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Introgression lines obtained from the cross between *Triticum aestivum* and *Triticum turgidum* (durum wheat) as a source of leaf and stripe (yellow) rust resistance genes

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Abstract: Introgression lines (ILs) were produced from crosses of common hexaploid bread wheat (*Triticum aestivum* L.) (AABBDD) cultivars with tetraploid durum wheat (*Triticum turgidum*) (AABB). These ILs were checked against diversity of crop-damaging fungal diseases and especially for leaf and stripe rusts, proved to be the most damaging biotic enemies in the world and especially in South Asia and the southern region of Pakistan. Microsatellite markers (STRs) were used to determine the frequency of translocations found on different chromosomes during the localization of durum wheat (*Triticum turgidum*). Two independent loci for stripe and leaf rust resistance were found on 2B and 5A during the molecular mapping. The major locus that accounts for 70% of quantitative or phenotypic variance of the trait present on the 5AS-5AL translocated chromosome was closely linked to STR markers Xwmc24, Xgwm95, Xwmc327, Xgwm644, Xgwm268, and Xgwm456 whereas the other minor locus contributing to the quality trait was found near Xgwm106 on translocated chromosome 2B. STRs located near these genes may be used for controlling the transfer of valuable traits in new wheat cultivars.

Key words: Stripe rust, leaf rust, *Triticum aestivum*, tetraploid wheat, STR marker

1. Introduction

Wheat is considered the second most yield-producing member among all cereal crops. Many biotic and abiotic factors that limit the production efficiency and yield are the major insulting components in the environment (Chen et al., 2014). Leaf (brown) rust and stripe (yellow) rust, caused by *Puccinia triticina* Eriks. and *Puccinia striiformis* Westend., respectively, are the most common fungal diseases of wheat in many countries where cereal crops are grown to get grains for daily use. Several genes that confer resistance to these rusts have been identified and used in breeding programs to reduce grain yield losses (Bariana et al., 2010). A majority of these designated resistance genes are race-specific and have become ineffective in combating current pathogen populations. In contrast, slow rusting (Herrera-Foessel et al., 2008) resistance, expressed at the adult plant stage, is quantitatively inherited and based on genes that have minor to intermediate but additive effects. Because it is often durable, such resistance should be of primary interest to most wheat breeders around the world.

The most common and deleterious diseases in many Asian countries like Pakistan, India, and Bangladesh are leaf rust, powdery mildew, stem rust, spot blotch, leaf blotch, and loose smut. Stripe and leaf rust are considered the most common and the most crop damaging diseases around the world (Ullah et al., 2014). The production of genetically resistant cultivars is the best and most reliable control method considered in the world of crop production. The duration of the efficacy of resistant genes deployed in wheat cultivars may remain up to a year and then change occurs because of overly rapid mutations in nature (Singh et al., 2005). Therefore, the search for new sources (donor) of resistant genes, including polygenic genotypes, should be an urgent task. Many wild relatives of wheat (diploids and tetraploids) carry high levels of resistance to diverse dangerous diseases. The major issue in transferring of genes from a donor to the common wheat genome is genome incompatibility and cytological instability of early hybrid generations. Thus, it is important to develop stable introgression lines (ILs) of common wheat with expression of the resistance from the wild species while retaining common wheat characteristics to be used as donor sources in breeding programs. Tetraploid wheat (*Triticum turgidum* (durum) (2n = 28, genome

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composition AABB)) is an excellent source of disease resistance, particularly against rust pathogens, i.e. stripe and leaf rust. Attempts to use *Triticum turgidum* as a source of pathogen resistance genes were undertaken earlier, as well. To date, two leaf rust resistance genes (*Lr18* and *Lr50*), three genes for resistance to stem rust (*Sr36*, *Sr37*, *Sr40*), and three genes for resistance to powdery mildew (*Pm6*, *Pm27*, *Pm37*) were transferred into common wheat from the *Triticum turgidum* genome.

Nowadays molecular markers are used for characterization and analysis of cereal hybrid genomes. Among different types of markers, microsatellite markers (STRs) are the most explanatory for the analysis of hybrids due to higher polymorphism level, codominant inheritance, chromosome specificity, and the ability of transfer between cultivated and wild species. STRs have been effectively used in analysis of genetic diversity of plants, construction of genetic mapping, and quantitative trait loci (Kumar et al., 2006; Zhang et al., 2008). The aim of our study was to investigate the resistance capability and sustainability of ILs against environment-insulting rust diseases. The data reported here in this study were created from *Triticum aestivum-Triticum turgidum* ILs evaluated against stripe rust and leaf rust in wheat at seedling and adult plant stages and detection of loci associated with resistance to leaf rust and yellow rust.

2. Materials and methods

2.1. Introgression lines

Seven common hexaploid (AABBDD) Pakistani wheat cultivars including Pasban-90 (PN90), Rawal-87 (RW87), Pirsabak-2004 (PS2004), Moomal-2002 (Mm2002), Suleman-96 (SM96), Zardana-93 (ZN93), and Chenab-70 (CN70) were crossed with tetraploid wheat (AABB) to get ILs. F_1 hybrids were backcrossed to the initial wheat cultivar with subsequent self-pollination of BC_1F_1 and following generations in the condition of isolation. In the BC_1F_4–BC_1F_7 generations selection was performed for cytologically stable (2n = 42) resistant plants, which served as a basis for development of ILs.

2.2. Evaluation of adult plant resistance to leaf and yellow rust pathogens

Resistance to leaf and yellow rusts at the seedling stage was tested under a controlled environment, i.e. in greenhouse conditions. Seedlings of 9–10 days old were inoculated with a suspension of *Puccinia* spp. (brown and stripe rust pathogen) and incubated at a temperature between 20 and 25 °C with a 10-h photoperiod and 80% relative humidity. Infection types of seedlings were scored 15–20 days after inoculation on a scale of 0–4 according to Roelfs et al. (1992). A seedling test for stripe rust resistance was carried out as described by Ullah et al. (2014). The adult plant resistance to leaf rust and yellow rust was estimated under natural infection with native populations of pathogens prevalent in Pakistan. Field experiments were performed as described by Ullah et al. (2014).

2.3. Genomic DNA extraction and molecular marker (STRs/microsatellite) analysis

DNA was extracted from 10-day-old seedlings of cultivars by the CTAB method (Porebski et al., 1997). Fresh leaves were cut from the plants and placed in 1.5-mL Eppendorf tubes. The tubes were subsequently dropped in liquid nitrogen for rapidly freezing the leaf material. The plant material was then crushed with a micropestle while inside the tube by adding 1 mL of preheated (65 °C) 2X CTAB solution. The homogenized leaf tissues were transferred to two 1.5-mL Eppendorf tubes and were incubated in a water bath at 65 °C for 30 min. Next, 0.5 mL of chloroform and isoamyl-alcohol (24:1) was added and tubes were inverted vertically 5–10 times, followed by centrifugation at 10,000 rpm for 10 min. After centrifugation, supernatant was transferred to fresh tubes and 0.6 volume of 3 M sodium acetate was added. Then 500 µL of cold isopropanol was added and mixed properly by inverting the tubes for a few minutes. The DNA was pelleted and washed with 70% cold ethanol.

The pellet was air-dried and resuspended in 40 µL of 0.1X TE buffer. Purified DNA samples were stored at −20 °C for further use. Purity of the dissolved DNA in the samples was analyzed by checking the concentration on a spectrophotometer measured to 50 ng/µL of DNA. STR markers (Xwmc24, Xgwm95, Xwmc327, Xgwm644, Xgwm268, and Xgwm456) were used for genetic mapping (Ganal and Röder, 2007). The 50-µL reaction mixture consisted of 60–70 ng of template DNA, 1.0 µL of Mg-free 10X PCR buffer (Fermentas), 0.6 µL (5 U/µL) of Taq DNA polymerase (Fermentas), 25 mM $MgCl_2$, 2.5 mM dNTPs (Sigma Chemical Co., St. Louis, MO, USA), and 30 ng of a single primer synthesized by Gene Link (Hawthorne, NY, USA). After 5 min of denaturation at 94 °C, amplifications were programmed for 40 consecutive cycles each consisting of 1 min at 94 °C, 1 min at 55 °C annealing, and 2 min at 72 °C, followed by a 7-min extension step at 72 °C (Gupta and Varshney, 2000; Mammadov et al., 2012). Quantitative trait loci (QTL) were detected using Map-Manager-QTX version $b_2$0 software. Regression analysis was used for finding associations between phenotypic and genotypic data (Lewis, 2007).

3. Results and discussion

Yellow (stripe) and brown (leaf) rust are major crop damaging diseases among all fungal diseases in all parts of the world where there is wheat production. Most of the races of the rust pathogen were able to overcome the resistant genes due to having rapid gene mutation processes. Therefore, the basic concept of this work was

the creation of lines resistant to both leaf and yellow rust. Twenty-eight known Lr/Yr resistant genes were overcome by the physiological races in northern areas of Khyber Pakhtunkhwa and the south of Sindh, Pakistan. Virulence to various leaf resistance genes, Lr2, 2c, 3a, 9, 11, 14b, 17, 18, 20, 21, 26, 30, 35, 36, 38, and 40, occurs with higher frequency (66%–98%). Much lower frequency (20%–60%) was observed for virulence to Lr2b, 3ka, 15, 16, 19, 24, 25, 44, and Lr46. On the basis of the obtained results 68 lines were selected with resistance to the native population of leaf rust and yellow rust. Adult plant reaction types of the *T. aestivum-T. turgidum* ILs were estimated over 2 years under different weather conditions. Among the lines 65% displayed the immune or resistant (R) reaction type, whereas others showed moderate resistance (MR) (Table 1).

3.1. Seedling stage resistance tests

All selected ILs were tested with a local bulk of urediniospores for reaction at the seedling stage. This survey showed that all the lines resistant to leaf and yellow rust at the adult plant stage exhibited resistance at the seedling stage. Under field conditions, diseases can be caused by different pathogens; therefore, it is important to develop varieties possessing resistance to more than one disease. The introgression lines were screened under field conditions for resistance to other fungal diseases in northern areas like Pir Sabaq, Khyber Pakhtunkhwa. Among the lines were found three lines with good resistance levels to yellow rust, twenty lines resistant to leaf rust, five lines resistant to both together, and 16 lines with limited rust resistance.

3.2. Molecular marker (microsatellite/STRs) analysis

Molecular marker analysis of *T. aestivum-T. durum* ILs was performed with the help of STRs. In total, 296 GWM and 8 GDM (Roder et al., 1998) and 9 BARC and 7 WMC (Gadaleta et al., 2012) primer pairs, mapped to the chromosomes of the *T. aestivum* and *T. turgidum* genomes, were used to estimate polymorphisms between the parental wheat cultivar and *T. turgidum*. On average, we used eight to ten markers for each of the chromosomes 1B, 3B, 4A, 4B, 6A, 7A, 7B, and all chromosomes of the D genome, and 25–30 markers for chromosomes 1A, 2A, 2B, 5A, 5B, and 6B. Each primer pair revealed 1–5 loci in the five parental common wheat cultivars, 3 alleles per locus on the average. Intervarietal polymorphism detected in our study was not very high but polymorphism involving null alleles (absence amplification in the *Triticum turgidum* genome) was observed for 70%–90% of markers depending on the genome, the highest polymorphism being found for markers of the A genome. Twenty out of 45 primer pairs (32%) mapped on the chromosomes of the D genome amplified fragments in *T. turgidum*. Three of them (Xwmc327, Xgwm644, and Xgwm268) were located on the *T. turgidum* chromosomes (4G, 5G, and 1G, respectively), while the chromosomal localization of the others is unknown. The absence of amplification of microsatellite markers in *T. aestivum-T. turgidum* ILs can be explained as substitutions or translocations, as well as by deletions in chromosomes of *T. aestivum*. In our study, the absence of PCR products for certain markers of chromosome 1B, which differs from its homolog 1G by a high level of rearrangements (Delgado-Salinas et al., 2006), can be considered as a deletion at the marker location site on chromosome 1B. This suggestion is supported by the data of Belyayev et al. (2013) obtained in the analysis of 47 *T. aestivum-T. turgidum* ILs by means of the C-banding technique (Belyayev and Raskina, 2013).

3.3. Chromosomal localization of tetraploid durum wheat genome in ILs

To determine the chromosomal location of the *Triticum turgidum* genome in the ILs, 130 polymorphic markers were used, including those not producing PCR fragments in *Triticum turgidum*. On the basis of the obtained data, the

Table 1. The number of the introgression lines resistant to leaf and stripe rust.

Wheat cultivar	Total no.	Leaf rust (*Puccinia triticina*)		Stripe rust (*Puccinia striiformis*)
		R	MR	R
PN90	28	25	3	8
RW87	7	2	5	1
CN70	14	10	4	4
Mm2002	7	3	4	5
SM96	12	5	7	9
Σ	68	45	23	27

R – resistant, MR – moderately resistant.

chromosomes containing substitutions and translocations were divided into two groups. The first group included chromosomes 1A, 2A, 2B, 5A, 5B, and 6B, in which introgressions occurred with high frequency (Figure 1). The second group included chromosomes 1B, 3A, 3B, 4B, and 7A with lower substitution and translocation levels (Table 2), whereas microsatellite marker analysis did not reveal substitutions or translocations in chromosomes 4A, 6A, and 7B in all investigated ILs. Analysis with microsatellite markers specific to the D genome suggested introgression of *Triticum turgidum* fragments into D chromosomes of four lines: the 5D, 6D, and 7D chromosomes of the lines derived from Pasban-90 (PN90) and the 7D chromosome of the line derived from Moomal-2002 (Mm2002). Intervarietal differences were observed both for chromosomal localization of introgression fragments and the number of introgressions. It was shown that the number of the fragments varied from three to eight, the greatest number being found in the lines obtained on the basis of cultivar PN90 (5.3 on the average), followed by RW87 (4.8), Mm2002 (4.2), CN70 (3.7), and SM96 (3.0). Three ILs differing in the number and chromosomal location of the *Triticum turgidum* genome fragments were selected for mapping of loci associated with resistance to leaf rust. IL-1 possessed three fragments in chromosomes 1A, 2B, and 5A; IL-2 was found in the lines obtained on the basis of cultivar PN90 (5.3 on the average), followed by RW87 (4.8), Mm2002 (4.2), CN70 (3.7), and SM96 (3.0). Three ILs differing in the number and chromosomal location of the *Triticum turgidum* genome fragments were selected for mapping of loci associated with resistance to leaf rust. IL-1 possessed three fragments in chromosomes 1A, 2B, and 5A; IL-2 was found to have five introgressions in chromosomes 1A, 2A, 2B, 5B, and 6B; and seven introgressions in chromosomes 1A, 2A, 2B, 5A, 5B, 6B, and 4B were determined in the genome of IL-3. The lines were crossed with susceptible cultivar RW87 for developing F_2 mapping populations. The leaf rust reaction was estimated in F_3 populations at the seedling and adult plant stages. For genotyping individual plants from the F_2 mapping populations, we used 104 polymorphic microsatellite markers, of which 37 markers were previously mapped in the *Triticum turgidum* genome (data not shown here). Linkage groups constructed for chromosomes containing introgressions indicated that the order of the microsatellite loci in ILs IL-1, IL-2, and IL-3 is in agreement with that of the chromosome 1A, 2A, 2B, 4B, 5A, 5B, and 6B maps described for the ITMI

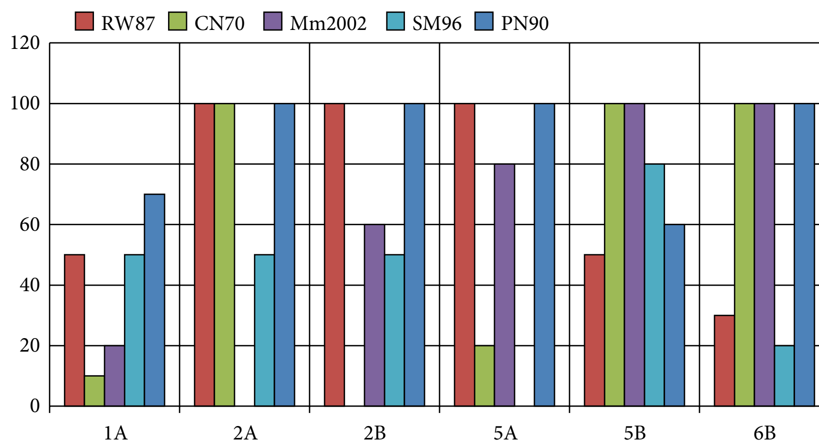

Figure 1. Frequency of substitutions and translocations on chromosomes 1A, 2A, 2B, 5A, 5B, and 6B in introgression lines obtained on the basis of different wheat cultivars.

Table 2. Frequency (%) of substitutions and translocations in chromosomes 1B–7B in ILs.

Wheat	Chromosome							
cultivar	1B	3A	3B	4A	4B	6A	7A	7B
PN90	15	10	30	0	25	0	0	0
RW87	10	0	0	0	0	0	0	0
CN70	25	0	15	0	10	0	0	0
Mm2002	10	0	0	0	15	0	0	0
SM96	15	0	0	0	0	0	40	0

wheat population (Cota et al., 2010; Zanke et al., 2014). Chromosomal localization of QTL associated with leaf rust resistance of IL-2 and IL-3 carrying five and seven introgression fragments, respectively, revealed three loci, QLr-2B, QLr-5A, and QLr-1A, located on chromosomes 2B, 5A, and 1A, respectively. The major locus on chromosome 2B was mapped to the marker interval Xgwm451-Xgwm115 and accounted for 64% of the expression of the trait on average (Table 3). The locus on chromosome 5A was located in the marker interval Xgwm327-Xgwm24. This locus controlled 11.5% of the phenotypic expression of the trait and originated from chromosome 2A. The third minor locus was mapped on the long arm of chromosome 1A, with a maximum near Xgwm24. Regression analysis detected three microsatellite markers on chromosome 2B with a high probability linked to the resistance to leaf rust: Xgwm711, Xgwm814, and Xgwm125. These markers were used as a background in composite interval mapping (CIM). The results of CIM showed that QTL on chromosome 2B did not affect the expression of the locus mapped to chromosome 5A and substantially inhibited the expression of the minor locus on chromosome 1A. This indicates that the loci QLr-2B and QLr-5A act independently, and they together control the trait by 75%. More precise localization of the resistance gene on chromosome 5A was performed by means of mapping the population developed on the basis of IL-1 containing three introgression fragments in chromosomes 1A, 2B, and 5A. The yellow rust resistance gene designated as *Yr2* was found to be located 7.2 cM away from microsatellite marker Xgwm106 (Figure 2). The mapping of the major resistance gene *Yr1* on the 5A

Table 3. Localization of the loci determining the leaf rust resistance of the ILs.

Chromosome			
	1A	5A	2B
Flanking markers			
	Xgwm 219-Xgwm 24	Xgwm327-Xgwm24	Xgwm451-Xgwm115
R^2	8.0	11.5	64.0
LOD	2.5*	3.5**	17.0**

R^2 – percentage of the variance of the trait associated with quantitative trait loci.
LOD – log likelihood ratio; *P < 0.01; **P < 0.001.

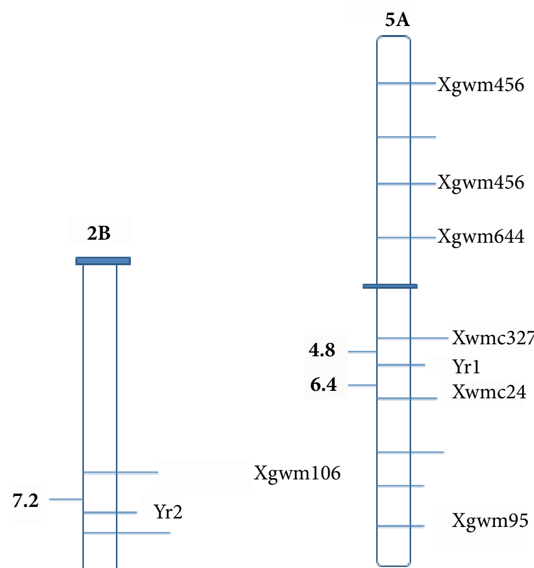

Figure 2. Genetic maps of the *Yr1* and *Yr2* regions on chromosomes 5A and 2B; the microsatellite marker names are indicated on the right side; genetic distances are given in cM on the left side.

chromosome showed that the gene was localized between markers Xgwm327 (4.8 cM) and Xgwm24 (6.4 cM) (Figure 2). Comparative analysis of PCR fragment sizes for three microsatellite markers demonstrated that DNA of resistant plants amplified fragments typical for *Triticum turgidum*, indicating that the resistance gene was derived from chromosome 5G. According to the literature, five leaf rust resistance genes, *Lr11*, *Lr17*, *Lr37*, *Lr38*, and *Lr45*, are located on chromosome 2A. All these genes did not originate from *Triticum turgidum* (Mayer et al., 2014a). So far, only two leaf rust resistance genes transferred from the *Triticum turgidum* genome are known. One of them, *Lr18*, was localized on the long arm of chromosome 5B and is associated with a *Triticum turgidum*-derived telomeric band (Rouse et al., 2014). The second, *Lr50*, was transferred from wild species *Triticum turgidum* subsp. *armeniacum* to the long arm of chromosome 2B and is linked with the microsatellite markers Xgwm644 and Xgdm327. The QTL responsible for leaf rust resistance of the *Triticum aestivum*-*T. turgidum*/*Ae. tauschii* IL was found to be located in the same chromosome region as *Lr50* (Leonova et al., 2008). It should be noted that in our ILs the translocated fragment in chromosome 2B did not possess loci associated with leaf rust resistance. Considering the origin and chromosomal location, it was possible to assume that *LrTt2* may be the same gene or a novel allele of *Lr18*. Molecular analysis of the near-isogenic Thatcher line RL6009 containing the *Lr18* gene with a set of Xgwm markers mapped to chromosome 5B indicated that the Thatcher line differed from the IL-2 and IL-3 lines both in translocation breakpoint and in the length of the PCR fragments amplifying with microsatellite markers (Leonova et al., 2011). In field examination it was demonstrated that ILs with the *LrTt2* gene exhibited immune or resistant types of reaction to the native population of leaf rust, whereas the Thatcher line with the *Lr18* gene displayed a susceptible reaction type. The results of microsatellite genotyping and pathogen resistance tests suggest that *LrTt2* may represent a new locus that originates from the 5G chromosome of *T. turgidum*. Thus, the obtained results have shown that selection of the *Triticum aestivum*-*Triticum turgidum* ILs for pathogen resistance after a single backcross leads to primary selection of genotypes containing, on average, from three to eight substitutions and translocations. Our results and the literature data indicate that subspecies of *Triticum turgidum* may carry leaf rust resistance genes differing both in chromosomal localization in the *Triticum turgidum* genome and their efficiency against leaf rust flora (Leonova et al., 2002; Liu et al., 2007; Mayer et al., 2014b; Wang et al., 2014). Microsatellite markers were shown to be an efficient tool for evaluation of intervarietal polymorphism, genotyping of hybrid genomes, and localization of gene/QTL.

The application of microsatellite markers allows for a more precise determination of the translocation regions in hybrids of common wheat containing alien chromosomal translocations as compared to cytogenetic methods. *Triticum aestivum*-*Triticum turgidum* ILs can be used for investigation and mapping of genes for pathogen resistance and as a source of resistance genes in breeding programs. The microsatellite markers closely linked with agronomic valuable loci may be used to transfer the loci from the ILs into other wheat cultivars.

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