorial mechanism.

Prehaustorial resistance would be the first defence mechanism to be encountered by an infection unit. Indeed, staining of the cells at the infection site in accessions with prehaustorial resistance indicates physiological activity of the plant cells when encountered by the pathogen. If the prehaustorial mechanism is not sufficiently strongly or quickly expressed, the infection unit will be able to enter the plant cell in which a posthaustorial defence mechanism may be elicited. If both pre- and posthaustorial resistance mechanisms are not expressed sufficiently, the infection unit continues to grow and colonise the plant tissue. In *T. monococcum* this colonisation would rarely result in a small sporulating pustule surrounded by necrosis as the infection type in *T. monococcum* varies between 1 and 3 (Table 1).

In this respect the prehaustorial resistance in diploid wheat appears to differ from partial resistance in barley and wheat. When early abortion without necrosis occurs in partially resistant barley or wheat almost all infection units that are not early aborted will produce normal pustules, except when, in addition to the genes for partial resistance, the plant also contains one or more effective genes for hypersensitivity resistance. The barley variety Cebada Capa, for instance, not only carries several genes for partial resistance which cause early abortion without necrosis in some infection units, but also the major hypersensitivity resistance gene *Rph7* (syn. *Pa7*) causing the late abortion of all other infection units (Niks and Kuiper 1983).

The level of prehaustorial resistance in the *T. monococcum* seedlings PI 272560, PI 428158, PI 518452, TmEinkorn and Tm2126/5 is higher than in Thatcher-*Lr34* seedlings (Rubiales and Niks 1995) and similar to that of Vada-*P. hordei* (Niks 1986). The prehaustorial nature of the mechanism may indicate a different class of resistance genes than the genes for hypersensitivity resistance, which are known to be ephemeral. The use of prehaustorial resistance in breeding programmes would broaden the pool of possibly durable forms of resistance to wheat leaf rust.

acknowledgements

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Chapter 3

Morphological and molecular characterisation confirm that *Triticum monococcum* s.s. is resistant to wheat leaf rust

Corine C. Anker, Jaap B. Buntjer and Rients E. Niks

abstract

The three diploid wheat species *Triticum monococcum*, *T. boeoticum* and *T. urartu* differ in their reaction to wheat leaf rust, *Puccinia triticina*. In general, *T. monococcum* is resistant while *T. boeoticum* and *T. urartu* are susceptible. However, upon screening a large collection of diploid wheat accessions 1% resistant *T. boeoticum* accessions and 16% susceptible *T. monococcum* accessions were found. In the present study these atypical accessions were compared with 49 typical *T. monococcum*, *T. boeoticum* and *T. urartu* accessions to gain insight in the host-status of the diploid wheat species for wheat leaf rust. Cluster analysis of morphological data and of AFLP fingerprints of the typical accessions clearly discriminated the three diploid species. *T. monococcum* and *T. boeoticum* had rather similar AFLP fingerprints while *T. urartu* had a very different fingerprint. The clustering of most atypical accessions was not consistent with the species they were assigned to but intermediate between *T. boeoticum* and *T. monococcum*. Only four susceptible *T. monococcum* accessions were morphologically and molecularly similar to the typical *T. monococcum* accessions. Results confirmed that *T. boeoticum* and *T. monococcum* are closely related but indicate a clear difference in host-status for the wheat leaf rust fungus in these two species.

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introduction

Triticum monococcum s.l., or diploid wheat, consists of three closely related species: *T. monococcum*, *T. boeoticum* and *T. urartu* (Jakubziner 1958). Diploid wheat is a valuable source of resistance genes for wheat breeding. The stem rust resistance genes *Sr21*, *Sr22* (The 1973), *Sr35* (McIntosh et al. 1984), some undesignated leaf rust resistance genes (Hussien et al. 1997) and the powdery mildew resistance gene *PmTmb* (Shi et al. 1996) have been successfully introduced to polyploid wheat from diploid wheat.

In a recent study the occurrence of resistance to wheat leaf rust, *Puccinia triticina* (syn. *Puccinia recondita* f. sp. *tritici*) was determined in 598 diploid wheat accessions (Anker and Niks 2001). The diploid wheat species clearly differed in their reaction to wheat leaf rust. Resistance was confined to *T. monococcum* with 84% resistant accessions whereas all *T. urartu* and 99% of the *T. boeoticum* accessions were susceptible to the wheat leaf rust isolate 'Felix'. The difference between the resistant and susceptible accessions was very clear with an infection type of 8-9 for the susceptible accessions and 0-3 for the resistant accessions on a scale of 0-9 (Anker and Niks 2001).

T. urartu does not easily intercross with both other species but the morphologically quite similar *T. boeoticum* and *T. monococcum* species intercross relatively easily allowing introgression of traits from one to the other species (Johnson and Dhaliwal 1976). Therefore, the identity of the accessions that reacted atypically to the wheat leaf rust should be verified before a proper statement about the taxon-specificity of the resistance can be made.

The aim of this study was to distinguish the three diploid wheat species based on morphological characters and AFLP markers and to verify the identity of the atypical susceptible *T. monococcum* and resistant *T. boeoticum* accessions. The results will provide more insight in the host status of the diploid wheat species for wheat leaf rust. The AFLP fingerprints will also provide information about the relatedness between the three diploid wheat species.

material and methods

plant material. Nineteen susceptible *T. boeoticum*, 15 resistant *T. monococcum* and 15 susceptible *T. urartu* accessions (hereafter referred to as 'typical accessions') were randomly chosen from at least 150 typical accessions of the same species (Table 1).

Table 1. Accessions of diploid wheat used for morphological and AFLP analysis*

Species	Accession Number	Leaf rust Reaction	Origin
Typical accessions based on leaf rust reaction			
T. boeoticum	538526	S	Turkey
	538556	S	Iraq
	538587	S	Iraq
	538607	S	Iraq
	538619	S	Turkey, Yozgat
ssp. *boeoticum*	427484	S	Turkey, Mardin
	427503	S	Turkey, Mardin
	427522	S	Turkey, Mardin
	427536	S	Turkey, Mardin
	427554	S	Turkey, Mardin
	427637	S	Iraq
	427664	S	Iraq
	427799	S	Iran
	427903	S	Iraq
	427977	S	Turkey, Urfa
	428002	S	Lebanon
ssp. *thaoudar*	352503	S	Switzerland
	352504	S	Switzerland
	352505	S	Switzerland
T. monococcum	10474	R	USA, Washington
	17657	R	USA, Washington
	167589	R	Turkey, Canakkale
	168806	R	USA, Kansas
	221329	R	Yugoslavia
	266844	R	UK
	277135	R	Not known
	295058	R	Bulgaria
	330550	R	UK
	352479	R	Turkey
	355524	R	Germany, W-stephan
	377666	R	Yugoslavia
	427927	R	Iraq
	428152	R	Belgium
	503847	R	South Africa
T. urartu	17664	S	Lebanon
	428184	S	Turkey, Mardin
	428194	S	Turkey, Mardin
	428204	S	Turkey, Mardin
	428214	S	Turkey, Mardin
	428234	S	Turkey, Urfa
	428254	S	Turkey, Mus
	428275	S	Lebanon
	428295	S	Lebanon
	428305	S	Lebanon

Table 1, continued

Species	Accession Number	Leaf rust Reaction	Origin
	428315	S	Lebanon
	428335	S	Lebanon
	428269	S	Syria
	538729	S	Turkey, Urfa
	538749	S	Lebanon
Atypical accessions based on leaf rust reaction			
T. boeoticum			
ssp. *boeoticum*	427447	R	UK
ssp. *thaoudar*	352502	R	Turkey
T. monococcum	94741	S	Ukraine, Kharkiv
	182461	S	Turkey, Bolu
	345133	S	Serbia, S. Klisura
	345186	S	Serbia, D. Kormilovo
	407604	S	Turkey, Ankara
	554529	S	Turkey, Izmir
	560719	S	Turkey, Siirt
	560721	S	Turkey, Siirt
	560722	S	Turkey, Van
	560723	S	Turkey, Mus
	560724	S	Turkey, Mus
	560725	S	Turkey, Mus
	560726	S	Turkey, Mus
	560727	S	Turkey, Mus
	560728	S	Turkey, Mus
	573520	S	Turkey, Eskisehir
	573521	S	Turkey, Bilecik
	573523	S	Turkey, Bilecik
	573524	S	Turkey, Bolu
	573525	S	Turkey, Cankiri
	573526	S	Turkey, Cankiri
	573527	S	Turkey, Ankara
	573528	S	Turkey, Ankara
	573529	S	Turkey, Ankara
	591871	S	Georgia
T. aestivum	Little Club	S	Own collection

*Accession numbers and indication of origin as used by the National Small Grains Collection, Idaho, USA. Leaf rust reaction according to Anker and Niks (2001)

The typical accessions were compared to two atypically resistant *T. boeoticum* and 25 atypically susceptible *T. monococcum* accessions (hereafter called 'atypical accessions') and *T. aestivum* accession 'Little Club'. All 76 diploid wheat accessions (Table 1) were obtained as part of a resistance study from the National Small Grains Collection, Idaho, U.S.A. (Anker and Niks 2001). Individual seeds were sown in 4 cm diameter pots in the greenhouse in December. Two-week-old seedlings were vernalised in a climate room at 6°C and 14 hours light per day for five weeks. Two plants per accession were transferred to 12x12 cm pots and grown in a non-heated greenhouse from February to May.

morphological characterisation. A morphological description of each of the two plants per accession was made at anther dehiscence of the first ear per plant from April to May. The 17 characters observed were selected from Johnson (1975), Kimber and Feldman (1987) and Percival (1921) (Table 2).

Table 2. Characters used for the morphological description

length of straw
length of ear
length of awns above the ear
length first awn of a spikelet
length second awn of a spikelet
length first tooth on sterile glume
length second tooth on sterile glume
length anthers
presence of a third awn (present: 1, absent: 0)
density (number of spikelets per 10 cm ear)
number of flowers per spikelet
number of rudimentary flowers
pubescence leaves (strong: 2, mild: 1, absent: 0)
pubescence nodes (strong: 2, mild: 1)
pubescence leaf ridges (longer hairs present: 1, absent: 0)
anthocyanin at base of the stem (present: 1, absent: 0)
flowering date (number of days till flowering of first ear)

DNA isolation and AFLP analysis. The two seedlings per accession were combined to collect one leaf sample of around 1 g for DNA isolation. DNA was extracted with the CTAB method (Van der Beek et al. 1992). The AFLP technique (Vos et al. 1995) was performed according to Van Eck et al. (1995) with modifications according to Qi and Lindhout (1997). The primer combination E33M58 was selected for its high number of amplification products and clear polymorphisms when tested on barley (Qi and Lindhout 1997) and on four diploid wheat accessions (Vaz Patto et al. 2000). Electrophoresis was performed in duplicate and only unambiguous fragments with a size range between 130 and 480 bp were scored as dominant markers.
data analysis. Both the AFLP data and morphological data were analysed using principal co-ordinate analysis in NTSYS-pc (Rohlf 1997) version 2.02. Morphological data were standardised before using the SIMINT module based on the average taxonomical distance to produce the similarity matrix. The AFLP similarity matrix was calculated using the SIMQUAL module with the Jaccard coefficient. DCENTER and EIGEN procedures were employed for

principal co-ordinate analysis. For comparison the data were clustered in dendrograms using the SAHN module with the UPGMA clustering method for both morphological and AFLP data.

results

Principal co-ordinate analysis of the morphological data revealed three distinct groups for typical *T. monococcum*, *T. boeoticum* and *T. urartu* accessions (Figure 1).

In general, the two plants of each accession clustered close together. The average, minimum and maximum values for the morphological traits of the typical accessions are presented in Table 3. Three morphological characters (presence of the third awn, number of rudimentary flowers and leaf pubescence) were sufficient for the clustering of the typical accessions of the three diploid wheat species.

Table 3. Average values and range for the morphological characteristics of the typical *T. boeoticum* (Tb), *T. monococcum* (Tm) and *T. urartu* (Tu) accessions. Traits are presented in the same order as in Table 2. In bold character-values that distinguished one species from the other two

	av. **Tb**	Range	av. **Tm**	Range	av. **Tu**	range
length, cm						
straw	81	54-108	81	50-108	60	39- 86
ear	7.0	5.5- 8.5	5.7	4.0- 7.0	7.8	5.0-9.5
awn	8.1	3.0-12.0	5.2	1.0- 9.0	4.5	3.0-9.0
awn1/sp	10.2	6.0-14.5	5.1	2.3- 8.8	5.5	2.0-9.3
awn2/sp	4.1	0.6- 8.8	0.2	0.1- 1.1	5.0	2.0-8.8
length, mm						
tooth1/gl	1.8	1.3- 2.1	**0.7**	0.5- 1.1	2.0	1.8-3.0
tooth2/gl	0.8	0.5- 1.0	0.5	-	0.5	-
anthers	4.0	3.5- 4.3	3.5	3.0- 4.0	**2.1**	2.0-3.0
other traits						
3rd awn	0	-	0	-	0.8	0/ 1
density	2.4	2.0- 2.7	4.1	3.3- 4.9	2.6	2.0-3.4
No. fl/sp	2	-	**1**	-	2	-
No. rud fl	1	-	**2**	-	1	-
leaves	2	-	0.3	0/ 2	**1**	-
nodes	2	-	1.2	1/ 2	1	-
ridges	1	-	0.3	0/ 1	0	-
base	0.1	0/ 1	0	-	0	-
flowering	82.4	75- 91	91.8	88-102	84.6	75- 91

Based on ear morphology (number of florets per spikelet and size of the teeth on the sterile glume) two atypical susceptible *T. monococcum* accessions were identified as *T. aestivum* (182461) and *T. durum* (94741) respectively. Four atypical *T. monococcum* accessions clustered with the *T. monococcum* group and the remaining with *T. boeoticum*. One atypical *T. boeoticum* accession (352502) resembled typical *T. boeoticum* morphologically. The two plants of the other atypical *T. boeoticum* accession (427447) differed morphologically and did not cluster together.

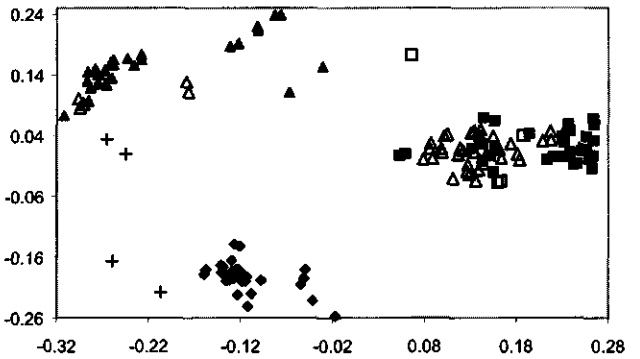

Figure 1. Principal co-ordinate plot of 70 diploid wheat accessions and one tetraploid accession (2 plants per accession) and two hexaploid wheat accessions (one plant per accession), based on morphological characteristics from Table 2 ; the first two principal co-ordinates accounted for 41.6% and 18.0% of the total variation respectively. ■ *T. boeoticum*, ▲ *T. monococcum*, ◆ *T. urartu*, + *T. durum* and *T. aestivum*; closed symbols typical accessions, open symbols atypical accessions.

AFLP patterns of typical *T. monococcum* and *T. boeoticum* were relatively similar while *T. urartu* was clearly different from these two species. As expected, the tetra- and hexaploid accessions showed a different pattern with more fragments than accessions of the diploid species (Figure 2). With primer combination E33M58 between 47 and 52 AFLP fragments were amplified per species. In total 64 fragments were used for the analysis of the diploid species and another eight fragments when the polyploid accessions were included. The number of monomorphic markers (present in all accessions of one species and absent in the other species) ranged from 20 to 24 per diploid

species and of polymorphic markers (present in some accessions of one species and absent in the other species) from 26 to 32.

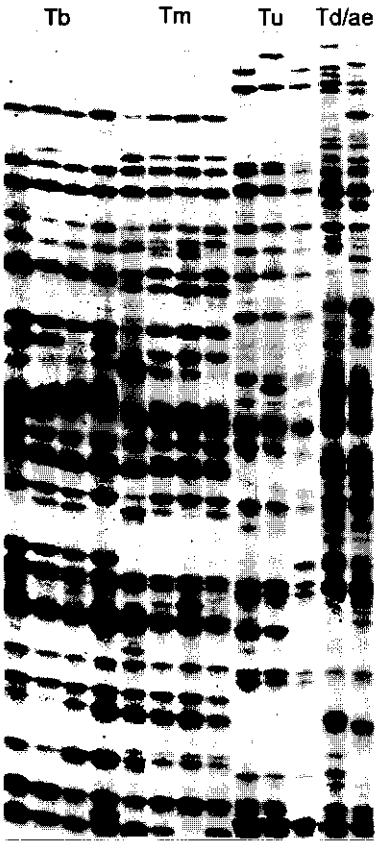

Figure 2. AFLP pattern of *T. boeticum* (Tb), *T. monococcum* (Tm), *T. urartu* (Tu), *T. durum* (Td) and *T. aestivum* (Tae).

None of the monomorphic markers present in typical *T. monococcum* accessions, was absent in all typical accessions of *T. boeoticum* and vice versa. Therefore, no species-specific markers for these species were generated using this primer combination. The distinction between these two species was restricted to polymorphic markers. Between *T. urartu* and typical *T. boeoticum* and *T. monococcum*, respectively, the percentage of polymorphism based on monomorphic markers was 21% and 25%. The rate of polymorphism within *T. boeoticum* was 59%, in *T. monococcum* 56% and in

T. urartu 42%. The three typical *T. boeoticum* ssp. *thaoudar* accessions had highly polymorphic AFLP patterns (43% polymorphism), two of these three AFLP patterns were also very different from the other *T. boeoticum* accessions. If the three typical *T. boeoticum* ssp. *thaoudar* were not taken into account 54% polymorphism was found in typical *T. boeoticum* accessions.
In the PCO analysis based on the AFLP data of all species, the three sets of typical diploid accessions and the polyploid accessions each formed a distinct cluster (Figure 3).

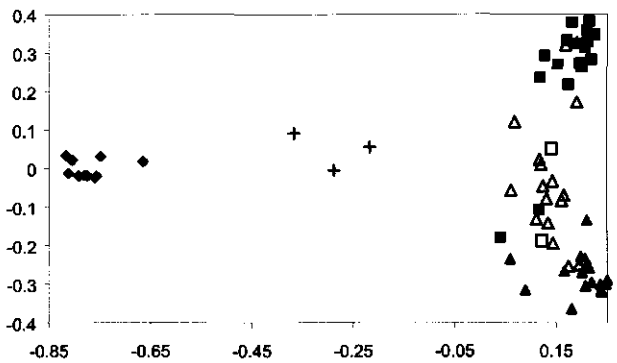

Figure 3. Principal co-ordinate plot of all accessions based on one AFLP fingerprint per accession; the two principal co-ordinates accounted for 29.7% and 12.7% of the variation respectively. ■ *T. boeoticum*, ▲ *T. monococcum*, ◆ *T. urartu*, + *T. durum* and *T. aestivum*; closed symbols typical accessions, open symbols atypical accessions.

However, two typical *T. boeoticum* ssp. *thaoudar* accessions (352504 and 352505) and most of the atypical *T. boeoticum* and *T. monococcum* accessions were placed in between the two typical clusters of these two species (Figure 4).

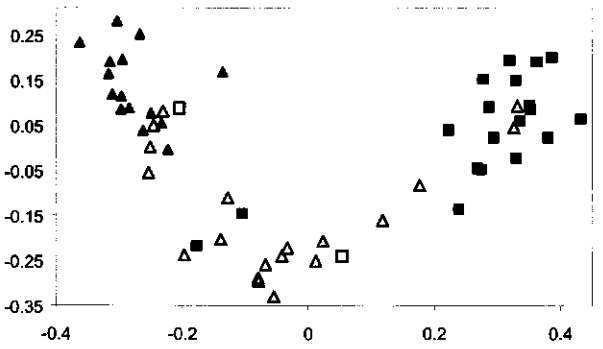

Figure 4. Principal co-ordinate plot of typical and atypical *T. monococcum* and *T. boeoticum* accessions, based on AFLP fingerprint as in Figure 3. The two principal co-ordinates accounted for 22.2% and 8.9% of the variation respectively. ■ *T. boeoticum*, ▲ *T. monococcum*; closed symbols typical accessions, open symbols atypical accessions.

Four atypical *T. monococcum* accessions (345133, 345186, 573529 and 591871) clustered within *T. monococcum* and two within *T. boeoticum* (560719 and 560721). These six accessions are the only atypical accessions for which the morphological data result in the same clustering as the molecular data. The groups formed in PCO analysis were confirmed with UPGMA analysis both for morphological and AFLP data (data not shown). The UPGMA analysis also showed that all accessions, save two *T. urartu* accessions, could be unambiguously discriminated based on the 72 amplified fragments (data not shown).

In summary, all three diploid wheat species, represented by the typical accessions, could be distinguished with PCO analysis based on morphology and on molecular data. The clustering of most atypical accessions, susceptible *T. monococcum* and resistant *T. boeoticum*, was not consistent with the species they were originally assigned to.

discussion

The data presented here showed that the 72 AFLP fragments obtained with one primer combination selected for high numbers of amplification products and high polymorphism rate, gave a clear separation of the three diploid wheat species *T. monococcum*, *T. boeoticum* and *T. urartu*. The use of DNA markers for the classification of different taxa is a relatively new development. In general, the clustering of accessions based on AFLP markers corresponds well with previous taxonomic classifications of the plant species studied (e.g. *Lactuca* spp.: Hill et al. 1996; *Manihot* spp.: Roa et al. 1997; *Solanum* spp.: Kardolus et al. 1998; *Gossypium* spp.: Pillay and Myers 1999; *Oryza* spp.: Aggarwal et al. 1999). Different numbers of polymorphic amplified fragments have been used to establish relatedness: from 273 by Mace et al. (1999) in Solanaceae to 1191 by Aggarwal et al. (1999) in rice. In the former study, major clusters obtained with each individual primer combination (nine to 49 markers) were similar to that with all primer combinations together.

Although the AFLP patterns of *T. monococcum* and *T. boeoticum* were distinct, species specific monomorphic markers were not found, indicating a close relatedness between these two species. Nevertheless, the two species could be unambiguously distinguished in the principal co-ordinate analysis based on polymorphic markers. *T. monococcum* and *T. boeoticum* patterns were quite different from the *T. urartu* pattern. This is in agreement with the findings by, amongst others, Ciaffi et al. (1997), Castagna et al. (1994), Dvorak et al. (1988) and Hammer et al. (2000) who applied gliadin patterns, RFLP markers based on single and repetitive sequences and microsatellite markers respectively. The poor crossability between *T. urartu* and either *T. monococcum* or *T. boeoticum*, almost always resulting in sterile F_1s, also indicates the distant relatedness between *T. urartu* and the other two species (Johnson and Dhaliwal 1976).

Based on the morphological and molecular data, two atypical accessions received as *T. monococcum* were actually polyploid wheats. The other atypical *T. monococcum* accessions could be divided in three groups. Two accessions resembled *T. boeoticum* morphologically and molecularly. Most

likely these accessions are typical –susceptible- *T. boeoticum* assigned to the wrong species in the germplasm collection.

The use of AFLP markers provided insight in the intermediary status of the 13 atypical *T. monococcum* accessions that morphologically resembled typical *T. boeoticum* accessions but molecularly clustered between *T. boeoticum* and *T. monococcum*. These accessions may represent progeny of hybrids that arose in the past. The offspring may have retained some of the *T. boeoticum* genotype, viz. for most of the morphological characters listed in Table 2, and for reaction to leaf rust, but also be genetically similar to *T. monococcum*. As both diploid wheat species cross relatively easily giving fertile offspring, hybridisation, and hence the genetic exchange, could easily have taken place in the region of common origin or even during propagation in the germplasm collection. Gene flow between closely related sympatric species has been observed for other genera as well (e.g. *Daucus*, Wijnheijmer et al. 1989; *Chenopodium*, Wilson and Manhart 1993).

Only four accessions resembled typical *T. monococcum* accessions both morphologically and molecularly but were susceptible to the wheat leaf rust fungus. Thus, 98% of the *T. monococcum* accessions tested before were resistant to wheat leaf rust instead of 84% (Anker and Niks 2001). The susceptible *T. monococcum* accessions could lack the resistance gene(s) to (one of) the avirulence gene(s) in the isolate. Niks (1988) and Heath (1991) presented a model and cited evidence that nonhost resistance in plants may be based on a very high allele frequency of one or a few resistance genes in the plant species and an equally high frequency of corresponding avirulence alleles in the pathogen. Histological observations in *T. monococcum* have shown that the majority of the accessions reacted to wheat leaf rust with a hypersensitive response (Anker and Niks 2001). This indicates that the species specific reaction to wheat leaf rust in diploid wheat is most likely based on a high allele frequency of one or more effective major genes in *T. monococcum* and the absence thereof in the closely related *T. boeoticum*, in the four susceptible *T. monococcum* accessions and in *T. urartu*. Hence, *T. monococcum* has almost a nonhost status to the wheat leaf rust (see Niks 1987).

To verify the model of a high allele frequency in both *T. monococcum* and the fungus for resistance genes and corresponding avirulence genes large-scale tests with different isolates would be necessary. A small-scale test with three leaf rust isolates and a mixture of isolates on two accessions each of typical and atypical *T. monococcum* and *T. boeoticum* showed that the typical *T. monococcum* and *T. boeoticum* were resistant and susceptible respectively. The atypical *T. boeoticum* were in general resistant and the atypical *T. monococcum* accessions showed a variable reaction depending on the inoculum used (data not shown, work by D.L. Long, Cereal Disease Laboratory). These results support the model described above.

The results presented here support the assumption of Anker and Niks (2001) that *P. triticina* isolate 'Felix' has species specific pathogenicity to the three diploid wheat species and that *T. monococcum* has almost a nonhost status to the pathogen. The results emphasise the need for verification of the identity of accessions in resistance surveys as was indicated by Niks (1987). This is especially true if seedlings of closely related species are tested and no distinction can be made based upon seed and seedling morphology.

acknowledgements

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Chapter 4

Genetics of prehaustorial resistance to wheat leaf rust in diploid wheat

Corine C. Anker and Rients E. Niks

abstract

F_2 segregation analyses and a temperature experiment were performed to establish the inheritance of prehaustorial resistance in *Triticum monococcum* accessions TmEinkorn and Tm2126/5 to *Puccinia triticina*. Temperatures between 12 and 28°C did not have a major influence on the macroscopic reaction of the parents and the F_1. The level of prehaustorial resistance (early abortion without necrosis) was influenced by temperature and highest at 12 and 18°C. Segregation ratios in the first and third or fifth leaves, the absence of F_2 plants that were as susceptible as the susceptible parent and the quantitative distribution of the level of prehaustorial abortion of infection units in the F_2 indicated the presence of several genes or QTLs for prehaustorial resistance.

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introduction

Diploid wheat comprises the three species *Triticum monococcum*, *T. boeoticum* and *T. urartu*. Of these three species, *T. monococcum* is a good source of resistance to the wheat leaf rust fungus *Puccinia triticina* (syn. *P. recondita* f. sp. *tritici*). Investigations by Niks and Dekens (1991) and Anker and Niks (2001) showed that, although resistance in seedlings did not show macroscopic differences, at the microscopic level remarkable differences could be seen. In some genotypes all infection units were aborted after the formation of a haustorium in association with a hypersensitivity reaction (posthaustorial resistance). In other genotypes more than half of the infection units were aborted before the formation of a haustorium (prehaustorial resistance). In most accessions a combination of prehaustorial and posthaustorial resistance was observed.

Niks and Dekens (1991) studied 13 diploid wheat accessions and identified two *T. monococcum* accessions with a high level of prehaustorial resistance in the seedling stage. These were crossed with a susceptible *T. boeoticum* and the F_2 was studied for the inheritance of the prehaustorial resistance (Zhang et al. 1992). Tests on at least 100 F_2 plants per resistant parent indicated a monogenic recessive inheritance (Zhang et al. 1992). In the same study no susceptible segregants were found in an F_2 of the two *T. monococcum* accessions (TmEinkorn and Tm2126/5) with a high level of prehaustorial resistance, indicating that these two genotypes harbour the same gene for resistance.

The prehaustorial resistant diploid wheat TmEinkorn to the leaf rust fungus is was not always fully resistant. It was intermediate in the study of Zhang et al. (1992) and intermediate to susceptible in one series and resistant in another series in the study of Niks and Dekens (1991). This suggests that the expression of resistance in TmEinkorn could be influenced by environmental factors.

The scope of this study was to study the influence of temperature on the expression of this resistance and to verify the inheritance of the prehaustorial resistance mechanism of diploid wheat to the wheat leaf rust fungus.

material and methods

plant material. Parents, F_1 and F_2 of the crosses Tb1486 (susceptible) x TmEinkorn (resistant) and Tb1486 x Tm2126/5 (resistant) made for the study of Zhang et al. (1992) were used for three experiments. TmEinkorn and Tm 2126/5 have a high level of prehaustorial resistance. In the second experiment *T. monococcum* accessions Tm272560, Tm428158 and Tm518452, with a similar high level of prehaustorial resistance as Tm2126/5 and TmEinkorn (Anker and Niks 2001) were included.

growing conditions. Seeds were sown in plant boxes (39x37 cm) and grown in a greenhouse compartment with temperatures between 19 and 26°C or in climate rooms at 12, 18, 21, 24 or 28°C continuously.

inoculation. First and third or fifth leaves were fixed horizontally and inoculated in a settling tower. Five mg of urediospores of leaf rust isolate Flamingo mixed with ten times the volume of *Lycopodium* spores was used per plant box resulting in an urediospore density of circa 300 cm^{-2}. After inoculation the plants were incubated overnight in a compartment at 100% humidity to ensure germination and penetration of the rust spores.

macroscopic observations. Ten to 12 days after inoculation the infection type (IT) was determined according to the scale of McNeal et al. (1971), with some modifications (Table 1). Plants were classified as resistant (R), intermediate (I) and susceptible (S) based on infection type (Table 1). If the resistant parent TmEinkorn did not have a fully resistant reaction, F_2 plants with a similar reaction were also classified as resistant.

Table 1. Adapted scale of McNeal et al. (1971) for wheat leaf rust infection types (IT) in diploid wheat seedlings

IT	description	interpretation
0	no visible symptoms	resistant (R)
1	very small, pinpoint necrotic flecks	,,
2	large necrotic flecks	,,
3	very small pustules surrounded by necrosis	,,
5	small and large pustules, necrotic and chlorotic flecks	intermediate (I)
7	large pustules with extensive chlorosis	susceptible (S)
8	large pustules with some chlorosis	,,
9	large pustules, little or no chlorosis	,,

microscopic observations. Leaves were sampled and stained according to Niks and Dekens (1991). The number of haustorium mother cells per infection unit and the presence of necrosis, indicated by plant cell autofluorescence at the infection site, were scored using fluorescence microscopy at 100x. All infection units with one to five haustorium mother cells were considered early aborted (Niks and Kuiper 1983). Infection units with more than five haustorium mother cells were considered established colonies.

experiments. First the F_2 segregation of both crosses was analysed in two series in the greenhouse to verify the 1:3 ratio for resistance found by Zhang et al. (1992). The reactions of the first leaves in the first series were tested at high greenhouse temperatures (continuous 26°C during daytime) while the third leaf in the first series and the first leaf in the second series were tested at moderate temperatures (average day temperature 20°C). Subsequently, the influence of temperature on the expression of resistance in accessions with a high level of prehaustorial resistance and the F_1 of both crosses was determined in climate rooms. Finally the F_2 segregation of the cross Tb1486 x Einkorn was analysed in the first and fifth leaf at 18°C, the optimal temperature for the expression of prehaustorial resistance.

results

F_2 segregation in the greenhouse. The two prehaustorially resistant *T. monococcum* accessions differed in their infection type upon inoculation with *P. triticina.* Tm2126/5 was always without symptoms (IT0) whereas TmEinkorn had small necrotic flecks (IT1) at high temperature or some sporulation (IT3-5) at moderate temperatures. At moderate temperatures the F_1 of Tb1486 x TmEinkorn was susceptible (IT8) and the F_1 of Tb1486 x Tm2126/5 had a moderately susceptible (intermediate) reaction (IT5).

The reactions of the F_2 in the first leaf differed very much between the two series (Table 2). In de first series (high temperatures) of Tb1486 x TmEinkorn resistant and susceptible plants occurred whereas in the second series (moderate temperatures) no resistant plants were found. The differences between the series of Tb1486 x Tm2126/5 in the first leaf were less extreme.

However, the F_2 shifted towards susceptibility in the series with the moderate temperatures. F_2 plants inoculated in the third leaf more often had a resistant reaction compared to the inoculation in the first leaf (Table 2). The difference in segregation ratios of the F_2s at the different temperatures suggest that at higher temperatures the resistance is expressed more effectively than at moderate temperatures. None of the F_2 plants were as susceptible as the susceptible parent.

Infection types were determined on the first leaf of F_3s of Tb1486 x TmEinkorn and Tb1486 x Tm2126/5 and compared with the infection type of the F_2 parent in the first leaf to study the genotype for resistance of the individual plants. Fully resistant F_2 plants (IT0-IT2) resulted in F_3 plants with resistant or intermediate reactions (IT0-IT5) whereas all susceptible F_2 plants produced F_3 lines that contained at least some resistant segregants. These results indicate a polygenic control of the resistance.

Table 2. Segregation ratios (number of resistant plants (R): intermediate (I) + susceptible plants (S)) of F_2 Tb1486 x TmEinkorn, inoculation with *P. triticina*

cross	series	R:I+S, 1st leaf	R:I+S, 3rd leaf
Tb1486xTmEinkorn	1	22:38 [a]	39:21 [b]
	2	0:58 [b]	
Tb1486xTm2126/5	1	30:13 [a]	38: 5 [b]
	2	13:26 [b]	

[a] high greenhouse temperature
[b] moderate greenhouse temperature

temperature studies. The infection types of the diploid wheat accessions and the F_1s of the two crosses are shown in Table 3. No or very little effect of temperature on the macroscopic expression of resistance was found as all genotypes remained in the same class (resistant, intermediate or susceptible) at all temperatures. The only exception is the F_1 of Tb1486 x 2126/5 that had a resistant to intermediate reaction at 12°C and an intermediate reaction at the other temperatures.

Microscopic observations on the five Tm-accessions showed that the proportion of early abortion without necrosis is highest at 12 and 18°C and decreases at higher temperatures in all accessions with prehaustorial

resistance (Figure 1; data TmEinkorn). In the F_1s the percentage of early aborted colonies without necrosis was lower, again with a tendency to be highest at 12-18°C (Figure 1; data F_1 Tb1486 x TmEinkorn).

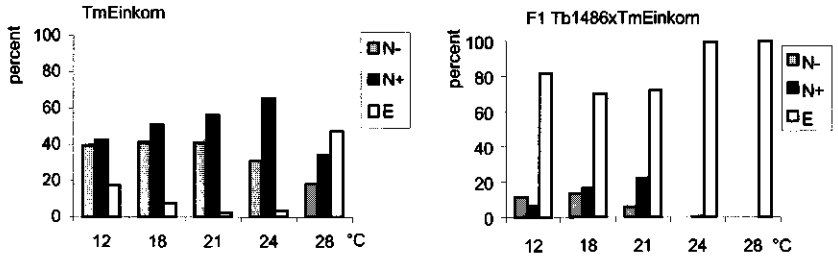

Figure 1. Percentage early abortion without (N-) and with (N+) necrosis and established infection units (E) in TmEinkorn (left) and F_1 Tb1486 x TmEinkorn (right) at different temperatures.

The colonies in the F_1 that were not early aborted continued to grow and most formed spores which resulted in an intermediate reaction (IT 5) for F_1 1486 x Tm2126/5 and a susceptible reaction (IT 7-9) for F_1 Tb1486 x TmEinkorn (Table 3).

Although the macroscopic reactions to the leaf rust infection remained the same at temperatures between 12 and 28°C, the proportion of early abortion without necrosis was highest at 12 and 18°C.

Table 3. The infection type of diploid wheat accessions and F_1 of Tb1486 x TmEinkorn and Tb1486 x Tm2126/5 at different temperatures in the first leaf stage

	12°C	18°C	21°C	24°C	28°C
Tb1486	9	9	9	9	9
TmEinkorn	0-1[a]	0	0	0	2/3[b]
Tm2126/5	0	0	0	0	0
F_1 Tb1486xTmEinkorn	9	9	8	7	7
F_1 Tb1486xTm2126/5	3-5	5	5	5	5
Tm272560	0	0	0	0	0
Tm428518	0	0	0-1/2	0/1/3	3/5
Tm518452	0	0	0	0	3

[a] x-y: score within the same leaf varies from x to y
[b] x/y: some plants score x, others y in the same accession

F_2 segregation at 18°C. The segregation ratio in the F_2 of the cross Tb1486 x TmEinkorn was tested at 18°C. Tb1486 was always susceptible (IT8-IT9) and in TmEinkorn the infection type in the fifth leaf was lower (IT0) than in the first leaf (IT1). The F_1 was susceptible in the first leaf (IT8-IT9) and had an intermediate reaction in the fifth leaf (IT5). The F_2 segregated with only 5% of the plants resistant in the first leaf. In the fifth leaf the segregation shifted to 58% resistant plants (Table 4). It seems therefore that plant development stage increases the expression of resistance in the cross Tb1486xTmEinkorn. No segregants as susceptible as Tb1486 were found in the F_2.

Table 4. Reaction types of F_2 Tb1486 x TmEinkorn to infection with the wheat leaf rust fungus in the first and fifth leaf at 18°C

	resistant IT 0 and 1[a]	intermediate, IT 5	susceptible, IT 7 and 8	Sum
1st leaf	11 (5)[b]	110 (49)	103 (46)	224
5th leaf	121 (58)	71 (34)	16 (8)	208

[a] IT 1 in the first leaf, IT 0 and 1 in the fifth leaf
[b] number of plants, percentage between brackets

The percentage of early aborted infection units without necrosis in TmEinkorn varied between 17 and 44% in the first leaf and between 21 and 71% in the fifth leaf. All other colonies aborted too. In the F_1 the percentage of early aborted infection units varied between 3 and 12% in the first leaf and between 4 and 32% in the fifth leaf. Most of the other infection units in the first leaf formed spores. In the fifth leaf the level of early abortion without necrosis was similar but more established colonies aborted before spore formation. In Tb1486 only established colonies, most of which started to form spores, were observed. The level of early abortion without necrosis varied from 0% to 50% (first leaf) and 0% to 65% (fifth leaf) in the F_2 plants. The correlation between the percentage of early abortion without necrosis in the first leaf and the fifth leaf of the F_2 was 0.68. A high percentage of early abortion without necrosis not necessarily implied a low infection type (Figure 2).

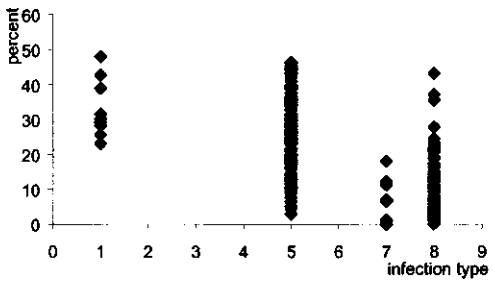

Figure 2. Level of prehaustorial resistance (percentage of early aborted infection units without necrosis; N-) in F_2 Tb1486 x TmEinkorn plants per infection type in the first leaf (n=223).

discussion

In the greenhouse experiment the segregation ratios in first leaf of the F_2s of both crosses shifted towards resistance at higher temperatures. Also the resistance of Einkorn in the first leaf was stronger at higher temperatures. The influence of temperature on the expression of rust resistance has been described for leaf rust, stem rust and stripe rust (e.g. Hyde 1982, Gerechter-Amitai et al. 1984, Gousseau et al. 1985). However, depending on the genotype studied and sometimes the isolate-genotype interaction a high temperature would induce either resistance or susceptibility.

It is not likely that stronger expression of resistance in the greenhouse at the higher temperature in this study was the result of temperature alone. The climate room studies on parents and F_1 of both crosses did not suggest a substantial effect of temperature on infection type. Although some changes of infection type at the lowest and highest temperatures were found for some accessions, classification of the genotypes (resistant, intermediate or susceptible) did not change in this experiment. The stronger expression of resistance in the greenhouse at the higher temperature might be induced by the shift between day and night temperatures, by a combination of high temperature and light intensity or maybe by other environmental factors. The effect of light in combination with high or low temperature on the expression of

resistance is reported for stripe rust and for leaf rust *Lr34* (Dyck and Samborski 1982, Ash and Rees 1994).

Contrary to the infection type, the ratio between early abortion with and without necrosis and established infection units was substantially affected by temperature in the climate room experiment. The level of early abortion without necrosis was highest at 12 and 18°C both in the resistant accessions and in the F_1s, although the overall level of prehaustorial resistance was much lower in the F_1. The microscopic data in this experiment showed that resistance was weaker at high temperatures even though the plants did not show different infection types.

In both the greenhouse and the climate room experiment the F_2 segregation ratios of Tb1486 x TmEinkorn shifted towards resistance in the fifth leaf. The reaction type of the F_1 changed from susceptible in the first leaf to intermediate in the fifth leaf and the infection type of TmEinkorn changed from IT1 in the first to IT0 in the fifth leaf in the climate room at 18°C. Also, the proportion of early abortion without necrosis was often higher in the fifth leaf compared to the first leaf, although the correlation was not very high (0.68). This indicates the influence of plant development stage on the reaction to wheat leaf rust infection. The effect of plant development stage on the expression of resistance has been reported for leaf rust, stripe rust and stem rust (e.g, Van Silfhout 1993, Knott 1997, Borner et al. 1999).

The greenhouse studies on both F_2 populations, nor the climate room experiment with the F_2 of Tb1486 x TmEinkorn confirmed the monogenic recessive inheritance of prehaustorial resistance reported by Zhang et al. (1992). In the microscopic observations of the first and fifth leaf of Tb1486 x TmEinkorn F_2 plants a quantitative distribution of early abortion without necrosis was found indicating a quantitative inheritance for prehaustorial resistance. We have therefore started experiments to determine QTLs for prehaustorial resistance in the seedling and older plant stage in the cross Tb1486 x TmEinkorn.

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Chapter 5

QTLs for wheat leaf rust resistance in diploid wheat confer prehaustorial as well as posthaustorial resistance

Corine C. Anker, Rients E. Niks, Piet Stam

abstract

T. monococcum is highly resistant to *Puccinia triticina*. In some accessions the resistance is mainly based on a non-hypersensitive type of resistance that causes abortion of most infection units just before the development of the first haustorium. Typically, the remaining infection units succeed in forming a haustorium and are arrested by a hypersensitivity reaction. To elucidate the genetics of prehaustorial resistance in diploid wheat, quantitative trait loci (QTLs) were mapped in a population of 72 RILs and in a population of 118 plants. In both populations a QTL was found on chromosome 5 for prehaustorial resistance. The same QTL also controlled posthaustorial hypersensitivity resistance. In each of the populations evidence was found for one or two additional QTLs that also controlled both resistance mechanisms. In barley cultivars with partial resistance to barley leaf rust there is no such association between pre- and posthaustorial resistance. The difference in the association of prehaustorial and posthaustorial resistance between barley and diploid wheat implies a class of resistance genes in diploid wheat different from the hypersensitivity resistance genes and different from the genes causing partial resistance in barley and cultivated wheat.

introduction

The diploid wheat species *Triticum monococcum* is highly resistant to the wheat leaf rust fungus, *Puccinia triticina* (Anker and Niks 2001, Anker et al. in press). Histologically, two types of resistance can be discerned in *T. monococcum*: prehaustorial and posthaustorial resistance (Niks and Dekens 1991, Anker and Niks 2001). Prehaustorial resistance precludes the formation of haustoria by the fungus although haustorium mother cells develop normally (Niks and Dekens 1991). Posthaustorial resistance results in plant cell necrosis due to a hypersensitivity reaction after the formation of at least one haustorium by the fungus (Niks 1983, Niks and Dekens 1991). Microscopic observations in a previous study indicated a quantitative inheritance for prehaustorial resistance in diploid wheat (Anker and Niks 2000).

Prehaustorial resistance is also found in partially resistant hexaploid wheat genotypes. Partial resistance in hexaploid wheat is either polygenic (Jacobs and Broers 1989) or monogenic (Rubiales and Niks 1995), has a quantitative expression and is assumed to be durable. The prehaustorial resistance in diploid wheat differs in some aspects from that in hexaploid wheat. The maximum level of early abortion without necrosis in partially resistant hexaploid wheat seedlings is in general lower than 15% and the infection type is compatible, i.e. not associated with plant tissue chlorosis or necrosis (Jacobs 1989, Rubiales and Niks 1995). The level of early abortion without necrosis in diploid wheat seedlings is, however, much higher (50-80% in some accessions; Niks and Dekens 1991, Anker and Niks 2001). Infection units that are not arrested prehaustorially will be arrested posthaustorially in association with necrosis resulting in complete resistance (Anker and Niks 2001).

The aim of the present study was to resolve the inheritance of the prehaustorial and posthaustorial resistance in diploid wheat.

material and methods

plant material Two populations were used to map the loci determining the pre- and posthaustorial resistance. One population consisted of 72 recombinant inbred lines (RILs, 13 F4:5, 55 F5:6 and 4 F6:7) from the cross *T. monococcum* DV92 (resistant) x *T. boeoticum* G3116 (susceptible; Dubcovsky et al. 1996). The other population consisted of 224 F_2 plants from the cross *T. boeoticum* 1486 (susceptible) x *T. monococcum* Einkorn (resistant). Both resistant parents are microscopically immune and have up to 45% early abortion without necrosis and 55% early abortion with necrosis in the seedling stage.

Seeds were sown in plant boxes (39x37 cm) and plants were grown in a climate compartment at 18°C continuously with 14 hours light per day, 40W/m^2. Five seeds each of five RILs, DV92 and G3116 were sown per box for the RIL population and twenty F_2, Einkorn and 1486 per box for the F_2 population. The susceptible *T. aestivum* cv. Little Club was included in each box.

inoculation Ten days after sowing, the first seedling leaves were inoculated in a settling tower. Five mg of urediospores of leaf rust isolate Felix mixed with about ten times the volume of *Lycopodium* spores was used for each plant box, resulting in a spore density of circa 300 cm^{-2}. After the inoculation, the plants were placed overnight at 100% humidity to ensure germination of the rust spores.

sampling and microscopic observations A four-cm-long central segment of each leaf was collected six days after inoculation. Per RIL three primary leaves were sampled. Leaf segments were stained following the procedure by Rohringer et al. (1977) using Uvitex 2B (Ciba-Geigy) instead of calcofluor. Fluorescence microscopy at 100x (Zeiss Axiophot, exciter filter BP 395-440, chromatic beam splitter FT 460 nm and barrier filter LP 420) was used for the observations. At least 30 infection units, i.e. mycelial structures originating from one urediospore that formed at least one haustorium mother cell, were observed per leaf segment. The number of haustorium mother cells and the presence of necrosis, indicated by plant cell autofluorescence at the infection site, were recorded. Infection units with at least one but no more than five

haustorium mother cells were considered early aborted, units with six or more haustorium mother cells were considered established (Niks and Kuiper 1983). The percentage prehaustorial resistance was calculated as the percentage of early aborted infection units without necrosis of the total number of counted infection units. The percentage of early aborted infection units with necrosis was calculated as the percentage early aborted infection units of all infection units without the early aborted infection units without necrosis. The lengths of ten established infection units per leaf segment were measured. Established infection units that were not early aborted had an approximately oval shape, parallel to the veins of the leaf.

macroscopic observations Nine days post inoculation, the infection type was determined according to the scale for seedlings of McNeal et al. (1971) with some modifications (Table 1). The about eight-cm long leaf stubs that remained after sampling, were used to evaluate infection type.

Table 1. Adapted scale of McNeal et al. (1971) for wheat leaf rust infection types (IT) in diploid wheat seedlings

IT	description	interpretation
0	no visible symptoms	resistant
1	very small, pinpoint necrotic flecks	,,
2	large necrotic flecks	,,
3	very small pustules surrounded by necrosis	,,
5	small and large pustules, necrotic and chlorotic flecks	moderately susceptible
7	large pustules with extensive chlorosis	susceptible
8	large pustules with some chlorosis	,,
9	large pustules, little or no chlorosis	,,

disease test fifth leaf After the evaluation of the infection type on the primary leaf, all F_2 plants and one plant per RIL were transplanted to a 12x12 pot and grown in a greenhouse compartment with a minimum day temperature of 19°C, a maximum of 26°C and a minimum day length of 16 hours. The fifth leaf of each plant was inoculated with a spore dispenser resulting in a spore density of circa 200 cm^{-2}.

statistics For the mapping data of the RIL population, the average value of the three first leaf segments per RIL was used. Spearman's rank correlation coefficient was calculated between the infection type in the first and fifth leaf

and between infection type and all other characters. Pearson's correlation coefficient was calculated for all other characters. The histological data were transformed (arcsine of the square root of the fraction for the percentage early abortion without and with necrosis, and log of the colony length + 1) to equalise the residual variances of the trait values. Because the transformed data indicated the same QTLs as the untransformed data, although the LOD scores were somewhat higher for the transformed data, the results of the untransformed data are presented.

map construction RIL population: AFLP markers were developed according to Qi and Lindhout 1997 using five PstMse primer combinations (P13M48, P13M61, P14M51, P14M58 and P15M54). Seventy-seven markers were obtained and 6 to 10 could be placed on each of the seven chromosomes. An integrated linkage map was constructed using the RFLP data obtained in the F_2 by Dubcovsky et al. (1996 or http://wheat.pw.usda.gov/ggpages/maps) as well as the AFLP data that were scored in the F_5-derived RIL population. To combine these data properly, an algorithm was used that enables the estimation of recombination rates between markers that were scored after different generations of selfing (Stam et al. 2001).

This integrated map served as a basis for QTL detection. However, since we used F_5-derived RILs for QTL mapping, for each RIL only those RFLP marker scores could be used that were homozygous in the ancestral F_2 plant of that RIL. This is because the zygosity status of a locus which was heterozygous in the F_2 cannot be predicted for the F_5 generation.

Since tightly linked markers that occur in clusters contribute little, if any, to the detection power and accuracy of QTL mapping, the least informative markers were removed from such clusters. Naturally, the majority of markers removed from the integrated map were RFLPs because of their poorer information content, as compared to the AFLPs. So, in the final integrated map used for QTL mapping, markers were distributed as evenly as the data allowed, having retained the most informative ones.

F_2 population: of the 224 F_2 plants used in the leaf rust reaction tests, 118 were selected as a mapping population based on the results from microscopy on the first leaf stage. Plants that combined a high level of early abortion without necrosis with either a very low or a very high level of early abortion

with necrosis and plants with a low level of early abortion without necrosis were overrepresented in the mapping population that also contained an intermediate group. The AFLP protocol was essentially performed according to Qi and Lindhout (1997) adapted to LI-COR automated sequencers and associated band-detection software (Myburg et al. 2001) with EcoMse and PstMse primer combinations E32M48, E32M49, E32M61, E33M51, E33M54, E33M58, E33M61, E35M48, E35M49, E35M51, E35M54, E35M61, E38M54, E38M55, E42M54, E42M60, P13M48, P13M49, P13M61, P14M51, P14M54 and P14M58. Two different plants of both parents and F_1 were included and each of these was put on gel in three replications to confirm the origin of the markers. 147 markers were obtained from Tb1486 and 126 markers from Einkorn. These were used to construct a maternal and paternal map of seven linkage groups with Joinmap version 3.0 (Van Ooijen 2000). The maternal map consisted of 99 markers spanning 353 cM in total, and the paternal map consisted of 91 markers spanning 483 cM in total. Linkage groups of the paternal map were between 44 and 115 cM and those of the maternal map between 12 and 81 cM. Some linkage groups could be assigned to chromosomes using 9 AFLP-markers that were mapped on the RFLP-map of the RILs and three AFLP markers of primer combination E35M48 that were mapped on the individual chromosomes of hexaploid wheat (Table 2; Huang et al. 2000). In this way three linkage groups could be assigned by means of two chromosome specific markers: linkage group 2 of the maternal map and group 4 of the paternal map were assigned to chromosome 5; linkage group 1 of the maternal map was assigned to chromosome 4 (Table 2). All other linkage groups had either one or no chromosome specific markers; these could not, or not reliably, be assigned to chromosomes.

The difficulties inherent to the estimation of recombination between dominant markers in repulsion phase (see e.g. Knapp et al. 1995, Maliepaard et al. 1997) and the resulting ambiguities in ordering such markers has prohibited the integration of the maternal and paternal maps. The addition of a number of codominant markers that can serve as 'bridges' will eventually enable a reliable integration of these two parental maps.

Table 2. Chromosome specific marker positions in the RIL and F_2 population* and chromosome specific markers according to Huang et al. 2000

chromosome	primer combination	RIL population	F_2 population
1	P14M51	306	308 p **
2	P14M58	185	187 p/ n.m.
3	P14M51	196	197 p/ n.m.
4	P13M61	329	329 m
	P14M51	231	231 p
	P14M58	248	248 m
5	P13M61	167	167 m
	P14M51	351	354 p
	P14M58	385	387 p
2	E35M48***	-	176 m
5	,,	-	81 m
6	,,	-	334 p

* discrepancies up to 3 bp can be caused by the different techniques used to obtain the AFLP markers, identity was always verified by comparing the patterns surrounding the markers
** p and m paternal and maternal map respectively; n.m. not mapped: insufficient linkage with *each of the linkage groups*
*** based on Huang et al. 2000.

QTL mapping QTL analysis was performed using the interval mapping module of MapQTL4.0 (Van Ooijen and Maliepaard 1996) with steps of 5 cM. Any peaks in the LOD profile above a threshold of 3.3 (RILs) and 3.7 (F_2; Van Ooijen 1999) were confirmed with the multiple QTL mapping (MQM) module of MapQTL4.0, taking the marker nearest to a peak as a cofactor. QTLs were classified as 'putative' in interval mapping when they were just below the significance threshold but placed at a position of a significant QTL for an other component of resistance. Support intervals with a LOD fall-off of 1.0 and 2.0 were read from the LOD profiles for each QTL. The Kruskal-Wallis test was performed with the same software to establish association between individual markers and the scores for infection type. A significance level of 0.001 was set to declare association.

results

The majority of the RILs was resistant to infection with leaf rust isolate Felix in both the first and the fifth leaf and no RIL was as susceptible as the susceptible parent G3116 (Figure 1a,b). In the F_2 on the other hand, most

plants were susceptible in the first leaf stage and more than half of the F_2 plants were resistant in the fifth leaf stage (Figure 1c,d).

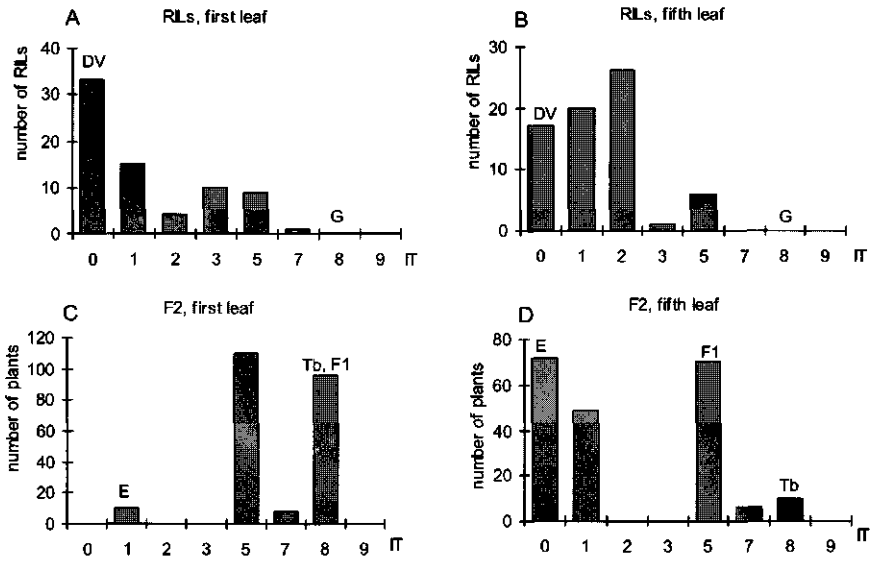

Figure 1. Frequency distribution of infection type in the first and the fifth leaf stage of the RIL and F_2 population (all plants). a: RIL first leaf stage, b: F_2 first leaf stage, c: RIL fifth leaf stage, d: F_2 fifth leaf stage. Average values of parents indicated with letters. DV: resistant parent DV92, G: susceptible parent G3116, E: resistant parent Einkorn, Tb: susceptible parent Tb1486.

Transgressive segregation was observed for the percentage of early abortion without necrosis, with the average values of ten RILs and 54 F_2 plants surpassing that of the resistant parent in the first leaf (Figure 2a,b). Transgressive segregation was also observed for posthaustorial early abortion with the average values of 21 RILs and 25 F_2 plants surpassing the average value of the resistant parent in the first leaf (Figure 3a,b). The frequency distribution of pre- and posthaustorial resistance in the first leaf of the F_2 plants selected for the mapping population is shown in Figure 2c and 3c. Overall, the results in the fifth leaf stage were similar although plants were more resistant in that stage.

There was no transgressive segregation for the size of the established infection units in either population. The percentage of established infection

units ranged from 0 to 100% in the RILs and from 16 to 100 % in the F_2 in the first leaf stage, and from 0 to 100% in both populations in the fifth leaf stage.

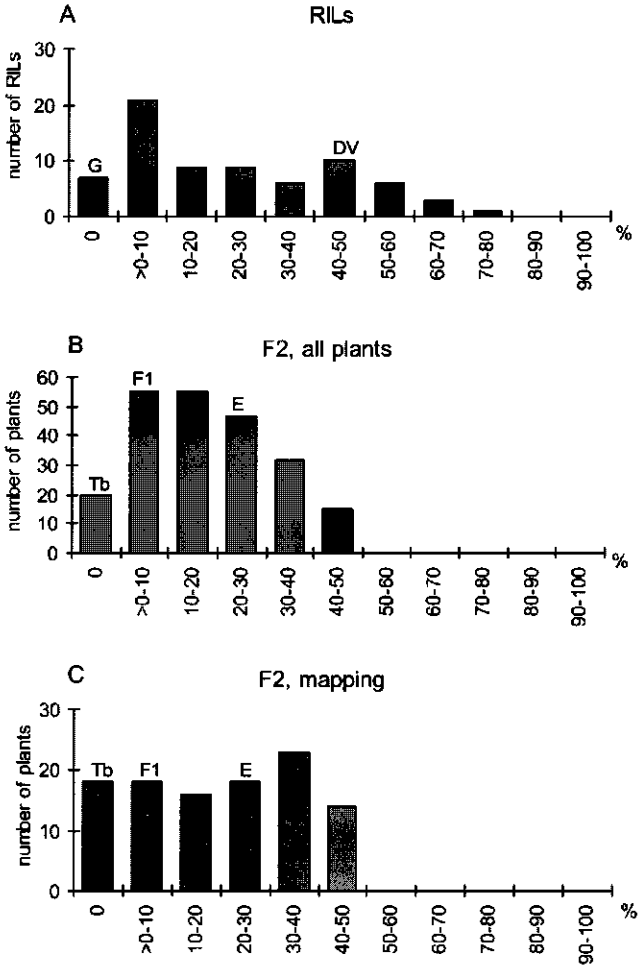

Figure 2. Frequency distribution of the percentage early aborted infection units without necrosis in the first leaf stage of DV92 x G3116 RILs and Tb1486 x Einkorn. Average values of parents indicated with letters. a: RILs, b: F_2, all plants, c: F_2, plants selected for mapping. DV: resistant parent DV92, G: susceptible parent G3116, E: resistant parent Einkorn, Tb: susceptible parent Tb1486.

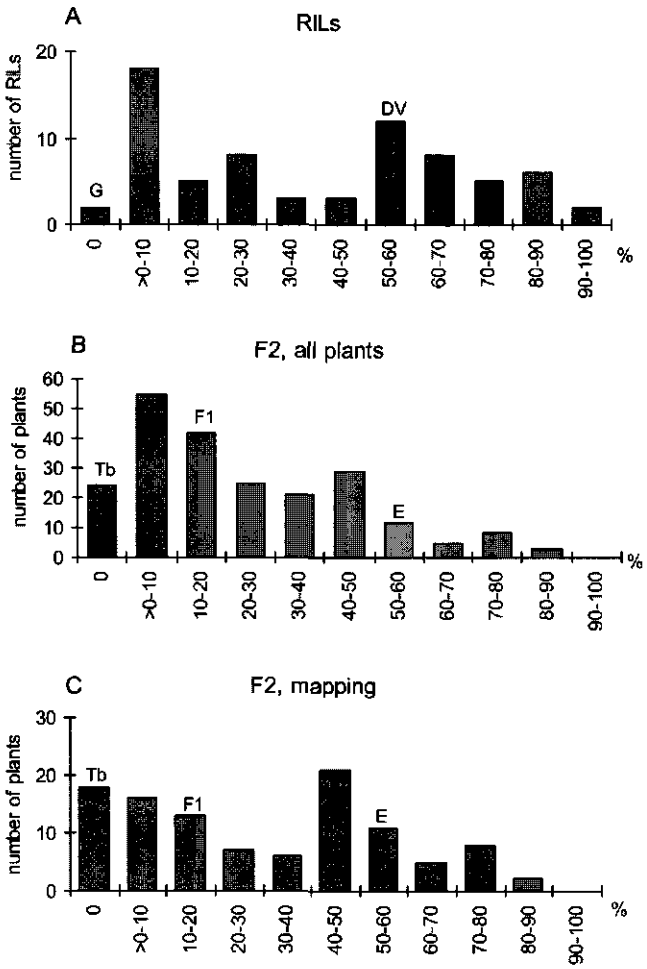

Figure 3. Frequency distribution of the percentage early aborted infection units associated with necrosis in the first leaf stage of DV92 x G3116 RILs and Tb1486 x Einkorn. Average values of parents indicated with letters. a: RILs, b: F_2, all plants, c: F_2, plants selected for mapping. DV: resistant parent DV92, G: susceptible parent G3116, E: resistant parent Einkorn, Tb: susceptible parent Tb1486.

Tables 3a and 3b show the correlations between the traits in the first and in the fifth leaf stage in the mapping populations.

Table 3a. Coefficients of correlation between macroscopic and microscopic variables scored for leaf rust resistance of RILs of DV92 x G3116

	IT5	%N-1	%N+1	%N-5	%N+5	mcs1	mcs5
IT1[a]	0.59[b]	-0.74	-0.81	-0.57	-0.63	0.76	0.73
IT5		-0.34	-0.54	-0.27*	-0.30*	0.73	0.62
%N-1			0.75	0.53	0.60	-0.55	-0.39*
%N+1				0.48	0.66	-0.67	-0.48
%N-5					0.51	-0.41	-0.37*
%N+5						-0.65	-0.61
mcs1							0.62

[a] IT = infection type, %N- = percentage early abortion without necrosis, %N+ = percentage early abortion with necrosis, mcs = mean colony size, 1 = first leaf, 5 = fifth leaf, values of the first leaf stage based on the average of three leaf segments; correlations between IT and other traits calculated using Spearman's rank correlation coefficient, all other correlations with Pearsons correlations coefficient.

[b] figures without * significant at 0.01, figures with * significant at 0.05, two tailed; percentages and IT based on 72 RILs, mcs based on 59 RILs in the first leaf stage, IT fifth leaf 70, percentages 59 and mcs 45 RILs

Table 3b. Coefficients of correlation between macroscopic and microscopic variables scored for leaf rust resistance for the F_2 plants of Tb1486 x TmEinkorn

	IT5	%N-1	%N+1	%N-5	%N+5	mcs1	Mcs5
IT1[a]	0.63[b]	-0.68	-0.73	-0.54	-0.58	0.75	0.43
IT5		-0.60	-0.71	-0.49	-0.69	0.68	0.66
%N-1			0.74	0.68	0.67	-0.79	-0.41
%N+1				0.51	0.65	-0.80	-0.38
%N-5					0.70	-0.66	-0.59
%N+5						-0.70	-0.64
mcs1							0.52

[a] as table 3a, values in the first leaf based on 1 leaf segment

[b] all values significant at 0.01 two tailed; IT, percentages and mcs in the first leaf stage based on 118 F_2 plants, IT fifth leaf 109, percentages 97 and mcs 71 plants

Early abortion without and with necrosis in the first leaf were significantly and positively correlated (r= 0.75, RILs; r=0.74, F_2) and both correlated negatively with infection type and mean colony size. Correlations between values in the fifth leaf stage were similar to those in the first leaf stage, albeit a little lower, as were correlation coefficients between traits in the first and the fifth leaf stage.

The infection type in the first and the fifth leaf stage was significantly associated with markers on chromosome 5 of each of the three maps (Kruskal-Wallis test; Figure 4). Significant association for infection type was also found on chromosome 4 of the maternal F_2.

One QTL that contributed to prehaustorial as well as posthaustorial resistance was found on all three maps. This QTL was located on the long arm of chromosome 5 of the RIL-map and on chromosome 5 of the parental F_2 maps (Table 4a,b,c), in the same region where the Kruskal-Wallis test indicated association with the infection type. The QTL was effective in the first and fifth leaf stage although no evidence was found for an effect of this QTL on early abortion without necrosis in the fifth leaf in the RIL-map. In the first leaf stage the same QTL also influenced colony size, for which in the fifth leaf a only a putative QTL was located on the paternal map of the F_2.

Table 4a. Chromosome number, peak position, LOD values, additive values, explained percentage of variance, closest marker and its position for QTLs on the map of DV92 x G3116, results of interval mapping

Trait	chrom	pos	LOD	add	% expl	nearest marker/pos
%N-1*	*3***	*142.4*	*3.5*	*10.6*	*23.4*	*Xabg55/143.0*
	5	57.8	4.0	14.3	36.7	Xcdo57/61.8
%N+1	*3***	*131.0*	*3.4*	*16.6*	*29.0*	*Xcdo189/131.0*
	5	61.8	7.1	24.6	59.4	Xcdo57/61.8
%N-5	*3***	*142.4*	*2.9*	*10.1*	*23.8*	*Xabg55/143.0*
%N+5	5	90.8	3.7	20.9	30.7	Xpsr120/91.6
mcs1	5	57.8	4.0	-0.1	39.5	Xcdo57/61.8
mcs5	-					

Table 4b. Trait, linkage group number, LOD values, additive values, percentage explained variance and nearest marker on the paternal map of F_2 1486 x Einkorn and chromosome number assigned to linkage group, results of interval mapping

Trait	lkg	chrom	pos	LOD	add	% expl	nearest marker/pos
%N-1*	4	5	28.2	11.3	18.5	72.1	E35M61-314/33.8
	*5***	*?*	*8.8*	*3.9*	*-8.4*	*16.4*	*E38M54-69/8.8*
%N+1	4	5	23.2	9.4	27.6	55.8	P14M51-354-5/18.2
	*5***	*?*	*8.8*	*2.6*	*-11.5*	*11.5*	*E38M54-69/8.8*
%N-5	4	5	39.2	7.0	18.7	43.3	E35M51-305/39.2
%N+5	4	5	28.2	9.0	37.9	57.1	E35M61-314/33.8
mcs1	4	5	28.2	12.3	-2.3	56.5	E35M61-314/33.8
mcs5	*4***	*5*	*33.2*	*3.4*	*-0.6*	*23.0*	*E35M61-314/33.8*

* %N-1, -5 = percentage early abortion without necrosis in the first or fifth leaf stage
%N+1, -5 = percentage early abortion with necrosis in the first or fifth leaf stage
mcs = mean colony size of established colonies
** putative QTLs in italics

Table 4c. Trait, *linkage group number*, LOD values, additive values, percentage explained variance and nearest marker on the maternal map of F_2 1486 x Einkorn and chromosome number assigned to linkage group, results of interval mapping

Trait	lkg	chrom	pos	LOD	add	% expl	nearest marker/pos
%N-1*	1	4	40.3	6.8	12.6	29.6	P14M54-142/40.4
	2	5	10.6	17.7	15.4	53.5	P13M48-360/10.6
%N+1	1	4	28.8	10.3	22.5	37.4	E35M51-419/28.8
	2	5	12.1	14.5	23.8	45.9	E42M54-459/12.1
%N-5	1	4	25.8	4.6	13.3	24.6	E35M48-580/25.8
	2	5	0.0	9.4	15.8	40.9	E35M61-258/0.0
%N+5	1	4	45.3	7.2	31.8	37.9	E32M48-235/45.3
	2	5	10.6	12.2	33.7	49.0	P13M48-360/10.6
mcs1	1	4	33.8	6.6	-1.9	38.4	P14M51-246/35.3
	2	5	10.6	18.3	-2.2	61.3	P13M48-360/10.6
mcs5	-						

* %N-1, -5 = percentage early abortion without necrosis in the first or fifth leaf stage
%N+1, -5 = percentage early abortion with necrosis in the first or fifth leaf stage
mcs = mean colony size of established colonies
** putative QTLs in italics

In the maternal map of the F_2, an additional QTL, for all the infection components except for the mean colony size in the fifth leaf, was located on linkage group 1 that was assigned to chromosome 4. On the RIL map as well as on the paternal map of the F_2 putative QTLs were identified (Tables 4a,b). The putative QTL on the paternal F_2 map (linkage group 5, position 8.8) is the only one of which the resistance-increasing allele is derived from the susceptible parent.

The interval mapping LOD-profiles for pre- and posthaustorial resistance on chromosome 5 of the three maps is shown in Figure 4. The LOD –2 support interval of the QTLs for pre- and posthaustorial resistance in the first and fifth leaf stage overlap on each of the three linkage groups. This indicates that, although the peaks of the LOD-profiles for the different traits are not always located on the same position, there is no evidence for more than one QTL per linkage group.

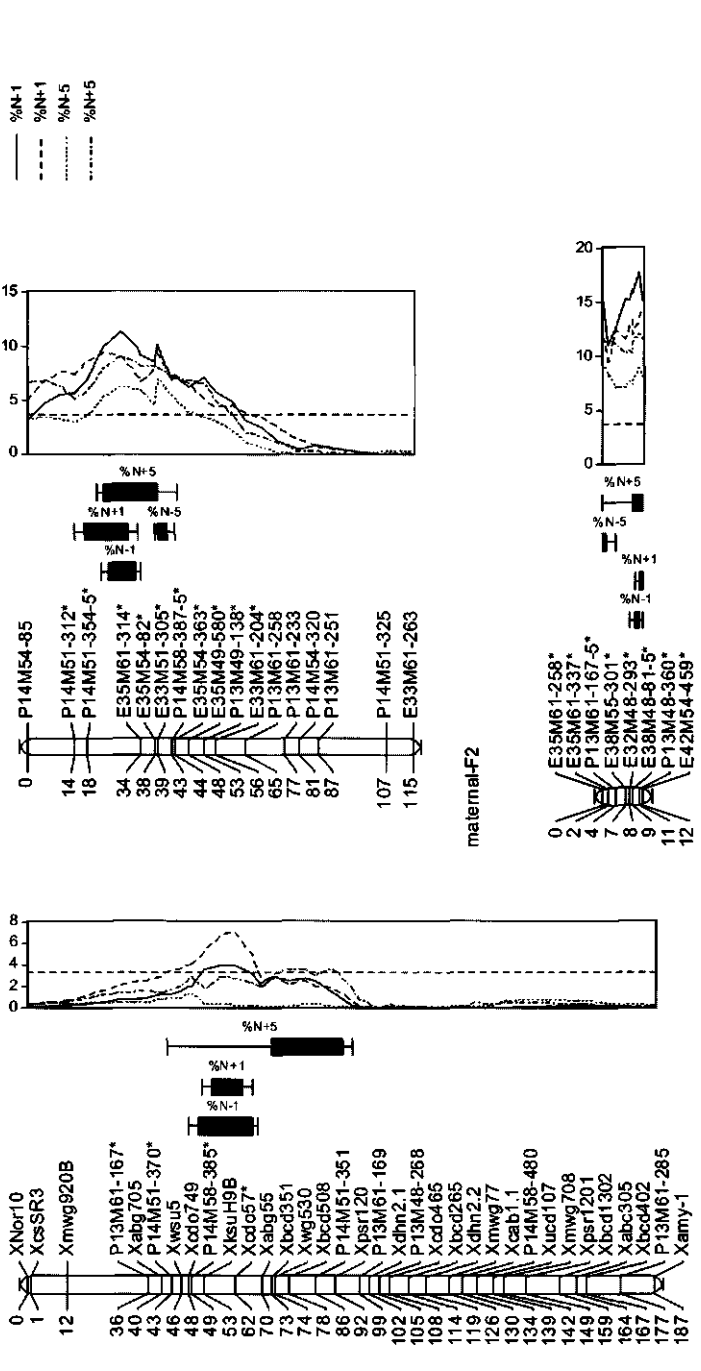

Figure 4. Lod profiles for early abortion without and with necrosis in the first and fifth leaf stage on chromosome 5 of the RIL map and the two parental F_2 maps. * = markers significantly associated with infection type in the first leaf stage, Kruskal-Wallis test.

discussion, conclusion

In the present study, a QTL was identified for resistance to wheat leaf rust on chromosome 5 in a RIL and in an F_2 population of diploid wheat. The QTL influenced the infection components early abortion without and with necrosis in the first and fifth leaf and the mean size of colonies in the first leaf. A similar QTL for all five infection components was found on the maternal map of the F_2 on chromosome 4. Furthermore, one putative QTL for pre- and posthaustorial resistance in the first leaf stage was identified in each population.

The LOD-2 support intervals for the QTL on chromosome 5 of each of the maps and on chromosome 4 of the maternal F_2 map indicate the presence of one pleiotropic QTL on each of these linkage groups, even though the LOD-profiles peaks were not always exactly in the same position. This strongly indicates that prehaustorial and posthaustorial resistance in the two studied diploid wheat populations are governed by the same genetic factors.

Qi et al. (1998) found QTLs that were plant development stage specific and QTLs that were effective in the first leaf as well as the flag leaf stage. The QTLs on chromosome 5 of all three maps and on chromosome 4 of the maternal F_2 map were expressed in both the first and the fifth leaf stage. The putative QTLs on chromosome 3 of the RIL-map and on linkage group 5 of the paternal F_2 map would seem to be specific for the seedling stage, but this remains to be verified. The putative QTL on chromosome 3 seemed to have an effect on prehaustorial resistance in the fifth leaf stage as well. The size of the data set might have influenced the results in the fifth leaf stage as the number of plants was somewhat reduced compared to the first leaf stage. In general, small data sets reduce the power to detect QTLs with small effects.

The QTL on chromosome 5 was found in both populations and mapped on all three maps. This indicates that an important factor for resistance to wheat leaf rust in the two diploid wheat populations is located on this chromosome. Whether or not the QTLs are located on the same position on the chromosome in both populations can only be established after the integration of, at least, the F_2 maps.

The QTL on chromosome 4 of the maternal F_2 map and the putative QTLs on chromosome 3 of the RIL map and on linkage group 5 (unassigned) of the

paternal F_2 map do not seem to have a counterpart in one of the other maps. It is possible that the unassigned linkage group 5 relates to chromosome 4 and thus to linkage group 1 on the maternal F_2 map in which case each population would have a population specific second factor for resistance. It is also possible that paternal linkage group 5 is not related to chromosome 4. In that case two additional QTLs might be present in the F_2 population. Linkage group 5 of the paternal F_2 map might also relate to chromosome 3 in which case the putative QTL found on this linkage group is similar to the putative QTL on chromosome 3 of the RIL map. Integration first of the parental F_2 maps and later of the maps of both populations will shed light on the identity of especially linkage group 5 of the paternal F_2 map and the number of additional factors in the two populations for resistance in addition to the one on chromosome 5. Based on the observed transgressive segregation for pre- and posthaustorial resistance in both populations at least one extra factor for resistance, in addition to the one on chromosome 5, would be expected in each of the populations.

The infection components in the first and fifth leaf stage showed a relatively high correlation in both populations. In tomato, maize, rice and barley QTLs for correlated traits often mapped to the same location (Xiao et al. 1996, Qi et al. 1998). An identical map position for QTLs of correlated traits may indicate either pleiotropic effects of single genes or tight linkage of several genes controlling the traits.

The present finding that QTLs may govern prehaustorial as well as posthaustorial resistance is remarkable. The prehaustorial resistance in diploid wheat differs in this respect from that in barley to barley leaf rust and that in hexaploid wheat to wheat leaf rust. In barley pre- and posthaustorial resistance are controlled by different genes (Qi et al. 1998). In polyploid wheat partial resistance to wheat leaf rust is either governed by single genes: *Lr34* on chromosome 7DS (Dyck 1987) and *Lr46* on chromosome 1B (Singh et al. 1998) or by a few genes with moderately large effects (Jacobs and Broers 1989). These slow-rusting genes confer a small amount of early abortion without necrosis in seedlings (12% at most; Jacobs 1989) but do not decrease the infection type nor do they lead to a hypersensitivity reaction (Rubiales and Niks 1995). The resistance in DV92 and in Einkorn, on the contrary, had a

relatively high level of early abortion without necrosis in seedlings (43% and 29% respectively) and was complete with no symptoms or very small necrotic flecks. The positive correlation between pre- and posthaustorial resistance implies that a high level of prehaustorial resistance reduced the chance of successful establishment of the remaining infection units. Those infection units that succeeded to form a haustorium aborted early in association with necrosis.

The results of the present study suggest the presence of three classes of resistance genes for wheat leaf rust in wheat. In addition to the *Lr* genes conferring hypersensitivity resistance and the *Lr* genes or polygenes conferring partial resistance, the QTLs found in this study, conferring pre- as well as posthaustorial resistance, would be the third class.

Within hexaploid wheat, comparative genetics using cDNA clones showed that most genes are triplicated on the A, B and D genomes (Devos and Gale 1997). The cDNAs generally cross-hybridised under high stringency conditions to wheat relatives such as barley and showed that gene orders are highly conserved between genomes (Devos and Gale 1997). The QTL detected in the present study on the centromeric region of the long arm of chromosome 5 in diploid wheat might therefore have a homologue (a locus with a similar function) on the polyploid wheat genomes or on the barley genome. On chromosome group 5 of polyploid wheat, one QTL for leaf rust resistance is known on chromosome 5AS (Messmer et al. 2000). The leaf rust resistance gene *Lr1* is located close to the telomere of chromosome 5DL (Feuillet et al. 1995) and *Lr18* is located on the telomere of 5BL (McIntosh 1983, McIntosh et al. 1995). Both *Lr1* and *Lr18* confer hypersensitive resistance (McIntosh 1995 and references therein). Comparing RFLP markers on barley maps and diploid wheat maps (Dubcovsky et al. 1996; Qi et al. 1998) we related the map position of the QTL identified on chromosome 5 of the RIL map in the present study to the centromeric region of barley chromosome 5HL. In barley three race-specific leaf rust resistance genes are known on 5HL, *Rph7, Rph9* and *Rph12*, located on the telomeric region (Qi et al 1998, 1999). Thus there does not seem to be any other leaf rust resistance locus known in polyploid wheat or barley that corresponds with the map

position of the QTL found on chromosome 5 of diploid wheat in the present study.

acknowledgements

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Chapter 6

General discussion

introduction

Diploid wheat is mentioned by many authors as a valuable source of resistance to wheat leaf rust, *Puccinia triticina* (e.g. Dhaliwal et al. 1986, Raupp et al. 1988). At least two different resistance mechanisms to wheat leaf rust can be found in diploid wheat: hypersensitivity or posthaustorial resistance and prehaustorial resistance (Niks and Dekens 1991). Hypersensitivity resistance to wheat leaf rust is common in cultivated wheat and often not durable (e.g. Park et al. 2000). Prehaustorial resistance is a resistance mechanism known from durably resistant interactions between cereals and cereal leaf rusts (Rubiales and Niks 1993, Niks and Rubiales 1995). Prehaustorial resistance to wheat leaf rust in diploid wheat might therefore also be durable and provide an interesting alternative for posthaustorial resistance in wheat breeding (Niks and Dekens 1991).
The research presented in this thesis was focussed on the frequency of resistance and prehaustorial resistance in diploid wheat, and the inheritance of this prehaustorial resistance.

species specific resistance

resistance in *T. monococcum*

Diploid wheat comprises three different taxa: *T. urartu, T. monococcum* and *T. boeoticum. T. urartu* is most likely the donor of the A genome of polyploid wheats (Dvorak et al. 1993). *T. monococcum* and *T. boeoticum* are very closely related and hybridise readily, resulting in fully fertile offspring (Johnson and Dhaliwal 1976). They are often considered subspecies or the cultivated and wild form of the same species, respectively (www.ksu.edu/wgrc/Taxonomy/taxintro.html). For clarity, the names of these three taxons were used throughout this thesis as if they were species.
The three 'species' can be distinguished based on morphological and molecular (AFLP fingerprint) differences (chapter 3). Resistance to *P. triticina* was restricted to *T. monococcum*; 98% of the accessions were resistant,

whereas all accessions of *T. boeoticum* and *T. urartu* were susceptible to the isolate used (chapter 2 and 3).

AFLP fingerprints showed that several 'atypical' accessions (chapter 2), i.e. susceptible *T. monococcum* and resistant *T. boeoticum*, were intermediates between these two taxa (chapter 3). Such intermediates could easily have arisen from spontaneous hybridisation in areas where both *T. monococcum* and *T. boeoticum* occur.

The finding that susceptibility and resistance to wheat leaf rust was almost perfectly associated with species identity is new and interesting. It suggests that *T. monococcum* is almost a nonhost to *Puccinia triticina*. By definition a nonhost is a plant species of which no individual is susceptible to a certain pathogen species. *T. boeoticum* and *T. monococcum* may represent a unique case where perfectly crossable species (or subspecies) differ in host status to a pathogen. Such a difference has not been reported before between two so closely related taxons. *Bremia* infection on *Lactuca sativa* (host) and *L. saligna* (nonhost) is an example of closely related taxons that differ in host status to a certain pathogen, but these two species are difficult to cross (Bonnier et al. 1992).

isolate specificity?

The question arises whether conclusions about the nonhost status of *T. monococcum* can be based upon results obtained with one wheat leaf rust isolate only (chapter 2). The clear differences in host status between the three species in this study do suggest that isolate-specificity does not play role in the reaction of the accessions to wheat leaf rust. It would be very coincidental if just this one isolate would distinguish the three species based on isolate specific reactions (chapter 2 and 3). The results of additional small-scale inoculation studies by Dr. David Long of the Cereal Disease Laboratory in St. Paul, Minnesota, U.S.A. (chapter 3), also indicated the absence of isolate specific reactions in the typical accessions. Further research should reveal whether the diploid wheat species react similarly to wheat leaf rust isolates collected in regions where diploid wheat occurs in the wild or has been cultivated since long, such as in south-east Turkey (Nessbitt and Samuel 1996, Vallega 1996).

nonhost

In nonhost combinations of cereals and cereal rust fungi the reaction of the plant is often a mixture of prehaustorial and posthaustorial resistance. Inoculation of wheat or barley with the rye leaf rust, for example, results in a high level of early abortion without necrosis and the remaining infection units are early aborted with necrosis (Niks and Dekens 1987, 1991). The ratio of prehaustorial and posthaustorial resistance in the reaction to the inappropriate rust fungus is plant genotype dependent (Niks and Dekens 1987).

The resistance mechanism in *T. monococcum* is also a combination of prehaustorial and posthaustorial resistance (chapter 2, 4 and 5). From this point of view *T. monococcum* would also appear to be a nonhost for *Puccinia triticina*.

host status difference between cultivated and wild 'form'

An intriguing question is why resistance to the wheat leaf rust fungus is confined to *T. monococcum*. Resistance to wheat leaf rust would seem beneficial to all three diploid wheat species unless they would never encounter the pathogen in the area of origin (roughly western Iran, northern Iraq, northern Syria and south-east Turkey; Nesbitt and Samuel 1996). Wheat leaf rust is, however, abundant in this area of origin.

It is estimated that domestication must have occurred in that region around 8000 BC (Nesbitt and Samuel 1996). There is little archeobotanic evidence for the domestication of *T. boeoticum* into *monococcum*, that has a tough instead of a brittle rachis and large instead of small seeds. Hillman and Davies (1990, in Nesbitt and Samuel 1996) have modelled wild cereal cultivation and estimated that under strong selective conditions a morphologically fully domesticated crop could be created in 25 years. If such a quick domestication has indeed been the case, evidence for the domestication of *T. boeoticum* into its cultivated form *T. monococcum* will very likely not be traceable.

Because the level of polymorphism in *T. monococcum* is similar to that in *T. boeoticum* (chapter 3, Hammer et al. 2000) it is likely that domestication started from a large number of *T. boeoticum* genotypes, maybe even in several places independently. Whether these *T. boeoticum* genotypes were carefully selected from exceptional resistant genotypes or whether resistance

in *T. monococcum* is a result of domestication would be difficult, if not impossible, to establish. Either scenario does not provide a plausible explanation for the absence of resistance in *T. boeoticum*.

prehaustorial resistance in *T. monococcum*

frequency of prehaustorial resistance

Truly high levels of prehaustorial resistance in *T. monococcum* accessions are rare (chapter 2). Only three out of 152 resistant accessions had 50% or more infection units that were aborted prehaustorially in the seedling stage. The majority of the *T. monococcum* accessions had levels of early abortion without necrosis between 0 and 40% (chapter 2). It was therefore fortunate that the resistant parent of the cross between DV92 and G3116, that formed the basis of an RFLP map of diploid wheat (Dubcovsky et al. 1996), had a relatively high level of early abortion without necrosis (45% early abortion without necrosis in the seedling stage; chapter 5).

Still the level of prehaustorial resistance to wheat leaf rust in diploid wheat is in general much higher than that found in cultivated wheat (Jacobs 1989, Rubiales and Niks 1995). The highest level of early abortion without necrosis reported in partially resistant wheat seedlings is 12% (in wheat lines carrying *Lr34* and wheat line Akabozu; Rubiales and Niks 1995).

inheritance of prehaustorial resistance

Prehaustorial resistance and posthaustorial resistance both occur in barley (against *P. hordei*) and in cultivated wheat (against *P. triticina*). In these species each resistance mechanism is regulated by independent genes (Niks and Kuiper 1983). The posthaustorial hypersensitivity resistance by is governed by *Rph* genes and most of the *Lr* genes, the prehaustorial resistance is governed by *Rphq* 'genes' (QTLs) in barley (Qi et al. 1998) and *Lr34*, *Lr46* or QTLs in wheat (Jacobs and Broers 1989, McIntosh et al. 1995). Combining the genes for prehaustorial and posthaustorial resistance in one genotype results in a phenotype with infection units that are early aborted

without necrosis and infection units that are early or later aborted with necrosis.

Based on these observations, an independent inheritance of the two mechanisms in diploid wheat was expected. Independent inheritance of the two resistance mechanisms was, however, not found. In both the RILs of DV92 x G3116 and the F_2 of Tb1486 x Einkorn, a high correlation between early abortion without necrosis and early abortion with necrosis was found in the first and in the fifth leaf stage (chapter 5). In the progeny of the two crosses no individual was found with a high level of early abortion without necrosis, in which the remaining infection units developed to sporulating uredia without a hypersensitivity reaction of the host plant tissue (chapter 5). The fact that quantitative trait loci (QTLs) for resistance in the RIL and the F2 population affected both pre- and posthaustorial resistance (chapter 5) confirmed the association between the pre- and posthaustorial resistance. The pleiotropic effect of the QTLs for resistance to wheat leaf rust in diploid wheat suggests that these QTLs might form a third class of resistance genes for wheat leaf rust, in addition to the hypersensitivity genes that confer posthaustorial resistance and the genes for partial resistance that confer prehaustorial resistance.

utilisation of prehaustorial resistance

durability

Transfer of prehaustorial resistance from *T. monococcum* to cultivated wheat could result in a new strategy in breeding for resistance to leaf rust in wheat. Non-durable resistance in wheat to wheat leaf rust is invariably based on hypersensitivity. Hypersensitivity resistance in the wheat-wheat leaf rust system acts in accordance with the gene-for-gene theory (Kolmer 1996 and references therein). The gene-for-gene theory implies that the pathogen produces an avirulence factor that is recognised by a resistance gene product in the plant (Hammond-Kosack and Jones 1997). One mutation in the avirulence gene could be sufficient to change the avirulence product in such a

way that it is not recognised by the plant any more: the pathogen has become virulent.

Durable resistance, on the contrary, is often based on non-hypersensitivity resistance and so far there has not been any case of non-hypersensitivity resistance that has been broken down completely. However, it should also be considered that a combination of factors in *T. monococcum* gives rise to complete resistance to *P. triticina*. Introducing just one or two factors of this combination into a susceptible background (wheat) might result in the loss of durability. This was the case with one of the factors for the (almost) nonhost resistance of rye to wheat leaf rust, *Yr9*. *Yr9* became ineffective after its introduction in wheat (Niks and Rubiales 1993; McIntosh et al. 1995).

The prehaustorial resistance from diploid wheat, associated with posthaustorial resistance, could represent a third type of resistance to wheat leaf rust that, based on QTLs and similar to nonhost resistance, might be durable.

introgression

Successful application of prehaustorial resistance from diploid wheat in cultivated polyploid wheats depends on several factors. First, prehaustorial resistance is governed by at least two, but possibly more, QTLs (chapter 5). Introducing more than one locus ('gene'/factor) always requires more work than introducing just one, although marker assisted breeding could greatly facilitate the introgression. In planning the transfer of the QTLs, the percentage of explained phenotypic variance and the additive effects should be taken into account. For prehaustorial resistance, the QTL on chromosome 5 with the largest effect is certainly the prime target for introduction.

Second, the introduction of traits from diploid wheat into polyploid wheat is not straightforward. In direct crosses between *T. monococcum* and hexaploid wheat, embryo rescue is required due to failing endosperm development. The hybrids obtained are often completely sterile (The and Baker 1975, Cox et al. 1991). One *T. monococcum* genotype is known in which two duplicated genes control female fertility in crosses with hexaploid wheat (Cox et al. 1991). The resistance of this genotype, PI 355520, is completely based on hypersensitivity (data submitted to Genetic Resources Information Network:

see reference GRIN). Additional crosses would be necessary to use that particular genotype as a bridge to transfer prehaustorial resistance to polyploid wheats.

The *Ph1* locus, on chromosome 5B of most tetra- and hexaploid wheat genotypes, negatively influences pairing and thus recombination between *T. monococcum* chromosomes and polyploid wheat A genome chromosomes (Dubcovsky et al. 1996). This and the above mentioned crossing difficulties necessitate a large number of crosses to be made to obtain sufficient starting material for the direct introgression of traits from diploid into hexaploid wheat (Cox et al. 1991).

It is possible to use the tetraploid *T. turgidum* as a bridging parent and avoid a direct cross between diploid and hexaploid wheat (Cox et al. 1991, Kerber and Dyck 1973). However, using such a bridge will disturb both the A and the B genome of the hexaploid wheat parent, thus requiring more backcross generations to recover the recurrent parent's phenotype (Cox et al. 1991). Also, the genes of the A genome are not always expressed in the presence of the B genome nor in the presence of both the B and D genome (Valkoun et al. 1986, Jacobs et al. 1996, Nelson et al. 1997).

Transfer of resistances from diploid wheat to polyploid wheat is feasible, despite all the aforementioned difficulties, as is shown by the resistance to powdery mildew, stem rust, leaf rust (monogenic) and Russian wheat aphid that were derived from diploid wheat and have already been incorporated in polyploid wheat (Potgieter et al. 1991, Valkoun en Mamluk 1993, Hussien et al. 1997, Shi et al. 1998).

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Summary

In modern wheat cultivars, resistance to wheat leaf rust, *Puccinia triticina*, is either based on hypersensitivity resistance or on partial resistance. Hypersensitivity resistance in wheat is monogenic, often complete and posthaustorial: it is induced after the formation of a haustorium by the pathogen in the plant cell. The localised plant cell death that follows prevents the uptake of nutrients by the pathogen. Although this type of resistance is widely used in wheat breeding, it is in general not durable as the pathogen overcomes the resistance with new variants. Partial resistance is mono- or polygenic, has a quantitative expression with a susceptible infection type and is assumed to be durable. A small proportion of the infection units in partially resistant wheat is aborted prehaustorially, before the formation of haustoria by the fungus, whereas the other infection units continue to grow and form pustules. Partial resistance is not associated with necrosis.

Diploid wheat is a close relative of tetra- and hexaploid cultivated wheats and can be used as a donor species in wheat breeding. Certain diploid wheat accessions show complete resistance to wheat leaf rust based on a high level of prehaustorial resistance as compared to hexaploid wheat. The remaining infection units are aborted in association with hypersensitivity. Transferred to cultivated wheat, the high level of prehaustorial resistance from diploid wheat might provide an interesting and possibly durable alternative for posthaustorial resistance in wheat breeding. The occurrence of resistance in diploid wheat, the level of prehaustorial resistance and the inheritance of prehaustorial resistance are the subject of this thesis.

Diploid wheat comprises three species, *T. monococcum*, *T. boeoticum* and *T. urartu*, that can be distinguished based on morphological and molecular characteristics. The frequency of resistance in diploid wheat was assessed by determining the reaction of about 200 accessions per species to the wheat leaf rust isolate Felix. Resistance and susceptibility to wheat leaf rust was almost perfectly associated with species identity. *T. monococcum* was almost completely resistant (98%), whereas *T. boeoticum* and *T. urartu* were completely susceptible.

The percentage of early aborted infection units without and with necrosis in resistant *T. monococcum* accessions was determined to assess the level of pre- and posthaustorial resistance, respectively. Resistant accessions with a

truly high level of prehaustorial resistance were scarce. Two percent of the accessions had a level of early abortion without necrosis of 50% or more in the seedling stage. The percentage of early abortion without necrosis in resistant seedlings ranged from 0% to 80% and all remaining infection units were aborted with necrosis.

The association between species identity and leaf rust reaction in diploid wheat and the combination of pre- and posthaustorial resistance in *T. monococcum* resemble nonhost resistance of cereals to inappropriate rust species, suggesting that *T. monococcum* is a nonhost for *Puccinia triticina*.

To elucidate the inheritance of prehaustorial resistance, two mapping populations were studied for their reaction to wheat leaf rust. One population consisted of 72 recombinant inbred lines (RILs, F_6) of the resistant *T. monococcum* DV92 and the susceptible *T. boeoticum* G3116. The other mapping population was an F_2 of 118 plants of the susceptible *T. boeoticum* Tb1486 and the resistant *T. monococcum* Einkorn. Both resistant parents had a relatively high level of prehaustorial resistance to wheat leaf rust. For the RIL population an F_2-based RFLP linkage map already existed to which AFLPs were added and for the F_2 population a maternal and paternal AFLP map were produced.

Two quantitative trait loci (QTLs) were identified: one on chromosome 5 of all three maps and one on chromosome 4 of the maternal F_2 map. The QTLs had a pleiotropic effect, governing prehaustorial as well as posthaustorial resistance, and were effective in the first and the fifth leaf stage. In addition, two putative QTLs, for pre- and posthaustorial resistance, effective in the first leaf stage only, were identified: one on chromosome 3 of the RIL map and one on linkage group 5 (unassigned) of the paternal F_2 map.

It is remarkable that the two different resistance mechanisms in diploid wheat are controlled by the same QTLs. In barley and cultivated wheat, genes for prehaustorial resistance and hypersensitivity resistance represent two different classes that map to distinct locations on the respective genomes. The QTLs from diploid wheat, identified in this study, might represent a third class of genes for wheat leaf rust resistance.

The pleiotropic effect of the QTLs for prehaustorial resistance from diploid wheat may prevent the introgression into cultivated wheat of prehaustorial

resistance alone. Still, the resistance from diploid wheat accessions with a high level of prehaustorial resistance, such as Einkorn and DV92, could prove useful in wheat breeding, conferring a possibly durable resistance similar to nonhost resistance.

Samenvatting

In moderne tarwerassen is resistentie tegen de bruine roestschimmel, *Puccinia triticina*, gebaseerd op een overgevoeligheidsreactie of op partiële resistentie. Overgevoeligheidsresistentie in tarwe is monogeen, meestal volledig en posthaustoriaal, d.w.z. geïnduceerd nadat de schimmel een haustorium heeft gevormd in de plantencel. Het daaropvolgende lokale afsterven van plantencellen verhindert de opname van voedingsstoffen door de schimmel. Over het algemeen is deze, in de tarweveredeling veelgebruikte, vorm van resistentie niet duurzaam omdat zij ineffectief wordt door nieuwe varianten van het pathogeen.

Partiële resistentie is mono- of polygeen, heeft een kwantitatieve expressie en is waarschijnlijk duurzaam. Een klein deel van de infectie-eenheden in partieel resistente tarwe aborteert prehaustoriaal, voordat de schimmel een haustorium heeft gevormd, terwijl de overige infectie-eenheden doorgroeien en puistjes vormen. Partiële resistentie is niet geassocieerd met afsterven van plantencellen.

Diploïde tarwe is nauw verwant aan de gecultiveerde tetra- en hexaploïde tarwe en kan gebruikt worden als donor in veredelingsprogramma's. Bepaalde diploïde tarwe-accessies zijn volledig resistent tegen bruine roest en hebben, in vergelijking met hexaploïde tarwe, een hoog aandeel prehaustoriale resistentie. De overige infectie-eenheden in dergelijke accessies aborteren in associatie met necrose. Introgressie van genen voor een hoog aandeel prehaustoriale resistentie uit diploïde in polyploïde tarwe zou een mogelijk duurzaam alternatief voor overgevoeligheidsresistentie in de tarweveredeling kunnen zijn. Dit proefschrift betreft het vóórkomen van resistentie in diploïde tarwe, het niveau van prehaustoriale resistentie en de overerving van prehaustoriale resistentie.

Diploïde tarwe omvat drie soorten, *Triticum monococcum*, *T. boeoticum* en *T. urartu*, die onderscheiden kunnen worden op basis van morfologische en moleculaire eigenschappen. Het vóórkomen van resistentie werd bepaald door van iedere soort ongeveer 200 accessies te inoculeren met bruine roest isolaat Felix. Resistentie en vatbaarheid waren bijna volledig soortafhankelijk. *T. monococcum* was bijna volledig resistent (98%) en *T. boeoticum* en *T. urartu* waren volledig vatbaar.

De percentages vroege abortie met en zonder necrose (afgestorven plantencellen) werden berekend in resistente *T. monococcum* accessies om respectievelijk het aandeel pre- en posthaustoriale resistentie te bepalen. Slechts twee procent van de accessies had meer dan 50% prehaustoriale resistentie in het zaailingstadium. Prehaustoriale resistentie in resistente zaailingen varieerde van 0 tot 80% en alle overige infectie-eenheden aborteerden in associatie met necrose.

Het feit dat de soort *T. monococcum* vrijwel geheel resistent is tegen *Puccinia triticina*, en dat de resistentie berust op een combinatie van pre- en posthaustoriale resistentie, suggereert dat de resistentie in *T. monococcum* een niet-waard resistentie is.

Twee splitsende populaties werden bestudeerd om de overerving van prehaustoriale resistentie te bepalen. De eerste populatie bestond uit 72 recombinante inteeltlijnen (RILs, F_6) van de kruising tussen de resistente *T. monococcum* DV92 en de vatbare *T. boeoticum* G3116. De andere populatie bestond uit een F_2 van 118 planten uit een kruising tussen de vatbare *T. boeoticum* Tb1486 en de resistente *T. monococcum* Einkorn. Beide resistente ouders hadden een relatief hoog niveau van prehaustoriale resistentie tegen bruine roest. Aan de reeds bestaande genetische RFLP kaart van de RIL populatie werden AFLPs toegevoegd en voor de F_2 populatie werd een moederlijke en een vaderlijke AFLP kaart gemaakt.

Twee genetische factoren ('quantitative trait loci', afgekort QTLs) voor resistentie werden geïdentificeerd: een op chromosoom 5 van alle drie de kaarten en 1 op chromosoom 4 van de moederlijke F_2 kaart. De QTLs hadden een pleiotroop effect: ze beïnvloedden zowel pre- als posthaustoriale resistentie. De QTLs waren effectief in het eerste en het vijfde bladstadium. Daarnaast werden sterke aanwijzingen gevonden voor een tweetal QTLs, voor zowel pre- als posthaustoriale resistentie, die alleen effectief zijn in het eerste bladstadium: één op chromosoom 3 van de RIL kaart en één op koppelingsgroep 5 (niet toegekend aan een chromosoom) van de vaderlijke F_2 kaart.

Het is opmerkelijk dat de twee verschillende resistentiemechanismen in diploïde tarwe worden gereguleerd door dezelfde QTLs. De genen voor prehaustoriale en posthaustoriale resistentie in gerst en gecultiveerde tarwe

vertegenwoordigen twee verschillende klassen die bovendien verschillende posities op de betreffende genomen innemen. De in dit onderzoek gevonden QTLs in diploïde tarwe vertegenwoordigen mogelijk een derde klasse van genen voor resistentie tegen bruine roest bij tarwe.

Het pleiotrope effect van deze QTLs voor prehaustoriale resistentie in diploïde tarwe verhindert mogelijkerwijs de overdracht naar gecultiveerde tarwe van uitsluitend prehaustoriale resistentie. Desondanks kan de resistentie van diploïde tarwe-accessies met een hoog niveau van prehaustoriale resistentie, zoals in DV92 en Einkorn, nuttig zijn in de veredeling van tarwe vanwege de mogelijk duurzame, op niet-waard resistentie gelijkende resistentie.

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Het proefschrift is af.

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curriculum vitae

Corine Caterijne Anker werd in 1969 op de dag voor moederdag geboren in Leiden. Na het VWO aan het Ashram College in Alpen aan den Rijn begon ze in 1988 met de studie plantenveredeling aan de toenmalige Landbouwuniversiteit in Wageningen. Hierbinnen koos ze de interoriëntatie tussen de studies plantenveredeling en planteziektenkunde met de afstudeervakken plantenveredeling, virologie, voorlichtingskunde en kruidkunde. In september 1994 studeerde ze cum laude af in de plantenveredeling en in de planteziektenkunde. Vervolgens werkte ze tijdelijk bij het Internationaal Agrarisch Centrum in Wageningen als cursusbegeleider bij de 25th *International Course on Applied Plant Breeding*. In november 1995 begon zij aan een promotieproject bij de vakgroep plantenveredeling van de Landbouwuniversiteit (inmiddels laboratorium voor plantenveredeling van Wageningen Universiteit) waarvan het voor u liggende proefschrift het resultaat is. Het onderzoek werd gefinancierd door Stichting Technische Wetenschappen, STW. Vanaf mei 2001 werkt zij bij het Productschap Tuinbouw in Zoetermeer als onderzoekscoördinator voor de bloembollensector.

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