Genetic analysis of seedling stripe rust resistance in the Australian wheat cultivar 'Batavia'

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ABSTRACT

Batavia, an Australian wheat cultivars has high level of seedling and adult-plant resistance to stripe rust in Australia. Batavia x Avocet S cross was used to study the seedling resistance of Batavia. Parental cultivars, F1 hybrids and genetic populations were scored for seedling resistance to pathotypes 110 E143 A^+ and 134 E16 A^+. The frequencies of seedling infection types of three F2 populations and a F3 population were a good fit to the expected segregation ratio for two genes. Chi-square values of individual F3 lines segregating for 12^{-c} and 23^{-c} displayed a good fit for single gene segregation. The data provide support for the hypothesis that two genes confer the seedling resistance in Batavia and putative stripe rust resistance genes were designated as *YrBat1* and *YrBat2* conferring seedling infection types of 12^{-C} and 23^{-C}, respectively. Interaction of these genes that were of intermediate IT shown in F1, produced a lower IT similar to the parent Batavia (IT;$1^{=CN}$) when combined in homozygous F3 lines.

INTRODUCTION

Stripe (yellow) rust caused by *Puccinia striiformis* West. f. sp. *tritici* (*Pst*) poses a major threat to wheat production in Australia (Wellings and McIntosh, 1990) that has been addressed by deploying resistant cultivars. Commercially successful resistant cultivars can be achieved by incorporation of effective seedling and adult-plant resistance genes in well-adapted and high yielding genotypes (McIntosh et al., 1995). To achieve this goal, an understanding of the genetic basis of resistance in current cultivars to prevailing *Pst* pathotypes will provide the knowledge necessary to recognize new sources of resistance and for deploying these resistances in effective gene combinations. Batavia is an Australian high yielding cultivar that has been resistant to the most prevalent *Pst* pathotypes in Australia for many years. The genetic basis of seedling and adult-plant resistance of Batavia is unknown. In the present study, investigations were sought on the inheritance of seedling resistance of Batavia to stripe rust in Australia.

MATERIALS AND METHODS

Pathotypes 110 E143 A^+ and 134 E16 A^+ were used in seedling and adult-plant tests of parental cultivars and genetic populations developed from the present study. Batavia was crossed to susceptible cultivar Avocet 'S' giving rise to 16 F1 hybrids. Field grown F1 plants were harvested individually to produce F2 populations. Three F2 populations, 70 seeds of each, were planted 5 cm apart directly in field. To determine the number of resistance genes, F2 plants were harvested individually to obtain F3 families.

Parental cultivars and thirteen F1 hybrids were scored for seedling responses against pathotypes 110 E143 A^+ and 134 E16 A^+ while four F2 populations and one F3 family were scored against pathotype 134 E16 A^+. Seedling infection types (IT) were recorded 14 to 17 days after inoculation using the 0, ; (fleck), 1 to 4 (McIntosh et al., 1995). Seedling infection types of 3 to 4 were considered high.

RESULTS

Pathogen isolates. 110 E143 A^+ and 134 E16 A^+ were used in seedling tests of parental cultivars and genetic materials. The combination of virulence factors of these pathotypes rendered seedling stripe rust resistance genes *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *YrSD*, *YrND* and *YrA* ineffective.

Seedling resistance in Batavia/Avocet 'S' cross. The 16 F1 plants tested against *Pst* pt. 110 E143 A^+ produced seedling IT of 2^+3^{-C}. It was evident that the gene(s) conferring seedling IT of 2^+3^{-C} in the F1 hybrids was partially dominant and effective to both *Pst* pathotypes.

F2 seedling plants were scored on an individual basis and plants were classified into one of four seedling IT groups viz. Resistant type 1 (IT ;$1^{=CN}$), Resistant type 2 (IT 12^{-C}), Resistant type 3 (2^+3^{-C}) and homozygous susceptible (IT 3^+4).

When 54 F2 plants of Population 1 were scored for seedling response to *Pst* pt. 134 E16 A^+, 28 plants showed IT of ;$1^{=CN}$, 15 were 12^{-C}, nine were 2^+3^{-C} and two were 3^+. The frequencies of seedling infection types were a good fit to the expected segregation ratio for two genes (χ^2 9:3:3:1= 3.218, *P* 3df > 0.5). Similarly, segregation for two seedling resistance genes was displayed by Population 2 (χ^2 9:3:3:1= 3.052, *P* 3df > 0.5) and Population 3 (χ^2 9:3:3:1 = 3.646, *P* 3df > 0.5). Homogeneity chi-square for all three F2 populations (χ2 homogeneity = 2.333, 0.90 < *P* < 0.80) showed a good fit to a digenetic segregation model. On this basis, the data for all three populations were pooled and the chi-

square conformed to 9:3:3:1 digenic ratio ($\chi 2_{\text{pooled}}$ = 6.667, *P* 3df > 0.10). Since the F2 data conformed to digenic segregation, an F3 genetic model was developed to assist the interpretation of observed phenotypes.

An independent F3 population was developed from residual seeds of the F2 populations 1, and this population was tested with *Pst* pt. 134 E16 A^+ at two temperature regimes: low at 15-18ºC and high at 22-25ºC. A total of 71 F3 families derived from F2 Population 1 were classified for disease response in order to predict F2 genotype. Genotype classes AABb, AaBB and AaBb could not be distinguished from each other. This was considered to be the result of failure to detect a susceptible plant because of limited sample size (AaBb) and the close gradation of similar ITs (AaBB, AABb). The chi-squared value for the seven phenotype response classes showed a good fit to a digenic segregation model (χ^2 1:1:2:1:2:1:8:3:6:10:8:6:3:35 = 5.423, *P* 6df > 0.50). The data provide support for the hypothesis that the seedling resistance in Batavia to *Pst* pt. 134 E16 A^+ is conferred by two genes.

DISCUSSIONS

Despite the potential race-specificity of seedling resistance genes, searching for new effective sources of seedling resistance and understanding how to manage and effectively deploy these resistances in breeding programs is likely to be of great benefit. Multipathotype test of Batavia demonstrated that Batavia was consistently resistant producing seedling ITs of $;^{N}$ to $1^{=CN}$ to eight *Pst* pathotypes used at seedling stage (data not presented). The combination of virulence factors of these pathotypes rendered seedling stripe rust resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr27*, *YrSD*, *YrND* and *YrA* ineffective. Seedling IT of $;^{N}$ and 1^{-CN} to pathotypes avirulent and virulent for *YrA*, respectively, was concluded to indicate the presence of *YrA* in Batavia.

Comparison of stripe rust seedling ITs of the resistant parent Batavia with its respective F1 hybrids in a cross with the susceptible parent Avocet 'S' and the frequency of response classes at F2, initially suggested that the seedling resistance of Batavia was incompletely dominant. However, there was no evidence for partial dominance in F3, and the IT distribution at F2 was then interpreted to indicate the respective ITs of the two single genes. Segregation analyses of three F2 populations indicated that two genes controlled the seedling resistance contributed by Batavia. Chi-square values of individual F2 populations and chi-square heterogeneity for the pooled F2 populations were accepted as evidence for digenic segregation. Seedling tests of an independently derived F3 population also confirmed digenic segregation, although there was no evidence for partial dominance in the F3 lines. Single gene families were identified among the segregating F3 lines. Chi-square analyses of individual F3 lines for each gene displayed a good fit for a single gene segregation. Chi-square homogeneities for single gene lines of each phenotypic seedling response were in agreement with segregation at single gene loci. Putative stripe rust seedling resistance genes were designated as *YrBat1* and *YrBat2* conferring seedling infection types of 12^{-C} and 23^{-C}, respectively.

It is imperative that wheat and its relatives continue to be examined for the presence of new resistance genes in order to diversify the genetic basis of resistance to wheat rusts (Kolmer, 1996). It can be anticipated that combinations of effective seedling resistance genes with race non-specific APR genes may provide longer lasting resistance. It will be important to evaluate the potential effectiveness of *YrBat1* and *YrBat2* as widely as possible to determine any potential vulnerability to pathogen populations. This can be achieved with the transfer of these genes into the Avocet near isogenic lines series that are being developed and distributed worldwide (Wellings et al., 2000).

It was stated that when two or more effective genes for resistance are present, the gene giving lowest infection type would determine the final resistance response (McIntosh et al., 1995). However, it is frequently observed that combinations of two or more resistance genes produce a lower infection type than the infection type conditioned by the most effective gene (Kolmer, 1992). For example, combinations of various leaf rust seedling resistance genes with *Lr13* and *Lr34* are observed to enhance these seedling resistances when they were tested against pathotypes of *Puccinia triticina* (*Pt*) avirulent for *Lr13* and *Lr34* (Kolmer, 1992; Schafer et al., 1963). It has also been demonstrated that combinations of 4-5 resistance genes based on minor, slow rusting genes to *Pst* with additive effects give high levels of resistance comparable to immunity (Singh et al., 2000). Similarly, transgressive segregation for resistance was obtained in intercrosses of three cultivars with incomplete resistance to stripe rust (Wallwork and Johnson, 1984). Race-specific genes in combination with such minor genes could further enhance their longevity (Singh et al., 2004). As indicated in the present study, interaction of the two seedling resistance genes (*YrBat1* and *YrBat2*) that were of intermediate IT, combined to produce a lower IT in the parent Batavia. This was also confirmed in the derived homozygous lines (*AABB*) where the cumulative effect of the two gene interaction provided a lower IT ($;1^{=CN}$) similar to the Batavia parent.

On the basis of the resistance response of Batavia to *Pst* pts. 110 E143 A^+ and 134 E16 A^+, it can be expected that the two gene combination would remain effective to other Australian *Pst* pts. However, to adequately test this hypothesis, the selected single gene lines should be further tested to an array of Australian *Pst* pathotypes. The single gene lines will also be useful to resolve relationships between the four APR genes with additive effects found in the present study.

The genetic ratio of 1:1 was found when a doubled haploid population Pelsart/ Batavia (34 resistant: 36 susceptible) was scored as seedlings against *Pst* pts. 104 E137 A^- and 108 E141 A^- in PBI- Cobbitty. This was concluded as evidence for monogenic segregation for a gene conferring ;1^{-CN} contributed from Batavia (Zahravi et al., 2003). This gene was initially located on the long arm of chromosome 7D using two flanking SSR markers *Xgwm437* and *Xgwm111*, which were associated with resistance responses. This gene has been designated as *Yr33* (McIntosh et al., 2003). However, one of the SSR markers that were reported to be in association with the seedling resistance of Batavia could not be reproduced (H.S. Bariana, pers. com.) and a genetic stock for the gene *Yr33* is currently not available (R.A. McIntosh, pers. com.). The present study showed digenic segregation at two loci corresponding for resistance to *Pst* derived from Batavia rather than monogenic segregation reported by Zahravi et al. (2003) and therefore the model proposed by Zahravi et al. could not be supported. The putative single gene lines obtained from the present study would be valuable stocks for use in marker analysis to determine chromosome location, assess the role of chromosome 7D and thus examine the relationship of *Yr33* with *YrBat1* and *YrBat2*.

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