Second Place Student Essay

Mapping of *Aegilops tauschii* Derived Leaf Rust Resistance Genes in Common Wheat

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ABSTRACT

Leaf rust, caused by *Puccinia triticina* Eriks, is one of the most serious diseases in wheat (*Triticum aestivum* L., AABBDD 2n=6x=42) production worldwide. Developing resistant cultivars is currently the most economical and effective approach to combating this disease. This experiment was designed to use molecular mapping to identify leaf rust resistance genes transferred from the wild goatgrass, *Aegilops tauschii* (DD, 2n=2x=14). The resistant lines in this experiment were developed from the *Triticum turgidum* line Do1 and the leaf rust resistance donor, *Ae. tauschii* accession TA1695. Microsatellite markers were used for chromosomal mapping and gene identification. During phenotypic screening, the population segregated for two different leaf rust resistance genes. One of the resistance genes was mapped on the short arm of chromosome 2D and may be the same as the previously mapped leaf rust gene *Lr39*. The closest marker identified in this experiment, *Xgdm35*, was located 12.6 cM from *Lr39*. While *Lr39* has already been incorporated into a developed germplasm, this research shows that *Ae. tauschii* accession TA1695 is also a source of this resistance gene. At this time, the other resistance gene in the segregating population has not been mapped. However, from preliminary screening, this gene looks to be a promising and valuable source of leaf rust resistance.

LEAF RUST is one of the most detrimental pathogens to wheat production in most major wheat (*Triticum aestivum* L., AABBDD $2n = 6x = 42$) growing areas of the world (Roelfs et al., 1992). The fungus *Puccinia triticina* Eriks [syn. *P. recondita* Roberga ex Desmaz. F. sp. *tritici* (Eriks. and E. Henn.)], which causes this disease, will infect susceptible lines and cause great yield and economic losses. Given the proper conditions, it has been noted that leaf rust alone can cause yield losses of up to 40% (Knott, 1989). Because of the great diversity of this pathogen and its ability to rapidly overcome the defense mechanisms of the host plants, new sources of leaf rust resistance are in constant demand.

Wild wheat relatives can be a valuable source of leaf rust resistance. By hybridizing wild wheat species with common wheat (*Triticum aestivum*), new leaf rust resistance genes can be introduced to the wheat genome. Many different wild species have been shown as potential gene donors. Included among these are *T. araraticum* (Brown-Guedira et al., 1997), *Aegilops markgrafi* (Peil et al., 1997), *T. tauschii* (Cox et al., 1994), and *T. timopheevi* (McIntosh, 1983).

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Aegilops tauschii Coss. [Syns. *Aegilops squarrose* L. and *Triticum tauschii* (Coss.) Schmal., DD, $2n = 2x = 14$], a wild grass, is a valuable source of leaf rust resistance. Leaf rust genes that have been transferred from *Ae. tauschii* and previously named include: *Lr21, Lr22, Lr32, Lr39, Lr41, Lr42*, and *Lr43* (Rowland and Kerber, 1974; Raupp et al., 1983; Kerber, 1987; Cox et al., 1994; Hussein et al., 1997). Continued use of this wild species in germplasm development should provide even more sources of leaf rust resistance.

Several hybridizations with *Ae. tauschii* accession TA1695 and *Triticum turgidum* line Do1 have been completed (G.L. Brown-Guedira, personal communication, 2002). Using this synthetic hexaploid, several subsequent backcrosses were made, which resulted in a segregating population for leaf rust resistance in a hexaploid background.

The objective of this project was to map the leaf rust genes transferred from accession TA1695 of *Ae. tauschii* using wheat microsatellite markers.

MATERIALS AND METHODS

Plant Material

Accession TA1695 of *Ae. tauschii* is highly resistant to diverse isolates of *Puccinia triticina* Eriks. The *T. turgidum* line Do1 was hybridized with TA1695 to give a synthetic hexaploid. This synthetic hexaploid was crossed with Jagger (F_1) and then backcrossed to give the BC_1F_1 (U3866A). At this time the plants were screened with *P. triticina* isolate MCDL (avirulence/virulence formula 2a, 2c, 3ka, 9, 11, 16, 18, 24, 30/1, 3a, 10, 17, 26). Resistant plants (U3866A-R2 and U3866A-R5, hereafter referred to as R2 and R5, respectively) were selected. These resistant plants were backcrossed with Jagger to give a BC_2F_1 (U4616 and U4619 from the R2 and R5 plants, respectively). Four resistant plants (U4616-1; and U4619-3, -4, and -5) were selfed to give the BC_2F_2, which was used as the mapping population (G.L. Brown-Guedira, personal communication, 2002). Population sizes for the mapping population are given in Table 1. The F_3 population was grown from selfed F_2 plants and scored to determine the heterozygous and homozygous plants in the F_2 population.

Phenotypic Screening

Using a direct application of urediospores in an oil immersion, the plants were inoculated with *P. triticina* isolate MCDL at the 2- to 4-leaf stage and placed in a mist-chamber for 24 hours. Reaction types were recorded one to two weeks after inoculation. The plants were scored resistant (R) or susceptible (S) in the F_2 population. The F_3 lines were screened with MCDL and classified as homozygous resistant (HR), homozygous susceptible (HS), or segregating (H) based on a

Table 1. The number of plants segregating R or S to inoculation with isolate MCDL of *P. triticina* for the four different BC_2F_2 populations used in mapping. The observed infection types (IT)† for each population are shown. The X^2 values for a segregating ratio of 3 resistant plants to 1 susceptible plants are also shown.

	No. of plants				
Line	Resistant/IT	Susceptible	Total	Ratio (R:S)	X^2 (3:1)
U4616-1	45/; - ;C	16	61	2.81:1	0.0476
U4619-3	41/;C-1C	15	56	2.73:1	0.0911
U4619-4	47/;C-1C	13	60	3.60:1	0.0392
U4619-5	49/;C-1C	12	61	4.08:1	1.0958
U4619 (all)	137/;C-1C	40	177	3.41:1	0.5834

† The seedling infection types are: 0 = no uredinia or other microscopic sign of infection, ; = no uredinia but small hypersensitive necrotic or chlorotic flecks present, 1 = small uredinia surrounded by necrosis, 2 = small to medium uredinia surrounded by necrosis or chlorosis, 3 = medium uredinia with or without chlorosis, 4 = large uredinia without chlorosis, C = more chlorosis than normal for the infection type.

minimum population of 16 for a confidence level of 99%. Selected F_3 lines were screened with *P. triticina* isolate PMNQ (avirulence/virulence formula 2a, 11, 16, 17, 26/1, 2c, 3a, 3ka, 10m 18m 24, 30). Reaction types were scored according to Roelfs et al. (1992).

Molecular Analysis

Molecular analysis was conducted on DNA extracted from F_2 plants. At the 4- to 8-leaf stage, young leaf tissue was collected in 1.5 mL polypropylene tubes, ground to powder in liquid nitrogen, and stored at −80°C. From the F_2 tissue, total genomic DNA was isolated according to the method of Riede and Anderson (1996). Concentration checks were performed with a 40:1 dilution of the concentrated DNA on a 0.8% GenePure agrose gel (GeneMate, Kaysville, UT, U.S.A). All gels were stained with ethidium bromide, visualized with a UV light, and photographed.

PCR reactions were conducted using a 25-µl cocktail of 2.5-µl 10X buffer, 2.5-µl $MgCl_2$, 2.5-µl dNTPs, 1.25-µl L&R primer, 0.2-µl Taq, 11.05-µl H_2O, and 5.0-µl DNA (10 ng/µl). The reactions were run on a MJ thermocycler (Watertown, Mass., U.S.A.) with the following cycles: 94°C for 3 min., 94°C for 1 min., 55°C (or proper annealing temp.) for 1 min., go to cycle 2 for 35X, 72°C for 10 min., and 4°C forever (indefinitely).

Whole genome marker screening was performed using bulk segregant analysis (Michelmore et al., 1991). The bulks were composed of 10 homozygous resistant F_2 plants and 13 homozygous susceptible F_2 plants in the U4619 population and 4 homozygous resistant and 7 homozygous susceptible F_2 plants in the U4616 population. Linkage analysis on the U4619 population was done using molecular markers *Xgwm296*, *Xgwm261*, *Xgwm455*, and *Xgdm35*. Bulk segregant and linkage marker selection was based on Röder et al. (1998) and Pestsova et al. (2000) microsatellite map of wheat. The 1DS, 2DS, and 3DS portions of the genome were screened first for both populations. Amplified DNA fragments were run on 2.3% GenePure agrose gels (GeneMate, Kaysville, UT, U.S.A) at 57V for 4–5 hours or 3% agrose gels at 60V for 6–8 hours.

Linkage maps were constructed by converting recombination frequencies to map units (cM) using MAPMAKER version 3.0 at LOD > 3.0 (Lander et al., 1987) and the Kosambi mapping function (Kosambi, 1944). Markers not meeting an LOD > 3.0 were placed as best fit using the MAPMAKER "try" command.

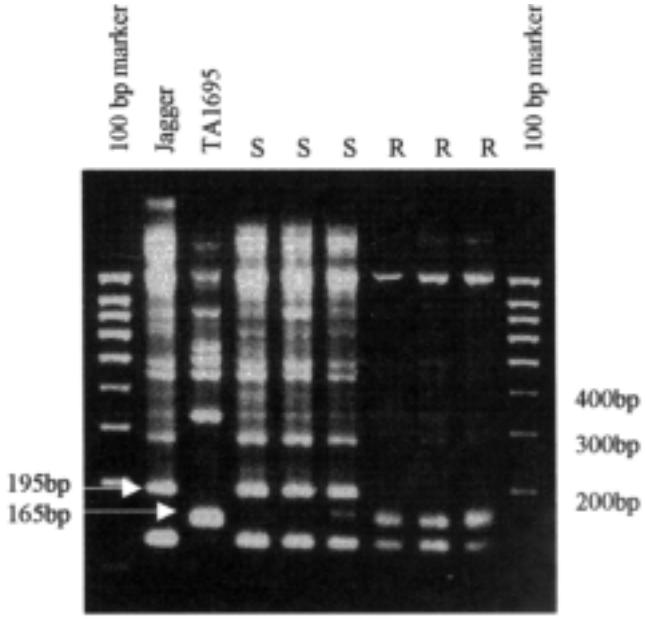

Fig. 1. Gel electrophoresis pattern of microsatellite *Xgwm455*. Shown are the leaf rust susceptible recurrent parent (Jagger) and the leaf rust gene donor, TA1695. Also shown are three susceptible derivatives (S) without a leaf rust resistance gene and three resistant derivatives (R) that are carrying a leaf rust resistance gene. The outside lanes are a 100bp ladder.

RESULTS

Phenotypic Screening

Leaf rust inoculations and reaction scoring indicated a segregation ratio of 3:1 for each of the F_2 populations (Table 1). When scoring the F_2 rust reactions with MCDL race, a difference in infection type was noted between the U4616 population and the three U4619 populations. Plants of the U4616 population had only a small chlorotic fleck and little if any pustule formation, indicating a strong hypersensitive reaction. Plants of the U4619 population had reaction types from ;C to 1C (Table 1) and consistently had slight to moderate sporulation surrounded by necrotic tissue. The U4619 hypersensitive reaction eventually killed the rust pathogen but was slow enough that a moderate amount of tissue was killed before the spread was stopped. Reaction types and the number of plants segregating into each class are shown in Table 1. Screening the two populations with race PMNQ of *P. triticina* resulted in a susceptible reaction (absence of any hypersensitivity) in the U4619 population and a resistant reaction that segregated in the U4616 population. The U4616 resistant reaction was an intermediate reaction of 2C wtih small uredinia.

Molecular Mapping

Because of possible differences in leaf rust genes between the U4616 and U4619 populations, two separate bulks were made for marker screening. Three markers, *Xgwm296*, *Xgwm261*, and *Xgwm455*, were polymorphic between the U4619 bulks but not in the U4616 bulks.

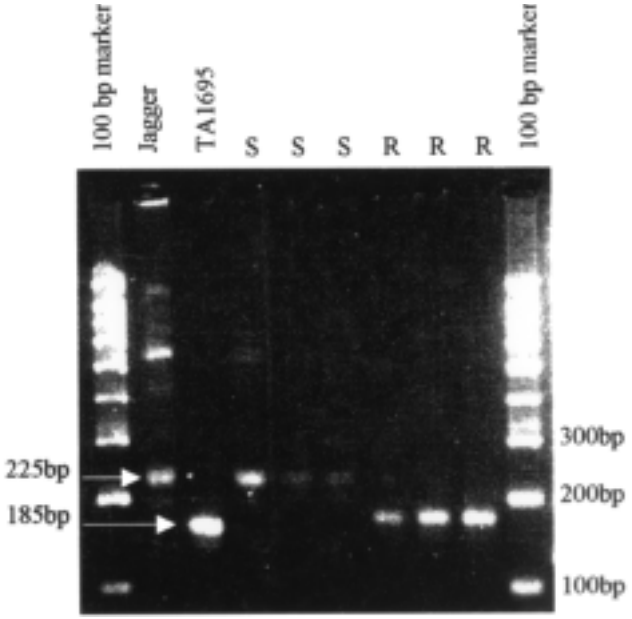

Fig. 2. Gel electrophoresis pattern of microsatellite *Xgwm35*. Shown are the leaf rust susceptible recurrent parent (Jagger) and the leaf rust gene donor, TA1695. Also shown are three susceptible derivatives (S) without a leaf rust resistance gene and three resistant derivatives (R) that are carrying a leaf rust resistance gene. The outside lanes are a 100bp ladder.

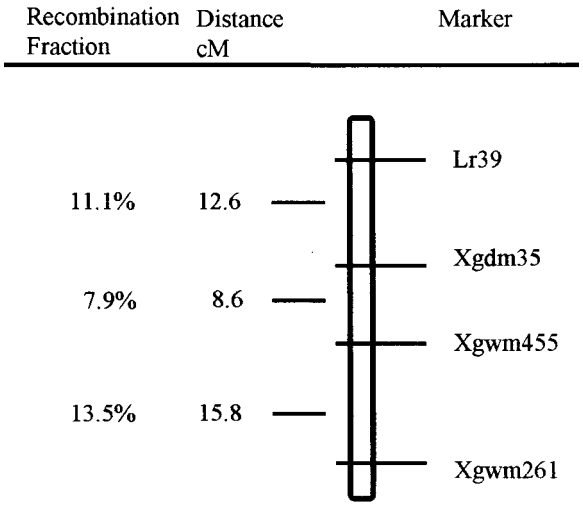

Fig. 3. The position of the leaf rust gene in the U4619R genome on the genetic linkage map of the distal portion of wheat chromosome 2DS.

Microsatellite primer pair *Xgwm261* was located at 37.0 cM and *Xgwm455* was 21.4 cM from the leaf rust gene in the U4619 population. The amplified bands for marker *Xgwm455* were 195bp and 165bp for Jagger and TA1695, respectively (Fig. 1). The marker *Xgwm296* was not used in the map construction because of a difficulty in scoring heterozygous genotypes. Once the location of the leaf rust gene was know to be on the short arm of homologous group 2 chromosomes, several other primer pairs specific to 2DS were run on the bulks. Primer pair *Xgdm35* was located 12.6 cM from the leaf rust gene in the U4619 population. Using this primer pair, *Xgdm35*, Jagger and TA1695 had amplified fragments of 225bp and 185bp, respectively (Fig. 2). The completed genetic linkage map of microsatellite markers *Xgwm261*, *Xgwm455*, *Xgdm35*, and the leaf rust gene in the U4619 population is shown in Figure 3.

None of the markers evaluated in this experiment were linked with the gene segregating in the U4616 population. These markers include: on 1DS: *Xgwm33*, *Xgwm106*, *Xgwm337*; on 2DS: *Xgwm210*, *Xgwm455*, *Xgwm296*, *Xgwm261*, *Xgwm515*, *Xgwm249*, *Xgwm30*; on 3DS: *Xgwm114*, *Xgwm71*, *Xgwm161*, *Xgwm183*, *Xgwm456*, *Xgwm497*, *Xgwm341*; on 7DS: *Xgwm350*.

DISCUSSION

Based on phenotypic reactions and genetic mapping, the leaf rust gene in the U4619 population is believed to be *Lr39*. Using physical and genetic mapping, the linked microsatellite markers *Xgwm296*, *Xgwm261*, and *Xgwm455* have been placed on the distal portion of the short arm of chromosome 2D (Röder et al., 1998). In a different population, *Lr39* and *Xgdm35* are closely linked with only one recombinant in over 100 plants (Sukhwinder-Singh, personal communication, 2002). While the U4619 population did have a much higher recombination fraction of 11.1% between the leaf rust gene and the microsatellite marker *Xgdm35*, the gene location can be accurately place on the distal portion of chromosome 2DS. This corresponds to the placement of *Lr39* in previous research (Raupp et al., 2001). Also, according to Raupp et al. (2001), the *Lr39* resistance reaction shows small uredinia surrounded by chlorosis when inoculated with MCDL and large uredinia without chlorosis when inoculated with PMNQ. The same reaction types were noted in this experiment. This research indicates that accession TA1695 of *Ae. tauschii*, in addition to accession TA1675 (Raupp et al., 2001), is a source of *Lr39*.

The phenotypic reactions and completed molecular analysis strongly indicate that line U4616 contains a single, dominant leaf rust gene that is different from *Lr39*. The presence of a single dominant gene is supported by the F_2 segregating ratio of 2.8:1. Support for the leaf rust gene in this population being different from *Lr39* is the contrast in resistance reactions; particularly in the screening with race PMNQ. The difference in genotypes was also supported by molecular analysis that indicated the leaf rust gene in U4616 is not linked with *Xgwm455* or *Xgdm35*. Based on the strong hypersensitive reaction of this leaf rust gene, it could be a valuable source of resistance for breeding resistant varieties.

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