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Wheat leaf rust (*Puccinia triticina*) reactions of hobbit ''sib''/bezostaja substitution lines

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Abstract: The wheat leaf rust reaction resulting from the substitution of individual chromosomes of the wheat cultivar Hobbit 'sib' with their homologues from Bezostaja were investigated using isolate WBRP 85-31, that is avirulent on both cultivars in the glasshouse and the field. Seedlings of most lines had low infection types (ITs), except the line with chromosome substitution 7A that had IT 3-. Significant decreases in leaf area infected were observed on substitution lines with chromosomes 2D, 5D and 5BL/7BL, whereas the substitution line with chromosome 1B had a significant increase in the level of infection as compared with that on Hobbit 'sib'. This provided a means of identifying chromosomes with resistance genes against leaf rust disease that can be transferred to cultivated lines for the control of the disease.

Keywords: wheat, Triticum aestivum, leaf rust, *Puccinia triticina*, chromosome substitution.

استجابة مرض صدأ أوراق القمح (*Puccinia triticina*) لصنف الهوبت وخطوط الاستبدال

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الملخص: تم دراسة استجابة مرض صدا أوراق القمح لاستبدال الكروموسومات الفردية لصنف Hobbit 'sib' من القمح مع مثيلاتها من (Bezostaja) باستخدام العزلة (WBRP 85-31) الغير شرسة على كلا الصنفين داخل البيوت المحمية والحقل. كانت نسبة العدوى في شتلاتمعظم الأصناف فيها منخفضة من الطراز (ITs) ما عدا خط الشتلات المستبدلة بها الكروموسومات (**A7**) وبها-IT 3 . وهناك انخفاض شديد في مساحة الأوراق ذات الكروموسومات المستبدلة (2D, 5D and 5BL/7BL) بينما الشتلات في خط الاستبدال كروموسوم (**B1**) شهد زيادة في مستوى الإصابات بالعدوى مقارنة مع Hobbit 'sib'هذا يقدم وسيلة تعريف بالكروموسومات التي تمتلك جينات المقاومة ضد مرض صدأ أوراق القمح التي يمكن نقلها إلى خطوط إنتاج للأصناف والتي من اجلها يتم السيطرة على المرض.

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Introduction

Wheat leaf rust caused by *Puccinia triticina* Eriks is one of the most severe diseases of the crop. It is said to occur wherever wheat is grown. Yield losses in wheat arising from infection by leaf rust vary considerably from traces to as high as over 90% depending on crop resistance and stage of growth (Chester, 1939; Brett, 2009). The use of resistant cultivars is the most effective and environmentally friendly way of protecting wheat crop against leaf rust (Singh et al., 2001; Wamishe and Milus, 2004; Martinez et al., 2005).

Intervarietal chromosome substitution lines, where individual chromosomes of one wheat cultivar are replaced with homologues from another, are often used in the genetic analysis of resistance. Single chromosome substitution and aneuploid lines of wheat are effective in identifying chromosomes that carry resistance genes with major effects. At times seedlings and adult plants that have race-specific leaf rust resistance genes express variation in their reaction to rust isolates in the field that cannot be explained by race specificity alone. Such reactions are said to be under the control of a number of genes with minor effects, the expression of which is influenced by the environment, genetic and/or external factors. However, using single chromosome substitutions lines it is also possible to identify the chromosome location of such genes.

Worland and Law (1991) used the wheat cvs. Hobbit 'sib' and Bezostaja A1 and their series of chromosome substitution lines to assess their reaction to yellow rust (*Puccinia striiformis*) disease of wheat. Cultivar Hobbit is known to have the yellow rust resistance genes *Yr1*, *Yr2* and *Yr3/4a* located on chromosome 2A, 7BS and 5BL, respectively and Bezostaja lacks any effective gene for seedling resistance to UK races of *P. striiformis*. In spite of this 14 of the 21 possible single chromosome substitution lines had significantly altered levels of resistance compared to the Hobbit `sib' control. Hobbit `sib' lines with chromosome substitution 2A, 5BL/7BL, 5D, 6B or 7D from Bezostaja were more resistant than Hobbit `sib' and Bezostaja.

Pink et al. (1983) reported that monosomics for the homoeologous chromosome group 5 of the wheat cv. Chinese Spring were more resistant to yellow rust than their corresponding tetrasomics. They also showed that the short arms of these chromosomes were responsible for the resistant reaction in this wheat cultivar. Also using monosomics of a wheat-rye recombinant line Sharma and Singh (2000) located the linked genes for leaf and stem rust resistance on chromosome 28. Intervarietal substitution lines for the homoeologous group 5 chromosomes of Chinese Spring with those of *Triticum spelta*, and the wheat cvs. Cheyenne, Hope, Cappelle Desprez, Lutescens, Synthetic and Ciano 67 had variable responses to yellow rust infection indicating allelic differences between these wheats. When the same lines were tested with powdery mildew (*Erysiphe graminis*) pathogen similar reaction pattern were observed, thus leading to the conclusion that resistance to both diseases is under the control of the same loci.

The leaf rust resistance gene *Lr14a* located on chromosome 7BL was reported in the wheat cv. Hope (McIntosh et al., 1967). Using chromosome substitution technique in Chinese Spring and Hope, Law and Johnson (1967) showed that chromosome 7B of Hope carries a leaf rust resistance gene that is likely to be *Lr14a*. This gene was found to be more effective in the Chinese Spring background and less so in the donor Hope, due to modifier genes in the homoeologous chromosomes 7A and 7D.

Chromosome substitution and monosomic lines were used to locate leaf rust resistance genes on specific chromosomes by various workers (McIntosh, 1988). Also Bariana & McIntosh (1993) identified the resistance gene *Lr37* on the short arm of chromosome 2A.

This study was therefore aimed at determining the chromosome(s) involved in the genetic control of seedling and adult plant resistance to leaf rust disease in Bezostaja using the Hobbit `sib'/Bezostaja chromosome substitution lines.

Materials and Methods

All the original seed stocks used in this study were provided by Worland, A.J. of the

Cambridge Laboratory, Norwich, NR4 7UJ. UK. The Hobbit `sib'/Bezostaja chromosome substitution lines for each chromosome were in duplicate, except that of chromosome 7A for which there was only one line. The substitution lines were developed by replacing individual chromosomes of Hobbit `sib' with their homologues from Bezostaja (Law and Worland, 1973). The chromosome substitution lines should therefore breed true in subsequent generations.

All the chromosome substitution lines and the susceptible check Armada were evaluated in the field in 1992/93 growing season in a single block with a single isolate (WBRP 85-31) due to shortage of seed stock. This isolate lacked virulence for the high temperature seedling gene *Lr17* and an adult plant gene also identified in Hobbit, possibly *Lr13* (Abdul, 1994) on chromosomes 2A and 2BS, respectively (McIntosh, 1988). It also lack virulence for the seedling gene *Lr3* reported in Bezostaja (Bartoš et al., 1969) located on chromosome 6B.

The substitution lines were assessed in hill plots of 30 by 30 cm in the 1992/93 growing season. The lines were allocated to the hill plots at random and at regular intervals a spreader plot of the susceptible Armada was included. As a result of delays in disease build up the spreader plot technique of inoculation was supplemented by direct spraying of wheat plants with their respective isolates in odourless kerosene using micro ulva sprayer (Micon sprayers Ltd, Bromyard, England). Disease scores were taken as percent Leaf Area Infection (LAI) on 30th June and 12th July, 1993. Analysis of variance was carried out on the percentage scores after angular transformation and the significance of differences between each chromosome substitution line and Hobbit 'sib' was determined.

Seedling tests were conducted in October 1993 on the chromosome substitution lines and Hobbit `sib', Bezostaja and the susceptible control Armada using seeds harvested from the field experiment. All tests were done in the glasshouse. Seedling reactions were recorded using the scale described by Stakman et al. (1962). Sometimes a pair of chromosome substitution line gave different ITs and both ITs were recorded separated by a slash (/).

Results

Analysis of variance showed significant differences between the chromosome substitution lines as well as the wheat cultivars in their leaf rust resistance (Table 1). The same level of significance were obtained from the analysis of variance with and without Armada (Table 1) therefore, the same least significance difference (LSD) value was used to test for differences between the chromosome substitution lines, Armada and Bezostaja and the control Hobbit `sib'.

Table 1. Analysis of variance for leaf rust reaction of Hobbit `sib'/Bezostaja chromosome substitution lines in the field.

Item	Degrees of freedom«	Mean Square
Date	1	8620.16***
	1	9622.40***
Line	22	263.92***
	23	316.71***
Interaction	22	118.91**
	23	124.32**
Residual	44	54.97
	44	54.97

Seedlings of Armada had IT 2+ in the glasshouse, while Hobbit `sib' and Bezostaja had ITs ; and 1-;, respectively. Also having similar IT or higher than the susceptible control were the chromosome substitution lines 2A, 2B, 5A and 7A (Table 2). One of a pair each of the chromosome substitution lines 2B and 5A had IT 2+, thus suggesting some differences between these lines in terms of their seedling leaf rust reactions. Hobbit `sib' and Bezostaja were more resistant to the leaf rust isolate WBRP 85-31 in the field than Armada (Table 2).

Table 2. Seedling reaction and mean percentage LAI of Hobbit 'sib'/Bezostaja chromosome substitution lines to isolate WBRP 85-31.

Line	Seedling (IT)	LAI (%)	Difference from Hobbit `sib'
HS* (Bezostaja 1A)	;1	5.55	-11.2
HS (Bezostaja 1B)	;1-	32.00	15.25*
HS (Bezostaja 1D)	;1-n	18.75	2.00
HS (Bezostaja 2A)	2+	13.85	-2.9
HS (Bezostaja 2B)	1/2+	17.00	0.25
HS (Bezostaja 2D)	1-;	1.58	-15.17*
HS (Bezostaja 3A)	1;	14.50	-2.25
HS (Bezostaja 3B)	1-;	15.00	-1.75
HS (Bezostaja 3D)	;1-	18.75	2.00
HS (Bezostaja 4A)	1-;	10.00	-6.75
HS (Bezostaja 4B)	;1-	17.05	0.3
HS (Bezostaja 4D)	2/1;	16.62	-0.13
HS (Bezostaja 5A)	2+/;1-	13.50	-3.25
HS (Bezostaja 5BS/7BS)	;1-	21.50	4.75
HS (Bezostaja 5D)	;1-	0.15	-16.6*
HS (Bezostaja 6A)	1-;	7.68	-9.07
HS (Bezostaja 6B)	1-;/;	4.55	-12.2
HS (Bezostaja 6D)	1-;/1	23.25	6.5
HS (Bezostaja 7A)	3-	20.50	3.75
HS (Bezostaja 5BL/7BL)	1-;	0.10	-16.65**
HS (Bezostaja 7D)	1-;	5.40	-11.35
Hobbit `sib'(HS)	1-;	16.75	0.00
Bezostaja	;	0.28	-16.47*
Armada	2+	37.50	20.75*

* HS = Hobbit `sib'

** = P< 0.01; * = P< 0.05 Significantly different from Hobbit `sib'.

All the chromosome substitution lines had altered LAI with lines 2D, 5D and 5BL/7BL showing significantly higher levels of resistance than the Hobbit `sib'. Of all the chromosome substitution lines only the line with chromosome 1B substituted was more susceptible than Hobbit `sib' by up to 15.25% LAI.

Discussion

Seedlings of all the chromosome substitution lines were resistant to isolate WBRP 85-31 in the glasshouse with the exception of those of 7A which had IT 3-. The high temperature seedling gene, *Lr17* located on chromosome 2A must have contributed to the IT 1-; on Hobbit `sib', since the substitution of this chromosome with its homologue from Bezostaja gave IT 2+. This reaction was either due to a resistance gene that is present on the 2A homologue of Bezostaja or another gene located on the remaining Hobbit `sib' chromosomes. The genetic background in which the *Lr17* gene is present is known to affect its expression (Abdul, 1994). Therefore the higher ITs 2+ and 3- recorded on substitution lines 2B, 5A and 7A in spite of the *Lr17* may be due to modifier gene(s) present on these chromosomes.

The resistant reaction Armada (IT 2+) differed from its usual susceptible ITs of 3 - 4. The Armada seeds harvested from the field were not used in the glasshouse investigation. It is therefore possible that the stock of Armada used in the field was wrong, which might be responsible for the differences.

In the field Hobbit `sib' had 16.75% LAI compared with the 37.5% on Armada thus establishing the effectiveness of the seedling and adult plant genes *Lr17* and *Lr13* identified in Hobbit (Abdul, 1994). All the chromosome substitution lines had leaf rust reactions that differed from that of Hobbit `sib' in the field, with lines 2D, 5D and 5BL/7BL having significantly higher levels of resistance. This indicated that chromosomes 2D, 5D and 5BL/7BL of Bezostaja have genes that confer higher levels of resistance to isolate WBRP 85-31.

The increased resistance observed on the chromosome substitution line 6A must have been conditioned by the *Lr3* gene, which has been reported in Bezostaja (Bartoš et al., 1969). Worland and Law (1991) reported that chromosomes 4B, 4D, 5BL/7BL and 5D of Hobbit `sib' promoted susceptibility to both yellow rust and powdery mildew diseases of wheat. In this study the replacement of chromosomes 5BL/7BL and 5D of Hobbit `sib' with those of Bezostaja were found to increase the level of resistance to leaf rust. The same chromosome substitution lines were shown to increase resistance against yellow rust (Worland and Law, 1991) and wheat powdery mildew (Worland, A.J. personal communication). It is most likely that these chromosomes carry resistance genes that could be used in the control of all three diseases.

The resistance gene *Lr34* associated with durable resistance to leaf rust (Dyck et al., 1966; Singh and Rajaram, 1992) is located on chromosome 7D (Dyck, 1991) and Bezostaja is reported as having durable resistance to leaf rust conditioned by the gene *Lr34* Kolmer *et al* (2008) and this is responsible for the increased resistance on chromosome substitution line 7D.

Only the line with chromosome 1B substituted was significantly more susceptible than Hobbit `sib'. Thus chromosome 1B of Bezostaja appeared to promote a high level of susceptibility. Hobbit is postulated as having an adult plant resistance gene that was effective against isolate WBRP 85-31 in the field (Abdul, 1994). This adult plant gene may be located on chromosome 1B, such that its substitution with a homologue from Bezostaja carrying alternate alleles resulted in increased susceptibility. However the 1B substitution line would still carry the *Lr17* gene that will confer some level of resistance in this line.

Conclusion

The presence of genes having minor or major effect in the different homologues chromosomes of Hobbit ‘sib’ and Bezostaja were identified through the substitution of chromosomes of Hobbit ‘sib’ with those of Bezostaja. At the seedling stage the environmentally sensitive gene *Lr17* was postulated in chromosome 2A of Hobbit ‘sib’. In the field Hobbit ‘sib’ chromosomes substituted with 2D, 5D, 5BL/7BL and 6A of Bezostaja showed higher level of resistance to leaf rust. This suggests the presence of resistance genes on these chromosomes from Bezostaja. The identification of these resistance genes in Hobbit 'sib' and Bezostaja can be used in the improvement of wheat resistance to leaf rust disease.

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