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A. Börner · M.S. Röder · O. Unger · A. Meinel

The detection and molecular mapping of a major gene for non-specific adult-plant disease resistance against stripe rust (*Puccinia striiformis*) in wheat

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Abstract A major gene determining non-specific adult-plant disease resistance against stripe rust (*Puccinia striiformis*) designated *Yrns-B1* was mapped by using a cross between 'Lgst.79–74' (resistant) and 'Winzi' (susceptible). Analyzing F_3 lines of two consecutive experimental years contrary modes of inheritance were observed due to the intermediate character of the gene and the difference in the disease pressure during the seasons. Using the disease scoring data of both experimental years independently two maps were constructed detecting *Yrns-B1* 20.5 and 21.7 cM, respectively, proximal to the wheat microsatellite (WMS) marker *Xgwm493* on the short arm of chromosome 3BS. The genetic relationships to other major genes or to quantitative trait loci controlling adult plant disease resistance against rusts in wheat are discussed.

Key words Adult-plant disease resistance · Gene mapping · Microsatellites · *Puccinia striiformis* · Wheat

Introduction

Resistance against airborne pathogens, like rusts, may be classified as specific or non-specific. Specific resistance is effective at the seedling and adult-plant stage although in some cases at the adult-plant stage only, and is assumed to follow a gene-for-gene relationship (Flor 1959). In many cases the combination of different specific resistance genes resulted in a remarkable progress of the stability of resistance in different geographical regions and over several years. Although the progress in breeding and research by the exploitation of these genes is evident, specific resistances are often overcome by rapid adaptation of the pathogen (Bayles and Stigwood 1994, 1995, 1996).

A different type of resistance genes was maintained in wheat breeding programs by assessing continuously the field resistance at different stages of plant development and at different locations and years. This type of resistance genes resulted in a durable non-race-specific, broad-range field resistance of adult plants.

Genetic studies of non-specific adult-plant resistance have so far been carried out only by a few research groups, including Barina and McIntosh (1995), Line et al. (1996), Law and Worland (1997) and Brammer et al. (1998). Although it is generally expected that non-specific adult-plant disease resistance is inherited quantitatively the authors were able to demonstrate that major genes on single chromosomes are involved in the expression of such a type of resistance.

The present paper describes the identification and mapping of one locus determining non-specific resistance against stripe rust by using a wheat line identified from a 25-year program of resistance breeding against rusts at the NORDSAAT breeding station at Böhnshausen. The expression of the adult-plant resistance of these lines was not influenced by any change of virulence since the 1970 s (Meinel and Unger 1998). For mapping, microsatellites or simple sequence repeats (SSRs) were applied. This type of molecular marker is genome-specific, appears to be evenly distributed over the wheat genome and shows a higher level of polymorphism compared to any other marker system (Röder et al. 1995, 1998). The utilization of wheat microsatellites (WMS) for mapping genes determining reduced plant height and vernalization response has already been demonstrated by Korzun et al. (1997, 1998). In those investigations, however, the chromosomal arm location of the target genes was already known, which was not the case in the present study.

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A. Börner (✉) · M.S. Röder
Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Correnstraße 3, D-06466 Gatersleben, Germany
e-mail: boerner@ipk-gatersleben.de
Fax: +49-39482-5155

O. Unger · A. Meinel
NORDSAAT Saatzuchtgesellschaft mbH, Hauptstraße 1, D-38895 Böhnshausen, Germany

Materials and methods

Plant materials, DNA isolation and marker analysis

The wheat breeding line 'Lgst.79–74' which has been included in adult-plant disease tests of the NORDSAAT breeding station at Böhnshausen since 1975 and showed a durable adult-plant resistance against stripe rust was crossed with the variety 'Winzi' known to be highly susceptible to the disease. One single F_1 plant was used to produce 160 F_2 seeds which were sown in the greenhouse. From 157 plants at least 150 F_3 seeds were obtained which were used for performing the disease tests. Leaves of 5–6-week-old F_2 seedlings were cut for DNA extraction according to the procedure of Anderson et al. (1992). Procedures of the WMS analysis and the WMS markers employed are described in Röder et al. (1998).

Disease tests and assessments

To test for the absence of specific resistance genes in the material seedling tests were performed in the greenhouse. Twenty seedlings per F_3 family, together with the parents, were inoculated with three stripe rust races containing the virulences 1, 2, 3, 6, 7, 9 and 17. The seedlings' reaction was described on a 0 (resistant) to 4 (highly susceptible) scale (McIntosh et al. 1995).

The adult-plant resistance was tested twice in the field by growing F_3 double rows (90 cm) during the seasons 1997 (157 lines) and 1998 (117 lines). The plants were inoculated with a mixture of actual stripe rust races containing the virulences 1, 2, 3, 4, 6, 7, 9 and 17 by using spreader rows. For classification the 'Coefficient of infection' described by Stubbs et al. (1986) was calculated. Plants classified as adult-plant resistant need to have a coefficient of <8.0 combined with a race non-specific susceptibility at the seedling stage. The coefficients of susceptible phenotypes range from 8.0 to 100. To test the goodness of fit of observed segregation ratios to theoretical expectations χ^2 analyses were applied.

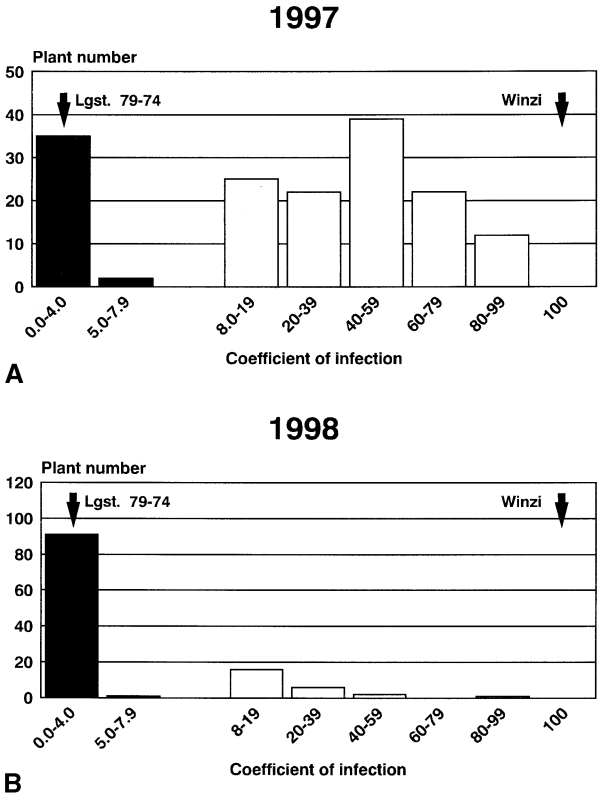

Fig. 1 F_2 segregation patterns for adult plant resistance against stripe rust of the cross 'Lgst.79–74'×'Winzi' based on analysis of F_3 scoring data of 1997 (**A**) and 1998 (**B**) field experiments. *Black and white bars* show the number of resistant and susceptible genotypes, respectively. The means of the parents are marked by the *arrows*

Results

Inheritance of adult-plant stripe rust resistance

The resistance tests done at the seedling stage in the greenhouse indicated that no specific resistance gene is present in either parent and, consequently, in the F_2 progenies. Two weeks after the infection all tested material was scored as highly susceptible.

The field tests were performed in 2 consecutive years. In both seasons bimodal segregation patterns were observed (Fig. 1). In 1997, when a generally high level of stripe rust infection was recorded, the 157 tested F_3 lines could be divided into 37 resistant and 120 susceptible ones. The observed segregation ratio was not significantly different from the expected 1:3 ratio for the monogenic inheritance (χ^2=0.17; P>0.60) of a recessive resistance gene. Somehow surprising was the result obtained in the following year when a lower level of pathogen infection was present. Here a segregation ratio of 92 resistant: 25 susceptible F_3 families was detected indicating again the presence of a single gene as tested by χ^2 (χ^2=0.82; P>0.30). The mode of inheritance of the resistance, shown to be dominant, was however contrary to that obtained with the same lines one season before.

Comparing the resistance level of the single lines tested in both years it is evident that all progenies which were classified as resistant in 1997 (37) were also resistant in 1998. On the other hand, all susceptible F_3 lines detected in 1998 (25) had already been found to be susceptible in 1997. Combining the results of both years, the expression of resistance of the heterozygous F_3 families can be considered as intermediate.

Chromosomal localization and gene mapping

The parental lines were tested with 283 PCR-amplified WMS. Scorable polymorphisms were observed for 67 WMS (24%). Testing of pools of ten susceptible and ten resistant F_2 plants did not result in a clear-cut polymorphism. Therefore, polymorphic WMS were screened on five susceptible and 12 resistant single F_2 plants for any linkage with the target gene. With this strategy linkage to *Xgwm533* and *Xgwm493* was discovered (Fig. 2). Both microsatellites were previously mapped on chromosome arm 3BS (Röder et al. 1998), indicating the location of the resistance gene on this chromosome arm. In order to differentiate the non-specific adult-plant resistance gene detected here from specific *Yr* genes the symbol *Yrns-B1* (Yellow rust non-specific resistance) is given throughout this paper. Unfortunately, no other WMS of chromosome 3BS were polymorphic in the 'Lgst.79–74'×'Winzi' mapping population.

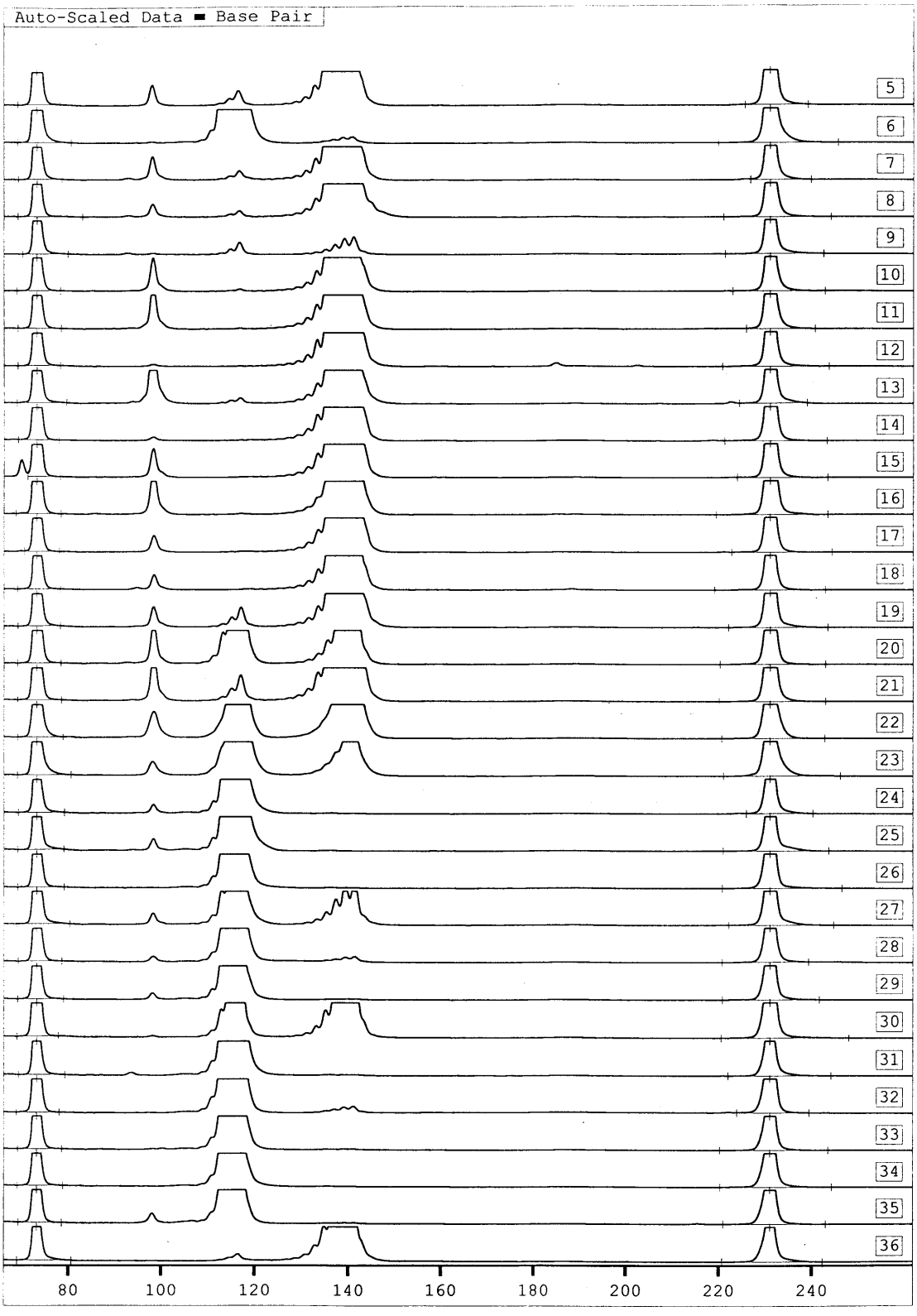

Fig. 2 Linkage analysis of wheat microsatellite WMS533 shown as a printout of the computer program 'Fragment Manager'. The bottom scale refers to bp units. The peaks at 73 bp and 231 bp are size standards. *Lane 5* susceptible parent 'Winzi'; *lane 6* resistant parent 'Lgst.79–74'; *lanes 7–21* susceptible F_2 plants; *lanes 22–36* resistant F_2 plants. The 115-bp and 138-bp fragments correspond to the resistant and susceptible alleles, respectively. Plants in *lanes 20, 22, 23, 27, 30 and 36* are recombinants

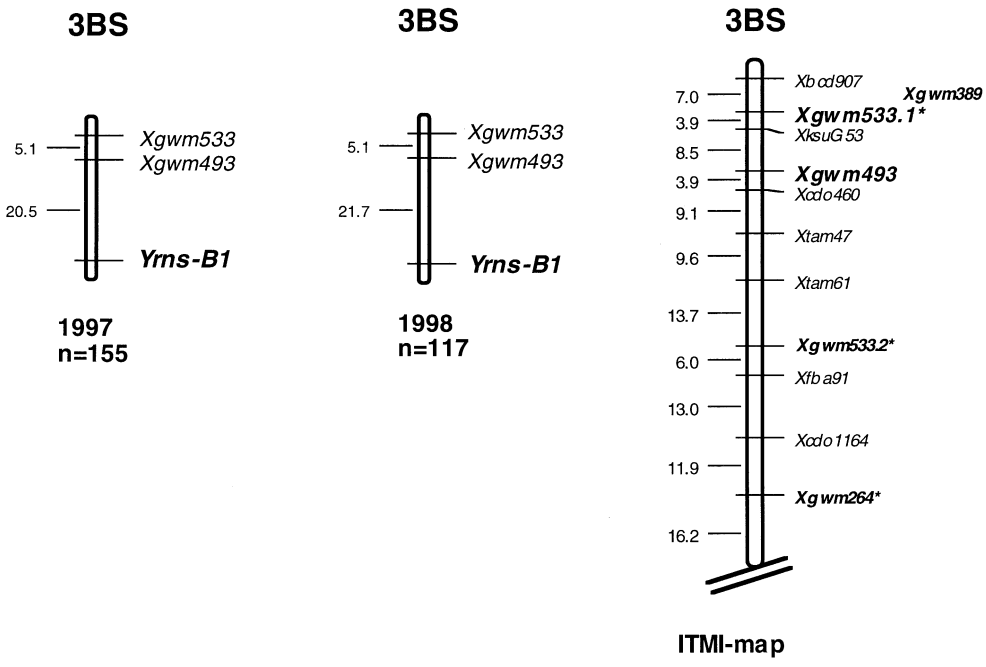

Fig. 3 Genetic map of chromosome arm 3BS of the population 'Lgst.79–74'×'Winzi' considering the disease scoring data of 1997 (left) and 1998 (center) in comparison to the published ITMI map (right) of Röder et al. (1998)

Two maps of the whole mapping population were constructed independently by using the disease scoring data of both experimental years. Figure 3 shows both maps in comparison with the 3BS microsatellite map of Röder et al. (1998). Although the mode of inheritance of the resistance gene was opposite in both years the map position of *Yrns-B1* corresponded well. The resistance gene was mapped 20.5 and 21.7 cM proximal to the WMS marker *Xgwm493* using the scoring data of 1997 and 1998, respectively.

Discussion

During recent decades, especially since the time when Flor (1959) discovered the gene-for-gene relation between virulence and resistance genes, enormous progress has been made in breeding rust-resistant wheat varieties. Based on this relationship new specific resistance genes were identified and could be quickly transferred into actual breeding material. This high level of resistance resulted in a concentration of varieties with certain specific resistance genes providing a high selection pressure to the pathogen so that after certain growing seasons new virulent races appeared. Examples of important stripe rust resistance genes which were overcome are *Yr2* (variety 'Heine VII'), *Yr9* (varieties 'Kawkas', 'Clement' or 'Sleipner') and *Yr6+Yr9* (variety 'Haven'). Analyzing the stripe rust epidemic which occurred in England in 1997 it was shown that due to the occurrence of new multiple virulences the highly effective resistances of *Yr17*, and even of the combination of *Yr6+Yr9+Yr17*, were overcome within 4 years between 1994 and 1997 (Bayles and Stigwood 1994, 1995, 1996).

To overcome the general problem of rapid adaptation of the pathogens, partial or non-specific adult-plant resistance should be employed in combination with the race-specific resistance genes. The genetics of non-specific resistance, characterized by susceptibility of the seedlings but resistance of the adult plants when inoculated with mixtures of actual races, was postulated to be mainly quantitative. However, the detection of highly comparable resistance behaviors in descendants of varieties showing high levels of non-specific resistance gave some indication that major genes may also exist (Meinel and Unger 1998). One such a gene, *Yrns-B1*, determining adult-plant resistance against stripe rust could be identified in the present investigation.

A prerequisite for the detection of resistance genes is an accurate disease assessment in the field. By using the coefficients of infection described by Stubbs et al. (1986) both the type of infection and its severity were considered. Due to the precise definition of the coefficients for resistant and susceptible plants the F_3 lines could be clearly discriminated in both experimental years. The observed contrary modes of inheritance due to the intermediate character of the gene and the difference in the disease pressure in the particular seasons may be one explanation of why in the past the genetics of non-specific adult-plant disease resistance was often described to be very complex.

The molecular mapping of agronomically important genes in wheat is mainly limited by the availability of polymorphic markers. With the development of WMS a new marker system became available showing a much higher level of polymorphism than any other. Using the 'International Triticeae Mapping Initiative' mapping population, derived from a cross between a synthetic wheat and the variety 'Opata', the level of polymorphism detected was about 80% (Röder et al. 1997). In the present study, however, it was surprising that only 24% of the tested WMS gave scorable polymorphisms.

A comparable low level of polymorphism was discovered by Börner et al. (1997) using a cross between a near-isogenic line of the French variety 'Bersee' with the Ukrainian variety 'Mironovskaya 808' for mapping the dwarfing gene *Rht-B1c* on chromosome 4B of wheat. There, only one out of four WMS was scorable. It may be concluded therefore that it is still necessary to continue the development of new WMS in wheat to compensate for the low level of polymorphism. For approaches using rapid, marker-assisted selection or a map-based isolation of this non-specific resistance locus in wheat, closely linked markers need to be identified.

Although, on average, only three polymorphic WMS per chromosome were detected we were able to map a non-specific adult-plant resistance gene for the first time within the Triticeae. Using replicated scoring data of 2 years *Yrns-B1* was mapped on the short arm of chromosome 3B. Line et al. (1996) were able to identify three quantitative trait loci for durable non-specific high-temperature adult-plant resistance to stripe rust in the wheat variety 'Stephens'. The major QTL could be associated with one RAPD marker. Using the fragment as a probe this marker could be localized on chromosome 3BS by employing cytogenetic tester stocks. It should be mentioned here that Robertson (1985) postulated that many, if not all, loci for which qualitative mutants have been found also have quantitative alleles. If this association is true, both the major QTL described by Line et al. (1996) and *Yrns-B1* could represent the same gene. To clarify this possibility further, investigations using common markers in both the 'Stephens' and the 'Lgst.79–74' mapping populations are necessary.

Beside 3BS at least one other chromosome was shown to carry a major gene(s) for adult-plant stripe rust resistance. Law and Worland (1997) detected major effects of the translocated 5BS-7BS chromosome. They suggested that the resistance gene(s) may be closely linked to the break point. Analyzing ditelosomic lines the authors could show that the major effect is due to a gene(s) on the 5BS chromosome arm.

For plant breeders in the near future major genes determining non-specific adult-plant resistance will become available not only against stripe rust. Plant material determining non-specific resistance against leaf rust (*Puccinia recondita*) was used for genetic studies by Börner et al. (1998). The authors detected monogenic segregation patterns highly comparable to those described in the present study. On the other hand, by performing a monosomic analysis Brammer et al. (1998) was able to demonstrate that chromosomes 1A and 4D carry major genes for adult-plant leaf rust resistance. By combining the research activities on disease resistance, applied genetics and molecular genetics further major genes and/or major QTLs for non-specific adult-plant resistance against several diseases will be discovered.

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