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Chromosomal location of leaf rust and stem rust resistance introgressed from *Triticum militinae* Zhuk. into common wheat

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Abstract

The leaf rust and stem rust resistance genes introgressed from *Triticum militinae* Zhuk. (2n = 4x = 28, genome, AAGG) into common wheat were assigned to the specific chromosomes through aneuploid analysis. Aneuploid analysis was done using two different and complete sets of monosomic series in the background of Lal Bahadur and Chinese Spring. The rust resistant selection, Sel. T 2600, carrying the resistance introgressed from *T. militinae* Zhuk. was crossed with individual monosomic plants and monosomic plants were identified cytologically. The genetic analysis of F_2 progenies derived from monosomic F_1 plants tested with the most virulent leaf rust pathotype 77-5 and the stem rust pathotype 40-1 at seedling stage indicated that the crosses Lal Bahadur 6B mono/Sel. T 2600 and Chinese Spring 1B mono/Sel. T 2600 as "critical line" for leaf rust resistance and the cross Chinese Spring 6B mono/Sel. T 2600 for stem rust resistance studies. The leaf rust and stem rust resistance of Sel. T 2600 were assigned to the chromosomes 6B and 1B, respectively, introgressed as two different independent translocations and under dominant monogenic control.

Key words : Wheat, *Triticum militinae*, rust resistance, monosomic analysis, chromosomal location.

Introduction

Wheat is attacked by several diseases, more so by fungal pathogens. Of these, stem rust or black rust (*Puccinia graminis* Pers. f sp. *tritici* Eriks. and Henn.), leaf or brown rust (*P. recondita* Rob. Ex. Desm. f sp. *tritici* Eriks. and Henn.) and stripe or yellow rust (*P. striiformis* West.) are known to cause significant yield losses to wheat crop. Major problems faced in stabilizing wheat production is to reduce the genetic vulnerability to important diseases prevalent in the Indian subcontinent [1]. Frequent spontaneous mutations lead to evolution of new virulent physiological races rendering the existing stem rust resistance genes ineffective. About 50 *Lr* genes providing resistance to leaf rust have been documented [1]. A large number of designated leaf rust resistance genes have their origin in *T. aestivum* and most of them have become ineffective to the prevalent Indian pathotypes [2]. There are about 47 genes from diverse sources, which have been identified for stem rust resistance [1]. However, the alien genes (*Sr26, Sr27, Sr28, Sr31, Sr33 and Sr38*) derived from related genera and species confer moderate to high resistance to the prevalent races of stem rust under Indian conditions [3].

Many useful traits, including resistance to diseases and pests, have been transferred to common wheat through wide hybridization [4] utilizing the wild relatives of wheat. The secondary gene pool of common wheat includes polyploid *Triticum/Aegilops* species, tetraploid wheats like *T. timopheevi* Zhuk. and *T. militinae* Zhuk. and diploid species belonging to the sitopsis and polyploid group of *Aegilops* species that have at least one genome in common with *T. aestivum*. Gene transfers from these species by homologous recombination is possible. These species have contributed several resistance genes that are being utilized in wheat improvement [5].

The tetraploid wheat species, *Triticum militinae* Zhuk. et Migush. (genome, AAGG) is a free threshing mutant of *T. timopheevi* Zhuk. and also known for its resistance to common fungal diseases of wheat [5]. Several attempts have been made to transfer disease resistance from this tetraploid species to common wheat [6, 7]. Cytologically stable selections of hexaploid types, namely, Sel. T 2600 and Sel. T216-1 were isolated from the wide cross involving *T. militinae* and common wheat. These selections exhibited a high level of resistance to rusts and powdery mildew [8]. The present investigation was aimed at location of genes for leaf rust and stem rust resistance transferred from *T. militinae* Zhuk. to bread wheat (*T. aestivum* L.) through monosomic analysis.

Materials and methods

Selection (Sel.) T 2600 was isolated from the cross (C 306 × *T. militinae*) × C 306 followed by five generations of selfing, It showed resistance to leaf and stem rusts at Wellington (altitude 1850 m amsl, 77° N) a hot spot location for rusts and other foliar diseases of wheat. A complete set of 21 monosomic lines (2n-1)

differing for the deficient chromosomes in the background of Chinese Spring (CS) developed by Sears and Lal Bahadur (LB) obtained from McIntosh, were raised in pots, examined cytologically and used to assign resistance gene(s) in Sel. T 2600 to specific chromosomes. The near-isogenic lines (NILs) and stocks carrying known genes, in use as differentials in India for pathotype analysis of leaf rust pathogen, *P. recondita* f. sp. *tritici* [9] and stem rust pathogen, *P. graminis* f. sp. *tritici* [9] were used to test purity of inoculum. The initial inoculum of pure uredospores of leaf rust pathotype 77-5 and stem rust pathotype 40-1 were obtained from DWR, Regional Station, Flowerdale, Shimla. The inoculum of each race was multiplied and used after ensuring the purity using differentials concerned.

To study the chromosomal location of rust resistance gene(s), cytologically identified individual monosomic plants of LB and CS series were crossed with the resistant stock, Sel. T 2600. The F_1s of all 21 different CS monosomics × Sel. T. 2600 and LB monosomics × Sel. T. 2600 crosses and F_2 population derived from cytologically identified monosomic F_1 of respective crosses were tested with selective leaf rust pathotype 77-5 and stem rust pathotype 40-1 at seedling stage. Specific chromosome carrying resistance gene(s) was identified by "critical line analysis". The F_2 population of non-critical monosomic lines could exhibit a normal segregation like disomic × disomic (CS × Sel. T. 2600 and LB × Sel. T. 2600) crosses while the F_2s of critical line (chromosome carrying resistance gene) would lead to a significantly distorted segregation ratios than normal ratios.

The chi-square (χ^2) test for testing goodness of fit was used to establish validity of observations in relation to expected values in segregating population on the basis of Mendelian segregation.

Results and discussion

The resistance in Sel. T 2600 was controlled by a single dominant gene as evidenced from the present investigation. If a single dominant gene confers resistance, then all the cytogenetically identified monosomic F_1 including the critical line will be resistant. In other words F_1 monosomic plants will be hemizygous resistant (R-). Selfing of these cytologically identified monosomic F_1 plants including the hemizygous ones will give rise to F_2 plants that are homozygous (RR) 24%, hemizygous (R-) 73% and nullisomics (--) 3%. Due to unequal gametic transmission rates, about 3 to 4 per cent of the progenies are of nullisomics that show susceptibility. As a result the frequency of resistant plants will be much higher (97R : 3S) than expected in the ratio favouring resistant category [10].

Leaf rust resistance to the pathotype 77-5: The segregation of F_2 progenies derived from monosomic F_1 plants with significant chi-square value in the critical cross, LB 6B mono × Sel. T 2600 ($P < 0.01$) and non-significant value in rest of the non-critical crosses ($p > 0.0.1$) indicated that the leaf rust resistance gene in Sel. T 2600 is located on the chromosome 6B. This was further ascertained from the test carried out with the Chinese Spring monosomic series (Table 1). The monogenic dominant control of resistance was also proved from the disomic × disomic cross and the pooled segregation ratio of 3R:1S from the non-critical crosses.

So far only three leaf rust resistance genes, *Lr3* and its alleles *Lr3bg* and *Lr3ka*, *Lr9* and *Lr36* have been assigned to chromosome 6B. The gene *Lr3*, *Lr3ka* and *Lr3bg* are ineffective to the pathotype 77-5. The gene *Lr36* is derived from *Ae. speltoides* and is not involved in the parentage of Sel. T 2600. Further confirmation is needed to prove the identity of resistance of T 2600 in comparison with *Lr9* which has come from *Ae. umbellulata* and may not have any allelic relationship.

Stem rust resistance to the pathotype 40-1: In order to locate the stem rust resistance in individual chromosome, monosomic lines of cv. Chinese Spring were crossed with Sel. T 2600. The F_2 progenies derived from the cytologically identified monosomic F_1 plants were tested with the stem rust pathotype 40-1. The segregation pattern in F_2 progenies fitted well into expected ratio of 3R: 1S except in the critical cross CS 1B mono × Sel. T 2600, where significant deviation of 97:3, than the normal ratio of 3:1 was noticed. The high significant chi-square value ($P < 0.01$) in the critical cross CS 1B mono × Sel. T 2600 indicated that the stem rust resistance gene in Sel. T 2600 is located on chromosome 1B (Table 1). Stem rust resistance genes such as *Sr14* from *T. turgidum* var. *dicoccum* [11] and *Sr31* from *Secale cereale* have also been assigned to chromosome 1B. Of these, *Sr31* is located on 1BL.1RS translocation involving rye and common wheat [12].

The independent translocation for leaf rust and stem rust resistance in Sel. T 2600 were determined from the segregation pattern in F_2 progenies upon simultaneous inoculation with, respective pathotypes 77-5 and 40-1. The segregation of F_2 population into four distinct classes gave a good fit to the expected ratio of 9:3:3:1 emulating that the resistance to stem rust and leaf rust in Sel. T 2600 were conferred by two different independently introgressed segments from the donor species, *T. militinae*. More than one translocations were reported in earlier studies by Enno *et al.* [8] and Jarve *et al.* [13]. Translocations of similar nature on chromosome 6B and 1B have also been reported in common wheat lines carrying introgressed

Table 1. Segregation of F_2 plants in critical and non critical lines (pooled) derived from F_1 monosomic between monosomic series of var. Chinese Spring and Sel. T 2600 and monosomic series of var. Lal Bahadur and Sel T. 2600 for resistance to leaf rust pathotype 77-5 and stem rust pathotype 40-1 in seedling stage

Cross	Number of plants tested			Expected ratio	χ^2	P value
	Resistant	Susceptible	Total			
Leaf rust						
LB 6B mono × Sel. T 2600	239	10	249	97:3	0.883	0.50-0.30
Pooled excluding LB 6B mono × Sel. T 2600	1214	372	1586	3:1	2.018	0.25-0.10
CS 6B mono × Sel. T 2600	83	1	84	97:3	0.944	0.50-0.30
Pooled excluding CS 6B mono × Sel. T 2600	1214	416	1630	3:1	0.236	0.75-0.50
Stem rust						
CS 1B mono × Sel. T 2600	48	2	50	97:3	0.171	0.70-0.50
Pooled excluding CS 6B mono × Sel. T 2600	1129	367	1496	3:1	0.174	0.75-0.50

$\chi^2 = 3.841$, P = 0.05

segments from *T. militinae* and *T. timopheevi* [7]. Partial genome affinities between the B genome of wheat, G genome of *T. timopheevi* and S genome of *Ae. speltoides* have been observed by several workers. It has been assumed that the chromosome of *B/G* genomes are frequently involved in spontaneous translocations and have acquired natural polymorphism for translocations. The frequent translocations and chromosome substitutions 6B(6G) were evidenced in *T. aestivum* × *T. militinae* or *T. timopheevi* crosses [7, 13, 14] attempted earliar.

The resistance genes identified and located on the chromosomes 6B and 1B for leaf rust and stem rust, respectively, could possibly contribute in maintaining diversification and exploitation of new sources of resistance to rust pathogens. Further, a better understanding about the exact physical location through cytogenetic techniques like C-banding and in situ hybridization combined with the molecular approaches of DNA marker aided techniques may lead to conclusive consideration in line with the present study.

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