Transfer of new leaf rust resistance genes from diploid *T. monococcum* and *T. boeoticum* to *T. aestivum*

Kaur S[1], Chhuneja P[1], Dhaliwal HS[2], and Singh K[1]
[1]*Dept. of Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana 141004, India*
[2]*Dept. of Biotechnology, Indian Institute of Technology, Roorkee, India*

ABSTRACT

The diploid 'A' genome progenitor gene pool of wheat, comprising three closely related species *T. monococcum* ssp *monococcum* (*T. monococcum*), *T. monococcum* ssp *aegilopoides* (*T. boeoticum*) and *T. urartu*, harbours useful genes for many economically important traits, including resistance to leaf rust. *T. monococcum* acc. pau14078 and *T. boeoticum* acc. pau 5088 are immune to all the leaf rust races at adult plant stage and so is the RIL population generated from the cross these two accessions. At seedling stage these lines were resistant to leaf rust races 77-2 and 77-5 but population segregated for resistance against race 104-2. *T. monococcum* and two RILs designated as RIL130 and RIL101 were crossed to susceptible *T. durum* parent, N59. Rust resistance was suppresses in the F_1. Further crossing and backcrossing with hexaploid wheat cultivars WL 711 and PBW 343 led to identification of resistant progenies. One BC_2F_2 population of the cross N59/RIL130//3*PBW343 when tested at seedling stage showed single gene segregation (489R: 142S $\chi^2 = 2.0$). Similarly another BC_2F_2 population of the cross N59/*T. monococcum*//3*WL711 also showed single gene segregation (153R:66S, $\chi^2 = 3.0$) at seedling stage. Both the populations are cytologically stable with 42 chromosomes indicating a stable leaf rust resistance gene transfer from *T. monococcum* to hexaploid wheat. Bulk segregant analysis of the BC_2F_2 population of the cross N59/*T. monococcum*//3*WL711 with 38 polymorphic SSRs indicated that the seedling resistance gene of *T. monococcum* is located on chromosome 5A. As none of the designated *Lr* genes map on 5A, the gene in question may be a novel one.

INTRODUCTION

Among the three rust diseases of wheat, leaf rust caused by *Puccinia recondita* Rob ex Desm. f. sp. *triitc*i is the most common, affecting the wheat production worldwide. An effective, economical and environmentally safe method to control epidemics of leaf rust is the cultivation of resistant cultivars. Until now more than 50 leaf rust resistance genes (*Lr1-Lr58*) have been identified (McIntosh et al. 2005; Kuraparthy et al. 2007, Chhuneja et al 2007) and most of these, especially the ones identified from the cultivated germplasm, are ineffective against the recently evolved pathotypes. This necessitates search for new sources of resistance. The wild relatives of wheat are rich sources of resistance genes particularly for biotic stresses. The diploid 'A' genome progenitor gene pool of wheat, comprising three closely related species *T. monococcum* ssp *monococcum* (*T. monococcum*), *T. monococcum* ssp *aegilopoides* (*T. boeoticum*) and *T. urartu*, harbour useful genes for many economically important traits (Feldman and Sears 1981;Dhaliwal et al. 1993; Hussien et al. 1997, Qiu et al. 2005) including resistance to leaf rust. Transfer of these genes into to hexaploid wheat generally require the use of *T. durum* as bridging species. However, presence of suppressor loci on the B genome of *T. durum* present a major hurdle in transferring useful variability from diploid to hexaploid wheats (Chhuneja et al 2008). Identification of DNA markers linked to the desirable genes at the diploid level could facilitate their transfer to hexaploid wheat (Yao et al. 2007). A spring type *T. monococcum* acc. pau14087 and *T. boeoticum* acc. pau 5088 has maintained high levels of resistance to a number of wheat diseases including leaf rust in Punjab (India) over years (Dhaliwal et al. 2003). This report describes the transfer of leaf rust resistance of *T. monococcum* and a RIL (RIL130 - derived from cross of *T. monococcum*/*T. boeoticum*) to bread wheat and its tagging with microsatellite markers using bulk segregant analysis.

MATERIAL AND METHODS

The plant material consisted of *T. monococcum* acc. 14087 (*Tm*14087), *T. boeoticum* acc. 5088 (*Tb*5088) and a set of 121 recombinant inbred lines (RILs) derived from their intercross. Mapping of leaf rust resistance was not possible because the parents and the population showed immunity to leaf rust under field conditions at adult plant stage. Hence efforts were made to transfer these genes to hexaploid wheat first. For this *Tm*14087 and RIL130 were crossed to a susceptible *T. durum* CV N59. The F_1 plants were crossed to WL 711 and a widely grown bread wheat cultivar PBW 343. The three-way F_1 thus generated was backcrossed to respective recurrent parents WL711 and PBW 343 to obtain BC_1F_1 generation. The BC_1F_1 plants resistant to leaf rust at seedling stage were identified, selfed and chromosomally stable BC_1F_2 plants identified. The BC_1F_2 and BC_1F_3 populations of N59/RIL130//2*PBW343 and BC_2F_2 and BC_2F_3 progenies of N59/Tm14087//3*WL711 were screened against leaf rust at seedling and against a mixture of pathotypes at adult plant stage. Chi-square (χ^2) test was applied to fit appropriate genetic ratio in F_2 and F_3 generations.

For mapping the leaf rust resistance genes transferred from *T. monococcum*, DNA was extracted from individual BC_1F_2 plants of the cross N59/RIL130//2*PBW343 and BC_2F_2 plants of N59/Tm14087//3*WL711.

For screening against leaf rust at seedling stage, race 77-5 (with avirulence/virulence formula P*Lr9, Lr18, Lr19, Lr24, Lr25, Lr28, Lr29, Lr32, Lr41, Lr45*/p*Lr1, Lr2, Lr3, Lr10, Lr11, Lr12, Lr13, Lr14, Lr15, Lr16, Lr17, Lr18, Lr20,Lr21, Lr22, Lr23, Lr26, Lr27+Lr31, Lr33, Lr34, Lr36, Lr37, Lr42, Lr43, Lr44, Lr46, Lr48, Lr49*) was used. For seedling tests, 10-15 seedlings of each parental line, and the segregating generations were sown in plastic trays containing mixture of farmyard manure and sandy loam in equal proportion. In each tray, six rows of experimental material and seventh row of susceptible cultivar Agra Local were sown. First leaf of seven-day-old seedling(s) of each plant was inoculated individually with uridiniospores - talc mixture keeping inoculum density of 6-8 uridiniospores over a microscopic field area of 2.92mm^2. The inoculated seedlings were incubated in a dark chamber maintained at 20°C±1°C at 100 percent relative humidity for 16 hours. After incubation, the trays were shifted to glass houses maintained at 20°C±2°C. Fourteen days after inoculation, the infection types on seedlings using the scale proposed by Stakman *et al* (1962). Disease severity for all parents and populations was assessed by growing adult plants of these in open experimental field during third week of November, which is the normal sowing time for wheat crop in Northern India. This material was repeatedly spray inoculated every evening with mixture of urediniospores of leaf rust races (including race 77-5), suspended in water (1 gram inoculum per 10 litres of water, using one drop of Tween-20 as dispersant). The inoculations started during first week of January and continued until rust started appearing on susceptible lines early February. Spreader rows were planted all around the population to ensure an effective disease spread.

At seedling stage plants with the disease reaction 0-2 were considered as resistant and the ones with 3 and above as susceptible. At adult plant stage observations on leaf rust severity were recorded as percentage of leaf area covered with rust according to a modification of the Cobb scale as described by Peterson *et al* (1948). The plants with disease severity 0 to 10MR were classified as resistant and the ones with 10MS or more were classified as susceptible.

RESULT AND DISCUSSION

TRANSFER OF LEAF RUST RESISTANT GENES

*Tm*14087 and RIL130 showed infection type of ; and ;1- respectively at the seedling stage and at adult plant stage in the field these were immune. The recipient hexaploid wheat cultivars WL711 and PBW343 were susceptible, giving an infection type of 33+ and 3 at seedling stage and leaf rust severity of 80S and 40S, respectively at the adult plant stage. *T. durum* cv N59 was also susceptible both at the seedling as well as the adult plant stages.

While transferring leaf rust resistance from *T. monococcum*, one major problem encountered was suppression of the resistance in F_1 of *T. durum* cv N59/ *T. monococcum* and *T. durum* cv N59/ *T. monococcum*/ //*T. aestivum* cv PBW343 (WL711). It was later confirmed that the loci suppressing resistance in F_1 generations are present on B genome chromosomes (Chhuneja et al 2008). Leaf rust resistant plants, however, were recovered in the BC_1F_1 generation of the crosses, *T. durum* cv N59/*T. monococcum*/ //PBW343 and *T. durum* cv N59/ RIL130// WL711. The resistant plants were further, either selfed or backcrossed to the recurrent bread wheat parent and BC_1F_2, BC_1F_3, BC_2F_2 and BC_2F_3 progenies generated. Segregation pattern of these progenies is presented in Table 1. At seedling stage BC_1F_2 progeny of the cross N59/RIL130//2*PBW343 segregated in 3:1 ratio ($\chi2$ = 2.0 and the BC_1F_3 progeny showed 1:2:1 segregation ($\chi2$ = 4.63). This confirms the transfer of a single dominant seedling resistance gene from RIL130 into hexaploid wheat cultivar PBW 343. Similarly, BC_2F_2 progeny of the cross N59/Tm14087//3*WL711 showed 3:1 segregation ($\chi2$ = 3.0) and the BC_2F_3 progeny showed 1:2:1 segregation ($\chi2$ = 0.99). This again confirms transfer of a single dominant gene from *T. monococcum* to hexaploid wheat. Allelic test for these genes is in progress to ascertain whether the gene transferred from RIL130 is same as that from *T. monococcum* or from *T. boeoticum*. Also the populations are being analysed for SSR markers for mapping of these genes. Bulk segregant analysis of BC_2F_2 progeny of the cross N59/Tm14087//3*WL711indicates that the gene for leaf rust resistance from *T. monococcum* may be located on chromosome 5A.

Table 1. Segregation pattern in various generations of the crosses N59/RIL130//2*PBW343 and N59/Tm14087//3*WL711

Progeny	Testing stage	Res	Seg	Sus	Total	$\chi2$
Cross	N59/RIL130//2*PBW343					
BC_1F_2 (2006-07)	Seedling	489	-	142	631	2.0
BC_1F_3 (2007-08)	Adult plant	156	264	122	542	4.63
Cross	N59/Tm14087//3*WL711					
BC_1F_2 (2006-07)	Seedling	153	-	66	219	3.0
BC_1F_3 (2007-08)	Adult plant	61	104	54	219	0.99

Res = resistant/ homozygous resistant, Sus = susceptible/ homozygous susceptible, Seg = segregating

REFERENCES

Chhuneja P, Kaur S, Goel RK, Aghaee-Sarbarzeh, Prasher M and Dhaliwal HS (2007) Transfer of leaf rust and stripe rust resistance from Aegilpos umbellulata Zhuk. To bread wheat (*Triticum aestivum* L.). Genet. Resour. Crop Evol., Evol. DOI 10.1007/s10722-007-9289-3.

Chhuneja P, Kaur S, Garg T. Ghai M, Kaur S, Prashar M, Bains NS, R K Goel RK, Keller B, Dhaliwal HS and Singh K (2008) Mapping of adult plant stripe rust resistance gene in diploid A genome wheat species and their transfer to bread wheat. Theor. Appl. Genet: 116:313-324.

Dhaliwal HS, Chhuneja P, Singh I, Ghai M, Goel RK, Garg M, Keller B, Röder M, Singh K (2003) Triticum monococcum—a novel source for transfer and exploitation of disease resistance in wheat. In: Proceedings of the 10th international wheat genetics symposium, Paestum, Italy, pp 346–349.

Dhaliwal HS, Singh H, Singh KS, Randhawa HS (1993) Evaluation and cataloguing of wheat germplasm for disease resistance and quality. In: Damania AB (eds) Biodiversity and wheat improvement. Wiley, London, pp 123–140.

Feldman M, Sears ER (1981) The wild gene resources of wheat. Sci Am 244:98–109.

Hussien T, Bowden RL, Gill BS, Cox TS, Marshall DS (1997) Performance of four new leaf rust resistance genes transferred to common wheat from *Aegilops tauschii* and *Triticum monococcum.* Plant Dis 81:582–586.

Kuraparthy V, Chhuneja P, Dhaliwal HS, Kaur S, Bowden RL, Gill BS (2007) Characterization and mapping of cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance genes *Lr57* and *Yr40* in wheat. Theor Appl Genet 114:1379–1389.

McIntosh RA, Devos KM, Dubcovsky J, Rogers WJ, Morris CF, Appels R, Anderson OD (2005) Catalogue of gene symbols: 2005 supplement. In: KOMUGI–Integrated Wheat Science Database. (http://www.grs.nig.ac.jp/wheat/komugi).

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can J Res 26:496–500.

Qiu YC, Zhou RH, Kong XY, Zhang SS, Jia JZ (2005) Microsatellite mapping of a *Triticum urartu* Tum. derived powdery mildew resistance gene transferred to common wheat (*Triticum aestivum* L.). Theor Appl Genet 111:1524–1531.

Stakman EC, Stewart DH, Loegering WQ (1962) IdentiWcation of physiological pathotypes of *Puccinia graminis* var. *tritici.* USDA Agri Res Serv No. E617 (Rev), p 53.

Yao G, Zhang J, Yang L, Xu H, Jiang Y, Xiong L, Zhang C, Zhengzhi Z, Ma Z, Sorrells ME (2007) Genetic mapping of two powdery mildew resistance genes in einkorn (*Triticum monococcum* L.) accessions. Theor Appl Genet 114:351–358.