Chromosome 6U from *Aegilops geniculata* Roth Carrying Powdery Mildew Resistance in Bread Wheat

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The production and isolation of a wheat—*Aegilops geniculata* disomic addition line and of monosomic substitution line resistant to powdery mildew at the seedling and adult plant stages are described. Cytological analysis revealed the presence of an acrocentric chromosome in the resistant derivatives. Polyacrylamide gel electrophoretic analysis indicated differences in the β -zone of the reduced by mercaptoethanol treatment seed protein pattern between the wheat parent cv. Trakya and the resistant derivatives. A component with a high staining intensity in the β -zone was assumed to be a marker for the chromosome 6 of *Ae. geniculata*. The isoenzyme activity at the Got-1 and Est-4 loci of the lines was lower than that of wheat. By analyzing the isoenzymes of the Amp-1 locus of the addition plants, a quantitative marker of *Ae. geniculata* 6U chromosome was identified. The progeny of the plants with the $2n=43$ and $2n=42$ chromosomes carrying the chromosome 6U added or mono-substituted for the wheat 6D chromosome segregated for powdery mildew resistance. The mono-and/or double conditions for this chromosome could be maintained by selecting resistant plants in the segregated populations.

Key Words: *Aegilops geniculata*, wheat, powdery mildew resistance, isoenzymes, chromosome 6U.

Introduction

Powdery mildew is a destructive foliar disease caused by a biotrophic parasitic fungus *Blumeria graminis* f. sp. *tritici* (syn. *Erysiphe graminis* f. sp. *tritici*) (Speer 1973). Although chemicals are effective against this disease, the use of host resistance has remained an economical and environmental safe device of control in practice.

Genes for powdery mildew resistance, i.e. *Pm* genes have been described at 33 loci in common wheat (Liu *et al.* 2002, Singrun *et al.* 2004, Zhu *et al.* 2005). Some of them have been utilized in commercial wheat varieties, but were soon overcome by new pathogen races. Strategies including cultivar mixing, diversification or gene pyramiding may increase the resistance to powdery mildew (Priestley and Bayles 1998, Hsam and Zeller 2002).

The genus *Aegilops* comprises 22 species, most of which are potential donors of resistance to foliar diseases (Van Stageren 1994, Valkoun and Bedo 2001, Zaharieva *et al.* 2003). One of them is *Aegilops geniculata* Roth (syn. *Ae. ovata* L.), a wild allotetraploid species ($2n=28$, UUMM). The species can be crossed to common wheat, and various derivatives such as addition, substitution and translocation lines have been obtained (Metten *et al.* 1977, Friebe and Heun 1989, Friebe *et al.* 1996, McIntosh *et al.* 2001). Up to

now, only the *Pm29* gene from *Ae. geniculata* had been transferred to common wheat (Zeller *et al.* 2002). However, the resistance could not be traced back to a specific chromosome, because of the high level of structural variation in the chromosome complements of the A and B genomes of the addition lines (Friebe and Heun 1989).

Identification of alien introgressions has been greatly facilitated by the use of seed storage protein patterns and isoenzymes as markers of alien genes in the wheat background and for determining the homoeology of the added or substituted alien chromosomes. Gliadin components and glutenin subunits were used as markers for powdery mildew or rust resistance due to putative introgressions from *Ae. turcomanica* (Vahl *et al.* 1999), *Ae. umbellulata* (Stoilova *et al.* 1999, Ozgen *et al.* 2004), *Ae. tauschii* (Pfluger *et al.* 2001), etc. Using isoenzymes as markers of nearly all the homoeologous chromosomes in wheat, Schmidt *et al.* (1993) and William and Mujeeb-Kazi (1996) developed biochemical diagnostics for *Ae. markgrafii* and *Ae. variabilis* chromosomes in the addition lines or amphiploids. Alcohol dehydrogenase (ADH), Est-5 and Got-3 isoenzymes encoded by genes of the homoeologous groups 4 and 3 chromosomes were found to be suitable markers for alien introgression from *Ae. ventricosa* and *Ae. geniculata* (Mena *et al.* 1989, Stoilova 2002). Finally the gliadins and many enzyme loci have been classified by their variability for possible location on the genetic map of wheat and for use in alien-wheat comparisons (Gale and Sharp 1988).

In the present study, we produced and characterized

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derivatives resistant to powdery mildew in a cross between *Triticum aestivum* cv. Trakya and *Ae. geniculata*. The chromosome morphology, protein patterns and Amp-1, Got-1 and Est-4 isoenzymes used as markers for the homoeologous group of chromosome 6 enabled to demonstrate that the alien acrocentric chromosome was a carrier for powdery mildew resistance in wheat.

Materials and Methods

Plant materials

Aegilops geniculata (2n=28, UUMM) accession 2233 from the collection of the Dobroudja Agricultural Institute – General Toshevo, Bulgaria, was used as a source of resistance to powdery mildew (*Pm*). The species was crossed as a male parent to *T. aestivum* cv. Trakya, a winter type cultivar registered in Bulgaria in 1977 and considered to be susceptible to powdery mildew (Petrova *et al.* 2000). First, the amphiploid (AD) was obtained by colchicine treatment of the F₁ hybrid. Backcross 1 (BC₁) plants were produced using the amphiploid as pistillate parent and Trakya as pollen parent. Two BC₁ plants expressing resistance to powdery mildew at the seedling and adult plant stages were emasculated and pollinated with cv. Trakya to produce BC₂ seeds. These backcross seeds were germinated and seedlings with 2n=43–46 chromosomes were transplanted and further evaluated for *Pm* resistance. Selfing of plants with 2n=44 chromosomes resulted in the production of a large number of BC₂F₂ plants, enabling the selection of individuals with resistance to powdery mildew. The following nullisomic-tetrasomic lines of the wheat cultivar Chinese Spring (CS) were used as subsidiary genetic systems: CSN6A/T6B, CSN6A/T6D, CSN6B/T6D, CSN6D/T6A, CSN6D/T6B and the addition line CS6U (addition of *Ae. umbellulata* chromosome 6U). These stocks were originally supplied by Dr. E. R. Sears and then maintained at the Institute of Genetics, Sofia, Bulgaria.

For chromosome identification, root tips of the seedlings were pretreated with a 1-bromonaphthalene solution and stained by the conventional Feulgen method.

Powdery mildew resistance

The resistance to powdery mildew caused by *Blumeria graminis* f. sp. *tritici* was screened at the seedling and adult plant stages. Plants at the two-three leaf stages were evaluated in the greenhouse in the natural infection background. Common wheat variety Sadovska ranozreika-4 as pathogen spreader and standard for high susceptibility to powdery mildew, was placed at several sites in the greenhouse. Mildew attack was recorded twice, once at the 2-leaf stage, and once at the 3-leaf stage. The ratings were performed using a scale from 0 (immune) to 9 (susceptible) (Tomerlin *et al.* 1984). Disease ratings of the plants were grouped into two classes- R (resistant) (0–6), and S (susceptible) (7–9).

The resistance at the adult plant stage was evaluated in a greenhouse under high natural disease epiphytotic conditions. The infection type (IT, 0–4) and the disease infection

severity (DIS) were estimated according to the method of Stakman *et al.* (1962). DIS was given in percentage from 0 to 100, where 0 corresponds to the lack of infection, and 100 to the maximum degree of infection. Disease was assessed as a visual estimate of the percentage of the leaf area covered by colonies of *Blumeria graminis* f. sp. *tritici* (*Bgt*). The leaf areas analysed involved the top three leaves of adult plants at 10-day intervals between growth stages 51 and 61. The plants subjected to the evaluation were grouped into resistant (R) with IT=0–2 and DIS=0–50, and susceptible (S) with IT=3–4 and DIS=60–100.

Biochemical methods

The proteins from wheat seeds were extracted and separated by polyacrylamide gel electrophoresis (PAGE) at pH 3.2 according to the method of Cooper (1987). The extraction medium consisted of pyronine G (0.05%) in 2-chloroethanol (20%) (w/v) containing urea (18%) and 2-mercaptoethanol (1%). The reduced by the 2-mercaptoethanol proteins were fractionated in 10% PAGE, using the discontinuous system of Laemmli (1970). Gels were stained with 0.03% (w/v) Coomassie Brilliant Blue R 250 in a methanol and acetic acid mixture (5:1) (v/v).

The enzyme systems aminopeptidase-1 (Amp-1), glutamate oxaloacetate transaminase-1 (Got-1) and esterase-4 (Est-4) were analyzed. Extracts for the analysis of the GOT and EST enzymes were prepared by grinding seeds at 4°C with 0.05 M TRIS-HCL buffer pH 7. 2 containing supplements—6 mM ascorbic acid, 6 mM cystein hydrochloride and 0.5 M sucrose (Rychter and Lewak 1969). The homogenates were centrifuged at 10,000 g for 20 min. The clear supernatants were analyzed by vertical PAGE using a 10% gel and discontinuous buffer system of TRIS-EDTA-boric acid, pH 8. 3 (Peacock *et al.* 1965). After electrophoresis, the gels were stained for GOT and α -naphthyl esterases by standard histochemical methods (Scandalios 1964, Shaw and Prasad 1970). The extracts for the analysis of aminopeptidase-1 (Amp-1) were prepared by grinding the seeds with 10 mM dithiothreitol (DTT) in distilled water at room temperature for 1 h (Koebner and Martin 1989). For the visualization of the aminopeptidase isoenzymes, 0.5 mg/ml N α -benzoyl-DL-arginine- β -naphthylamide (BANA) was used as a substrate. The investigation was performed using a Biotech Hoefer SE 600 apparatus (Pharmacia, Uppsala, Sweden) and an electrophoretic power source (EPS) 600. The gel plate with 15 wells measured 180/160/1.5 mm.

For biochemical studies of cv. Trakya and *Ae. geniculata*, average seed samples were used. Individual kernels derived from selfed BC₂F₂ plants with 2n=44 chromosomes corresponded to the plants referred to as addition plants and seeds derived from selfed BC₂F₂ plants with 2n=42 and 43 chromosomes and the presence of the *ac* chromosome corresponded to the plants referred to as substitution plants. The chemicals were supplied by Chemapol (Prague, Czech Republic) and Serva (Heidelberg, Germany).

Results

In total 54 BC₁ plants were investigated and two plants expressing the resistance were selected and backcrossed. The two selected BC₂ plants (2n=44) were also resistant to powdery mildew at the seedling and adult plant stages, compared with the wheat parent (Table 1) and all the plants in their selfed progeny were resistant to powdery mildew. Disomic addition plants accounted for about 65% of the progeny (data not shown). Comparison of the karyotypes revealed that the acrocentric chromosome (*ac*) in the resistant derivatives was similar to the *ac* chromosome in the *Ae. geniculata* parent and the F₁ hybrid (Trakya × *Ae. geniculata*) (Fig. 1, Fig. 2 and Fig. 3). Wheat satellite chromosomes 1B and 6B were also visualized and distinguished from the alien 1U and 5U satellite chromosomes, as well as from the *ac* chromosome in the hybrid (Fig. 2). The cytological analysis revealed the presence of an acrocentric pair of chromosomes in all the checked plants with 2n=44 chromosomes. This chromosome may be a carrier of resistance genes to powdery mildew. Plants with 2n=42 and 43 chromosomes were isolated to produce a disomic substitution for the alien chromosome. Their progeny segregated for the *Pm* resistance (Table 1). Individuals with the acrocentric chromosome under the double or monosomic condition expressed always *Pm* resistance, irrespective of their chromosome complement.

Mitrophanova (1976) demonstrated that the gliadin components from the β- and α-zones in the cv. Chinese Spring are controlled by chromosomes of the first and sixth homoeologous groups. Almost all the components of the β-zone are under the control of two or three chromosomes of the wheat genome. By analyzing the phenotypes of the CSN6D/T6A, CSN6D/T6B lines and cv. CS, we observed that the slowest moving component from the β-zone of the euploid form (arrowhead) was controlled by chromosome 6D (Fig. 4, tracks 1–6). The wild species *Ae. geniculata* (track 8) displayed a clear banding pattern. A component specific for *Ae. geniculata* with a high staining intensity (arrowhead in the figure) was detected in both, the amphiploid

and the addition (tracks 10–12) and mono-substitution (tracks 13–15) plants. Compared with the pattern of cv. Trakya, the components of the β-zone of the addition plants (tracks 10–12) were more prominent.

Based on the electrophoretic pattern of the isoenzymes at the Got-1 locus, the cv. Trakya and *Ae. geniculata* differed in the number of isoenzymes, four and two, respectively (Fig. 5, tracks 2, 3, 9, 10). From the quantitative viewpoint, the Got-1 pattern in the addition (tracks 5–8) and substitution plants (tracks 11–13) was similar to that of wheat, but the activity was lower. The isoenzyme nearest to the anode showed a fuzzy pattern in the resistant addition and mono-substitution plants (arrow in the figure), while in the cv. Trakya the pattern was clear.

Based on the banding pattern of the Est-4 locus, three isoenzymes were recognized in cv. Trakya (Fig. 6, track 5). In the pattern of *Ae. geniculata*, two isoenzymes with a low activity which corresponded to their homoeologous in the wheat, were visualized. The phenotype of the addition (tracks 1–4) and mono-substitution (tracks 7–10) plants was similar to that of wheat at the Est-4 locus, although three among the isoenzymes displayed a lower activity (arrowhead in the figure).

At the Amp-1 locus in the CS6U addition line (Fig. 7,

Fig. 1. Chromosome complement of *Ae. geniculata* accession 2233 (2n=28): arrows point to the acrocentric 6U and satellite 1U and 5U pairs of chromosomes.

Table 1. Incidence of powdery mildew in parents and derivatives from the cross *T.aestivum* × *Ae.geniculata* under greenhouse conditions

Genotypes	Line no.	2n	Parentage	Powdery mildew reaction		Chromosome number and powdery mildew reaction in selfed progeny		
				Seedling ¹⁾	Adult plant ²⁾	2n	Seedling	Adult plant
Parent	–	42	<i>Triticum aestivum</i> cv. Trakya	S	S	42	S	S
Parent	–	28	<i>Aegilops geniculata</i> accession 2233	R	R	28	R	R
Amphidiploid	AD	70	C ₁ Trakya × <i>Ae.geniculata</i>	R	R	68–70	R	R
Disomic addition	12-4	44	BC ₂ Trakya × <i>Ae.geniculata</i>	R	R	42–44	R	R
Disomic addition	12-5	44	BC ₂ Trakya × <i>Ae.geniculata</i>	R	R	43–44	R	R
Mono-substitution	12-z	43	BC ₂ F ₂ Trakya × <i>Ae.geniculata</i>	R	R	42–44	S, R	S, R
Mono-substitution	2-4	42	BC ₂ F ₂ Trakya × <i>Ae.geniculata</i>	R	R	41–42	S, R	S, R

¹⁾ R=resistant (ratings 0–6), S=susceptible (ratings 7–9);

²⁾ Infection type (IT) and disease infection severity (DIS) were estimated for powdery mildew resistance of the adult plant stage: R=resistant (IT=0–2, DIS=0–50); S=susceptible (IT=3–4, DIS=60–100); Susceptible standard cv. Sadvoska ranozreika-4 showed S type of reaction to powdery mildew at both stages.

Fig. 2. Chromosome complement of F_1 (Trakya $\times$ *Ae. geniculata*): $2n = 35$; arrows point to the wheat and alien satellite and acrocentric 6U chromosomes.

Fig. 3. Chromosome complement of a disomic addition line (Trakya $\times$ *Ae. geniculata*): $2n = 44$; arrows point to the acrocentric 6U chromosome pair derived from *Ae. geniculata*.

track 2), an isoenzyme with a high activity was visualized, compared with the very low activity in CS. It is, therefore, suggested that the isoenzyme examined in the CS6U line is under the control of chromosome 6U and could be used as a marker. The patterns of cv. Trakya and *Ae. geniculata* revealed a clear specificity (tracks 3, 4). The band showing a high staining intensity in CS 6U was detected in the two parents, although its intensity in *Ae. geniculata* was considerably high. The isoenzyme activity of the addition plants (tracks 5, 6) was as high as that of the alien species, while the activity of the substitution plants (tracks 7, 8) did not differ from that of wheat.

Discussion

The few *Pm* genes that have been transferred to a hexaploid cultivar, could be relatively exploited in breeding programs. However, transfer of the *Pm* resistance gene, from species with the U and M genomes to the AABBDD genetic

Fig. 4. Reduced by mercaptoethanol seed protein pattern of wheat lines with the addition and mono-substitution of chromosome 6 of *Ae. geniculata* (8): addition plants (10–12); substitution plants (13–15); cv. Trakya (7); cv. CS (6); AD (9). Subsidiary genetic systems: CSN6A/T6B (1); CSN6A/T6D (2); CSN6B/T6D (3); CSN6D/T6A (4); CSN6D/T6B (5); arrowheads point to the specific component for *Ae. geniculata* (8) detected in the resistant addition and substitution plants.

Fig. 5. Glutamate oxaloacetate transaminase (GOT-1) seed isoenzyme pattern of wheat lines with the addition and mono-substitution of chromosome 6 of *Ae. geniculata* (3, 10); cv. Trakya (2, 9); AD (4); cv. CS (1); arrowheads point to the isoenzymes with a fuzzy pattern in the addition (5–8) and substitution (11–14) plants due to the influence of chromosome 6 of *Ae. geniculata* (3).

background of wheat has been found to be very difficult (Zeller *et al.* 2002). The *ac* chromosome in the resistant lines seemed to be that of the alien species used. Based on its unique morphology, this chromosome could correspond to chromosome 6U of *Ae. geniculata*. Friebe *et al.* (1995) showed that the U genome chromosomes of *Ae. geniculata* were similar in size, arm ratio and C-banding pattern, compared with those of the diploid progenitor species *Ae. umbellulata*. These similarities were used to assign the U genome chromosomes in the CS addition lines to the various homoeologous groups of wheat. Moreover, the 6U chromosome of *Ae. umbellulata* was extremely acrocentric, with a long-to-short arm ratio of 5:1 (Friebe *et al.* 1995). Furthermore, the lack of aberrations in the *Ae. geniculata* chromosomes during the isolation of the addition lines was emphasized (Friebe *et al.* 1999). During the investigation, we observed

Fig. 6. Esterase (Est-4) seed isoenzyme pattern of wheat lines with the addition and mono-substitution of chromosome 6 of *Ae. geniculata* (6); cv. Trakya (5); arrowheads point to the lower isoenzyme activity in the addition (1–4) and substitution plants (7–10) due to the influence of chromosome 6 of *Ae. geniculata* (6).

Fig. 7. Seed aminopeptidase (Amp-1) isoenzyme pattern in wheat lines with the addition and mono-substitution of 6U chromosome of *Ae. geniculata* (4, 9); cv. Trakya (3, 11); addition plants (5–6); substitution plants (7–8); cv. CS (1); addition line CS6U (2, 10); The higher activity of the isoenzyme (arrowheads) is due to the presence of chromosome 6U of *Ae. geniculata* (4).

that the *ac* chromosome pair was present only in the plants with $2n=44$ chromosomes, and in a few cases, in the genotypes with $2n=43$ and 42 chromosomes. The experimental data supported the statement of Friebe *et al.* (1999) that the 6U^s chromosome was only present under monosomic conditions. Thus, we suggest that the *ac* chromosome added to and mono-substituted in the cv. Trakya background corresponds to the 6U chromosome transferred from *Ae. geniculata*. In the mono-substitution plants, the two satellite chromosomes 1B and 6B were easily recognized. Thus, chromo-

somes 6A or 6D may have possibly been substituted for the alien *ac* chromosome. The plants with $2n=43$ and 42 chromosomes that carry the *ac* chromosome 6U under monosomic conditions segregated for *Pm* resistance. The mono-and/or double conditions for this chromosome could be maintained in segregated populations by selecting the resistant plants. The plants with $2n=44$ and 43 chromosomes showed an identical protein phenotype, which indicated that one and the same chromosome was added or substituted. The higher staining intensity of the slowest moving β -component, controlled only by chromosome 6D might indicate that the chromosome that had been added to or mono-substituted in the plants was a chromosome belonging the group 6 of *Ae. geniculata*. Therefore, we considered that the β -component specific for *Ae. geniculata* that was observed in all the resistant lines could be used as a marker for alien chromosome introgression. Previously, it had been demonstrated that the Got-1 and Est-4 isoenzymes were biochemical markers of the sixth homoeologous group of chromosomes in wheat (Hart 1975, Ainsworth *et al.* 1984). The lower activity of the Got-1 isoenzymes, especially the fastest moving one, and Est-4 isoenzymes in the addition and mono-substitution plants was probably due to the influence of chromosome 6 of *Ae. geniculata*. Using nulli-tetrasomic lines of the cv. CS, Koebner and Martin (1989) determined that the 6D chromosome of wheat was responsible for the genetic control of the second of the three Amp-1 isoenzymes. The higher activity of the second Amp-1 isoenzyme in the CS6U line compared with that of the euploid CS cultivar supported the assumption that the 6U chromosome of *Ae. geniculata* was involved in the genetic control. By analogy, the activity of the homoeologous isoenzyme of *Ae. geniculata* in the addition plants indicated the presence of chromosome 6U. This conclusion was in agreement with the cytological results showing that the *ac* chromosome in the derivatives might correspond to the 6U chromosome from *Ae. geniculata*. It appeared that the Amp-1 isoenzyme encoded by the 6D wheat chromosome was homoeoallelic to the 6U chromosome of *Ae. geniculata*. The nearly identical phenotype of the mono-substitution plants with that of wheat may be ascribed to the fact that the alien 6U chromosome did not genetically compensate for the 6D wheat chromosome or that the investigated plants lacked the alien chromosome as a result of segregation. The results for the reduced seed proteins showed the presence of chromosome 6 of *Ae. geniculata* in the mono-substitution plants and the influence of the same chromosome on the isoenzyme activity of the Got-1 and Est-4 loci. Therefore, we considered that the first assumption could account for the Amp-1 phenotype of the mono-substitution plants.

In summary, the results from the cytological and biochemical investigations suggested that the 6U chromosome of *Ae. geniculata* was added and mono-substituted for the wheat 6D chromosome in the resistant derivatives and may be a source of resistance to powdery mildew.

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