A novel leaf rust resistance gene transferred from *Aegilops caudata* L. to *Triticum aestivum* L. maps on chromosome 5D

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ABSTRACT

Leaf rust and stripe rust are the two most important foliar diseases of wheat worldwide. Host genetic resistance is the most economical and environmentally friendly approach to reduce the losses due to rust diseases in wheat. The germplasm of non-progenitor *Aegilops* species have been found to be an invaluable source for new resistance genes. The transfer and mapping of a new leaf rust resistance gene from *Ae. caudata* L. is reported here. A leaf rust resistant accession PAU #3556 of *Aegilops caudata* was crossed with *T. durum* and an amphiploid synthesized for transferring leaf rust resistance to *T. aestivum* through induced homoeologous pairing. Leaf rust resistant introgression lines (ILs) were developed through backcrossing with a leaf rust susceptible cultivar WL711. An F_2 population derived from one of the introgression lines segregated for a single leaf rust resistance gene at the seedling (208R:78S; χ^2 =0.79) and adult plant stage (219R:67S; χ^2 =0.37). The testing of F_3 progenies confirmed the transfer of a single gene for leaf rust resistance. Bulk segregant analysis was conducted using 101 polymorphic D-genome specific SSR markers. The SSR markers *Xcfd18*, *Xcfd189*, *Xcfd78 and Xfd81* detected *Ae. caudata* specific alleles in the parental introgression line and the resistant bulks. All these markers mapped on distal region of 5DS indicated a terminal translocation. Analysis of the F_2 population mapped *Ae. caudata* leaf rust resistance gene on chromosome 5DS with *Xcfd18* as the closest linked marker.

INTRODUCTION

Wild species of wheat are a reservoir of variability for several biotic and abiotic stresses. Genes especially for disease resistance transferred from wild species have provided an insurance cover for sustaining wheat production globally. Among the wheat diseases, leaf rust, stripe rust and stem rust are the most important worldwide. A total of 60 genes for leaf rust, 40 for stripe rust and 45 for stem rust have been designated so far and out of these 30 genes for leaf rust, 12 for stripe rust and 18 for stem rust resistance have been introgressed from wild progenitor and non-progenitor species (McIntosh *et al*. 2007). Although a number of genes have been introgressed from wild *Triticum* and *Aegilops* species, only a few have been commercially exploited due to substantial linkage drag. The T1BL.1RS translocation carrying complete IRS arm from *Secale cereale*, is the most successful example of utilization of the alien variability in wheat (Hoisington *et al*. 1999).

Most of the commercially exploited genes condition hypersensitive reaction and interact with the pathogens in gene-for-gene fashion. Deployment of such genes over large areas leads to the development of new virulence in the pathogen. A large number of these genes have been rendered ineffective due to the emergence of virulent races of the pathogens necessitating the constant search and transfer of new and effective sources of rust resistance to counter balance the continuous evolution of rust populations. Wild progenitor and non-progenitor species of wheat are highly valuable source of additional resistance genes (Gale and Miller 1987; Jiang *et al* 1994; Friebe *et al.* 1996; Singh *et al* 1998; Singh *et al* 2007). In our laboratory leaf rust and stripe rust resistance genes have been transferred from *Aegilops geniculata* (*Lr57* and *Yr40*), *Ae. triuncialis* (*Lr58*) and *Ae. umbellulata* (*LrU1, LrU2, YrU1*) (Kuraparthy *et al* 2007a,b; Chhuneja *et al*. 2007). Two new stripe rust resistance genes from *T. monococcum* and *T. boeoticum* have been mapped and transferred to cultivated wheat (Chhuneja *et al.* 2008).

Ae. caudata, a non-progenitor diploid species with the CC genome, was also found to be an excellent source of resistance for various diseases. In the present study we report the transfer of a leaf rust resistance gene from *Ae. caudata* accession pau#3556 to hexaploid wheat and its inheritance and mapping in hexaploid wheat background.

TRANSFER OF LEAF RUST RESISTANCE FROM AE. CAUDATA

A leaf rust and stripe rust resistant *Ae. caudata* acc. pau3556, was used for developing a synthetic amphiploid with *T. durum* cv. WH868. The amphiploid was crossed with Chinese Spring {CS(*Ph*^{I})} to induce homoeologous pairing. The F_1 plants were crossed with a leaf rust susceptible genotype, WL711(NN). The resistant F_1 plants from the cross *T. durum* WH868-*Ae. caudata*//CS*Ph*^{I}/3/WL711(NN) were then selfed and homozygous resistant introgression lines were selected in advanced self generations. In the segregating generations, selection for leaf rust resistance was carried out by screening at the seedling stage against two most virulent and prevalent leaf rust pathotypes (121R63-1 and 21R5) using the standard inoculation procedure (Nayar *et al.*, 1997). The adult plant screening was conducted against a mixture of pathotypes and data was

recorded according to modified Cobb's scale (Peterson *et al.* 1948).

Recipient parent WL711 (NN) and CS (Ph^I) were susceptible to the leaf rust pathotypes 121R63-1 and 21R5 at the seedling stage. WL711 developed 60S-80S leaf rust at the adult plant stage while CS (Ph^I) developed 20S leaf rust. Hexaploid and cytologically stable homozygous WL711-*Ae. caudata* introgression lines, however, were resistant at the seedling as well as adult plant stage (Fig. 1).

Fig. 1. Leaf rust reaction of the WL711-*Ae. caudata* introgression lines and F_2 plants derived from the cross WL711-*Ae. caudata*/PBW343

INHERITANCE STUDIES FOR LEAF RUST RESISTANCE

An F_2 population was developed by crossing a selected WL711-*Ae. caudata* introgression line with a widely grown wheat cv. PBW343 for studying the inheritance of leaf rust resistance transferred from *Ae. caudata*. The F_1 of WL711-*Ae. caudata*/PBW343 was resistant at both seedling and adult plant stage indicating that the resistance was controlled by a dominant gene. The F_2 population segregated into 208R:78S ($\chi^2_{3:1}$=0.79) and 219R:67S ($\chi^2_{3:1}$=0.37) at the seedling and adult plant stage, respectively (Table 1). Progeny testing in F_3 also showed 1:2:1 segregation, thereby confirming that single dominant gene transferred from *Ae. caudata* conditioned leaf rust resistance.

	Resistant	Susceptible	Total	χ^2
Leaf rust-seedling[1]	208	78	286	0.79
Leaf rust-Adult plant[2]	219	67	286	0.37

Table 1. Segregation of leaf rust (*P. triticina*) resistance in an F_2 from the cross of WL711-*Ae. caudata* introgression line with *T. aestivum* cv. PBW343

[1]Seedling screening was done with leaf rust pathotype 121R63-1

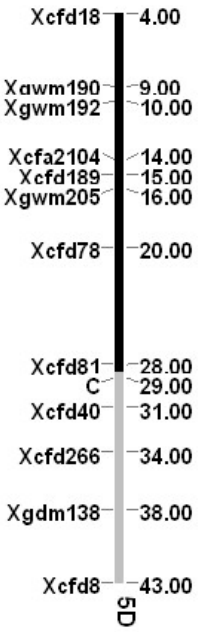
[2]Adult plant screening was done with a mixture of pathotypes 109R31-1, 121R63-1 and 21R5

MOLECULAR CHARACTERIZATION OF *AE. CAUDATA* INTROGRESSION LINES

For characterizing the introgression carrying leaf rust resistance gene of *Ae. caudata* transferred to bread wheat, bulk segregant analysis based on the F_2 population was conducted. The DNA of the parents, WL711-*Ae. caudata* introgression line and F_2 plants was isolated using CTAB method. The DNA of the 10 resistant and 10 susceptible F_2 plants was bulked to generate resistant 'R' and susceptible 'S' bulks. For molecular analysis only D-genome specific SSR markers were used because in the F_1 derived from the cross of *T. durum-Ae. caudata* amphiploid with CS (Ph^I) only C-genome chromosomes of *Ae. caudata* and D-genome chromosomes of CS were present as univalents and hence had higher probability of pairing. A total of 168 SSR markers were tested on the parents and 101 markers were polymorphic which were amplified on the parents, wheat-*Ae. caudata* introgression line, 'R' and 'S' bulks. *Ae. caudata* specific introgressions were detected on chromosome 4D, 5D, 6D and 7D in the wheat-*Ae. caudata* introgression line. However, only 5D mapped SSR markers *Xcfd18*, *Xgwm190, Xcfa2104, Xcfd189*, *Xgwm205*, *Xcfd78* and *Xfd81* showed *Ae. caudata* specific amplification in the introgression line as well as the resistant bulks indicating that the leaf rust resistance gene of *Ae. caudata* has been transferred to wheat chromosome 5D.

Graphical genotyping of the introgression line showed that the almost complete short arm of chromosome 5D has been transferred from *Ae. caudata* with translocation break point near the centromere (Fig. 2).

Figure 2. Graphical genotyping of a WL711-*Ae. caudata* introgression line for chromosome 5D based on SSR markers. Black areas indicate *Ae. caudata* specific introgression and grey areas indicate wheat specific alleles. Map distances are according to the Somers *et al.* 2004.



MAPPING OF *AE. CAUDATA* LEAF RUST RESISTANCE

SSR markers *Xcfd18*, *Xcfd189*, *Xcfd78* and *Xfd81* showing introgression in the resistant bulks were amplified on the F_2 population for studying the co-segregation of these markers with the leaf rust resistance gene transferred from *Ae. caudata* (Fig. 3). A total of 254 F_2 plants were analysed with these four markers. PCR products for *Xcfd18, Xcfd78 and Xcfd189* were resolved on Agarose gel while *Xcfd81* was resolved on 8% PAGE. All the markers co-segregated with the leaf rust resistance. *Ae. caudata* is a non-progenitor species and hence no recombination was expected within the introgressed segment.

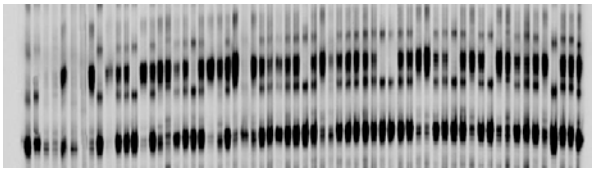


Fig. 3. PCR amplification of the F_2 population derived from the cross WL711-*Ae. caudata* IL/PBW343 for SSR marker *Xcfd81*. PCR products were resolved in 8% PAGE and visualized on the LICOR sequencer. Lane 1-WL711; Lane 2-PBW343; Lane 4-WH868, Lane 5-WL711-*Ae. caudata* IL and Lane 6-64: F_2 plants.

During the present investigation a leaf rust resistance gene has been transferred from *Ae. caudata* pau3556 to cultivated wheat and the introgression carrying this resistance has been mapped to wheat chromosome 5D.

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