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The incorporation and characterization of powdery mildew resistance from *Aegilops longissima* in common wheat (*T. aestivum* L.)

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Summary. In the progeny of a hybrid between monotelosomic line 3B of 'Chinese Spring' wheat and 'Chinese Spring' – *Aegilops longissima* ditelosomic addition line 'G' a cytologically stable strain was selected consisting of 20 wheat chromosome pairs, one pair of telosomic chromosome 3BL and one pair of telosomic longissima chromosome 'G'. Inoculating 'Chinese Spring' – *Aegilops longissima* addition and substitution lines with ten different powdery mildew isolates, partial resistance was observed. The infection grade as well as the number of spores/cm^2 leaf area were significantly reduced.

Key words: Mildew resistance – *Aegilops longissima* – Substitution line – Wheat breeding

Introduction

Many wild species related to *Triticum* possess agriculturally beneficial genes useful in wheat breeding. The incorporation of such genes can be achieved by exploiting homoeologous recombination between wheat and the alien chromosome which carries the useful genetic variation. Several procedures are available for incorporating those variations, including 1) backcrossing of wheat and wheat-alien hybrids deficient for chromosome 5B which suppresses homoeologous chromosome pairing; 2) using *A. speltoides* to prevent activities of the *Ph* locus on chromosome 5B (Riley and Chapman 1964); 3) backcrossing F$_1$ hybrids to a *Ph-1* mutant in which the 5B suppressor gene of homoeologous pairing has been deleted (Sears 1977) or 4) transferring segments of alien chromosomes to wheat chromosomes by mutagenic treatment (Driscoll 1968; Knott 1983). Genes from related species could also be transferred into wheat by the establishment of lines with single pairs of alien chromosomes possessing desirable genes, either added to the full wheat genome, or substituted for individual wheat chromosome pairs. The purpose of this paper is to describe the transfer of powdery mildew resistance from *Aegilops longissima* ($2n = 14$) into common wheat via aneuploid manipulation, and the characterization of this resistance.

Material and methods

The monotelosomic ($20'' + t'$), ditelosomic ($20'' + t''$) and double ditelosomic ($20'' + t_S'' + t_L''$) 3B lines of wheat cv 'Chinese Spring' (CS) employed were selected by E. R. Sears, Columbia, Mo, USA. The CS – *A. longissima* disomic addition line 'G' ($21'' + G''$) resistant to powdery mildew was provided by M. Feldman, Rehovot, Israel. The donor of the alien chromosome was *A. longissima* line No. 7011 collected from the Negev desert (Israel).

In 1984 725 single pustule isolates of *Erysiphe graminis* f. sp. *tritici* were collected within the southern part of the Federal Republic of Germany. They were characterized by their reaction on the primary leaves of near-isogenic lines of cv 'Chancellor' (provided by J. G. Moseman, Beltsville, Md, USA) possessing different resistance genes, i.e. *Pm*1, 2, 3a–c, 4a–b and 6, and some cultivars with further resistance genes, i.e. *Pm*5, 7, 8, and 9 (alone or in combination). The most distinctive mildew isolates were maintained; ten of these were used for analysing the resistance of a CS – *A. longissima* addition and a substitution line in relation to CS.

Meiosis was studied in Orcein-Feulgen stained squashes of pollen mother cells (PMCs) from anthers fixed in acetic-alcohol. Chromosome counts were made from squashes of root-tip cells pretreated with mono-bromonaphthalene and stained by the Feulgen technique.

Three primary leaf segments (each 3 cm long) of each of the above mentioned genotypes were placed in dishes of agar containing 50 ppm benzimidazole. For inoculation spores formed on infected leaves of cv 'Caribo' were dispersed using a settling tower (Aslam and Schwarzbach 1980) giving an inoculation density of 180–250 spores/cm^2 (checked simultaneously on exposed slides). The whole experiment (10 inoculations with different isolates) was repeated three times.

The following assessments were done when disease symptoms on the susceptible control cv 'Caribo' were well-developed: infection type (Nover 1972), infection grade (modified following Horsfall and Barratt 1945) and number of formed spores/cm^2 leaf area counted under microscope using a hemocytometer. The latter two traits were used for the analysis of variance: two-factorial experiment repeated three times. The multiple comparisons of means were done by Tukey-tests (Weber 1980; Steel and Torrie 1960).

Results

In the progeny of disomic CS – *A. longissima* addition line 'G' ($21'' + G''$) one plant was extracted containing a telosomic chromosome in addition to the wheat complement ($21'' + t_G'$). This telosome turned out to be a complete arm of chromosome 'G' of *A. longissima.* Several lines possessing two telosomes in addition to the complete wheat genome were selected from selfed plants. Ditelosomic ($21'' + t_G''$) and disomic addition ($21'' + G''$) plants with an arm or complete chromosome 'G' of *A. longissima* have a brittle rachis.

Since Chapmam (personal communication) has observed brittle rachis in 'Holdfast' wheat – *A. bicornis* substitution lines $3S^b(3A)$ and $3S^b(3B)$ it has been assumed that *A. longissima* chromosome 'G' is homoeologous to wheat group 3. Therefore, monotelosomic 3BL lines of CS were crossed with ditelosomic addition plants. F_1 hybrids ($20'' + 1' + t' + t_G'$) possessing one wheat and one alien telosome were selfed. One plant was found containing 20 chromosome pairs plus two telosomic univalents ($20'' + t' + t_G'$) in metaphase I of PMCs within the resulting F_2 generation. Several lines were extracted which had $40 + 4t$ in root-tip cells (Fig. 1a) and $20'' + t'' + t_G''$ in meiosis when these plants were selfed. Their rachis was fragile. *A. longissima* plants having 44 chromosomes ($20'' + t'' + t_G''$) were crossed to disomic, ditelosomic 3BS, ditelosomic 3BL

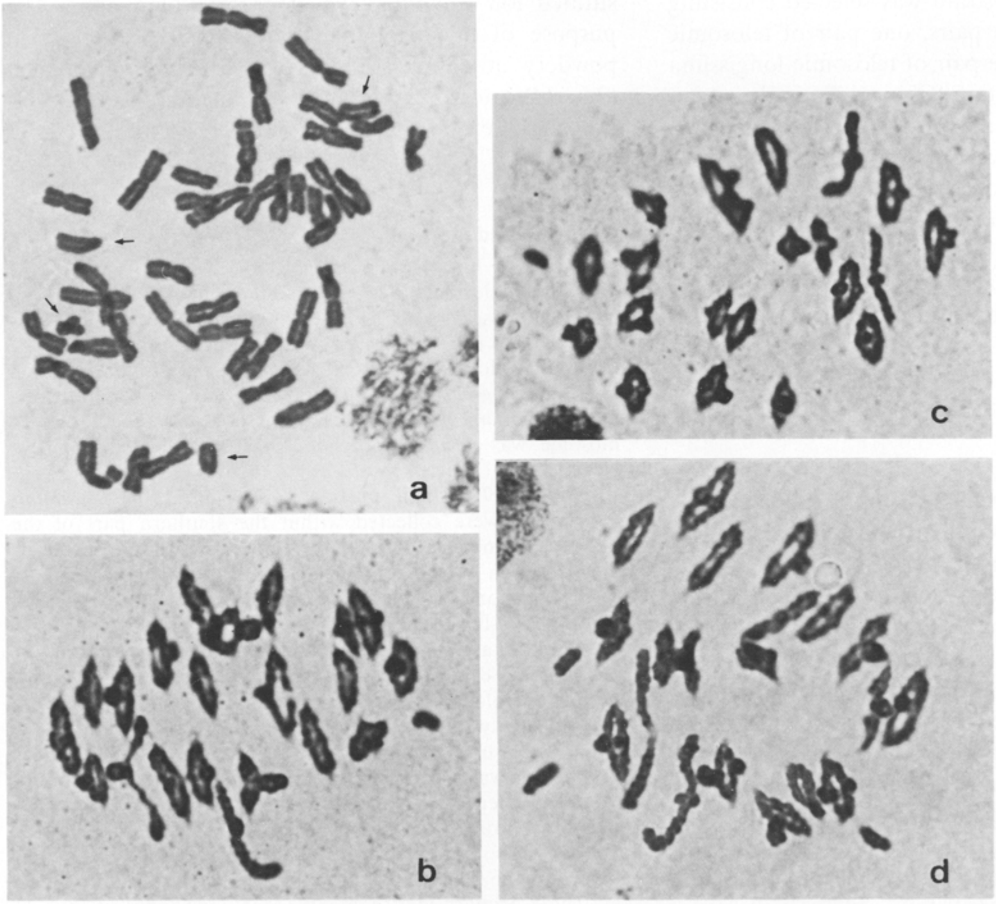

Fig. 1. **a** Root-tip cell of 'Chinese Spring'-*A. longissima* substitution line 'G' with $2n = 40 + 4t$; telocentric chromosomes marked by *arrows;* **b** Metaphase I (MI) in PMC's of F_1 hybrid between disomic 'Chinese Spring' and substitution line 'G' showing $20'' + tl'' + t'$; **c** MI cell of F_1 hybrid between CS DT-3BL (long) and substitution line 'G' with $20'' + t'' + t'$; **d** MI cell of F_1 hybrid between CS DT-3BS (short) and substitution line 'G' with $20'' + t' + t' + t'$

Table 1. Chromosome pairing in hybrids of disomic 'Chinese Spring' (CS), CS double ditelo 3B, CS ditelo lines 3BL (long) and 3BS (short) with CS-*A. longissima* substitution line 'G' ($20'' + t' + t'' + t_G''$)

Line	No. of plants	Chromosome configurations in MI of PMC's			
		$20''$ $+ 1'$ $+ tl''$	$19''$ $+ 3'$ $+ tl''$	$20''$ $+ 2'$ $+ tl'$	$18''$ $+ 5'$ $+ tl''$
CS disomic × substitution 'G'	4	98	6	1	1
		$20''$ $+ t' + t'$ $+ t''$	$20''$ $+ t' + t'$ $+ t' + t'$	$19''$ $+ 1' + 1'$ $+ t' + t'$ $+ t''$	$18'' + 1^{IV}$ $+ t' + t'$ $+ t''$
CS DDT 3B × substitution 'G'	3	64	1	2	1
		$20''$ $+ t'$ $+ t''$	$19''$ $+ 1' + 1'$ $+ t' + t''$	$20''$ $+ t' + t'$ $+ t'$	$19''$ $+ 1' + 1'$ $+ t' + t' + t'$
CS DT-3BL × Substitution 'G'	2	50	1	2	1
CS DT-3BS × Substitution 'G'	2	–	–	74	3

Table 2. Infection grade (1st no.) and infection type (2nd no.) of 13 wheat lines/cultivars possessing different resistance genes after inoculation with ten mildew isolates (mean values of three independent experiments)

Mildew isolate	Host gene/s												
	Pm 1[a]	2[a]	3a[a]	3b[a]	3c[a]	4a[a]	2+6[b]	8[c]	1+2 +9[d]	–[e]	–[f]	t_G[g]	t_G[h]
CA-2	00	00	00	00	00	00	00	54	00	44	44s	14vs	14vs
CA-6	74	00	00	00	00	64	00	74	00	74	74	44	24s
CA-8	74	00	00	00	24s	64s	00	74	00	64	64s	34s	14s
CA-14	34s	00	00	14s	54	34s	00	74	00	74	74	44	34
CA-42	14s	00	00	00	14	14s	00	74	00	64	74	44	24
MH-0	54	64	00	00	44s	64	64	00	00	64	74	34	34s
MH-3	00	64s	00	00	34s	54	00	00	00	64s	54s	14s	14s
MH-16	74	64	00	00	24	54	00	74s	44s	64	54	14s	00
KA-10	00	74	74	14s	74	84	64	74	00	84	84	34s	24s
KA-46	00	00	00	00	24s	00	00	74	00	64	74	24	24s

[a] 'Chancellor' near-isogenic line; [b] 'Maris Huntsman'; [c] 'Disponent'; [d] 'Normandie'; [e] 'Caribo'; [f] CS; [g] CS-*A. longissima* addition line; [h] CS-*A. longissima* substitution line

Table 3. Spores/cm^2 leaf area of CS ($21''$), CS-*A. longissima* addition- ($21'' + t_G''$) and -substitution line ($20'' + t'' + t_G''$) inoculated with ten mildew isolates (mean values of three independent experiments)

	$21''$	$21'' + t_G''$	$20'' + t_L'' + t_G''$
CA-2	13,515	3,305	1,166
CA-6	34,156	14,473	7,664
CA-8	61,892	4,713	8,477
CA-14	92,779	10,310	15,300
CA-42	161,415	26,684	6,401
MH-0	52,008	6,271	4,184
MH-3	18,006	3,440	2,784
MH-16	49,940	3,620	1,815
KA-10	126,470	13,217	7,650
KA-46	40,009	8,913	7,649

and double ditelosomic 3B (DDT-3B) CS lines to demonstrate the incorporation of alien variation.

From the cross, CS×substitution plants, F_1 progenies were obtained containing 41 complete chromosomes and two telosomes. In metaphase I of PMC's of these plants $20'' + tl'' + t_G'$ configurations (Fig. 1b) occurred mostly, indicating the presence of one *Aegilops* telosome and one wheat telosomic chromosome pair in the substitution line (Table 1).

Pairing in PMC's in 40+4t chromosome hybrids between DDT-3B lines of CS and substitution plants was also analysed. The most common chromosome configuration was $20'' + t'' + t' + t_G'$ (Table 1). This shows that the substitution plants contain one 3B telosomic chromosome pair (short or long) from wheat and one *Aegilops* telosomic chromosome pair substituting for the corresponding 3B telosome.

The identification of the substituted telosomes was achieved by crossing substitution plants with CS ditelosomic lines of 3BL and 3BS, respectively. As shown in Table 1, in F_1 hybrids of crosses with ditelosomic 3BL lines of $20'' + t_L'' + t_G'$ configurations (Fig. 1 c) were mostly observed, and $20'' + t' + t_S' + t_G'$ configurations (Fig. 1 d) were mainly found in crosses with ditelosomic 3BS plants. This leads to the conclusion that in the substitution line, the telosome pair 3BL of wheat is present while the telo pair 3BS was replaced by one telo pair of chromosome 'G' of *A. longissima.*

The mildew isolates showed a wide variation in their virulence for the different wheat lines/cultivars (Table 2). Only infection type 0 (no pustules) or type 4 pustules not limited by a necrotic reaction of the host were observed. Intermediate infection types, i.e. 1, 2 or 3, characterized by different hypersensitive reactions, were hardly ever found. Variability in size of type 4 pustules was indicated by the letter(s) 's' (small) or 'vs' (very small). Sometimes the mycelium was difficult to see with the naked eye. Therefore, a genotype characterized by infection type 0 may show slightly growing mycelium, e.g. type 4 vs under changed conditions.

The characterization of resistance was ascertained by assessing the infected leaf area, expressed as the infection grade (Table 2). CS, the addition and the substitution lines gave significantly different mean infection grades (CS: 6.30; CS – *A. longissima* addition line: 2.60 and CS – *A. longissima* substitution line: 1.70; for multiple comparison of these three mean values each representing 30 assessments differences >0.84 are significant), i.e. the *A. longissima* telosomic chromosomes led to a higher resistance. For further characterization of these lines epidemiological parameters were used: no differences were found in the latent period but there was variation in the number of spores/cm^2 leaf area (Table 3). Depending on the high variability in this trait (differences $> 17{,}303$ are significant) only CS could be proved to be highly susceptible in relation to the CS – *A. longissima* addition and substitution lines (CS: 65,019; CS – *A. longissima* addition line: 9,495; CS – *A. longissima* substitution line: 6,309). However, this trait does not provide a significant difference between addition and substitution lines, in contrast to the analysis of the infection grades.

Discussion

The findings clearly show that one arm of *A. longissima* chromosome 'G' is able to substitute for wheat chromosome arm 3BS. Ceoloni (1983) and Ceoloni and Galili (1984) using a similar CS – *A. longissima* ditelosomic addition line 'G' reported that a structural gene coding for phosphodiesterase isozyme was found on the short arm of *A. longissima* chromosome 'G' linked with genes for mildew resistance and brittle rachis, respectively. In *Triticum aestivum* a triplicate set of phosphodiesterase genes has previously been reported to be located on each of the short arms of wheat group 3 chromosomes (Wolf et al. 1977). In addition, Hart and Tuleen (1983) described structural genes for three further isozymes on *A. longissima* chromosome 'G', namely Est-S^{l}1 (esterase-1), Tpi-S^{l}1 (triosephosphate isomerase-1) and Got-S^{l}1 (glutamic-oxaloacetic transaminase-3). Est-1 and Tpi-1 are orthologous to sets of genes located on the short arms of wheat chromosomes 3 (Bergman 1972; Pietro and Hart 1982) and Got-3 is orthologous to sets of genes located on the long arms of wheat group 3 chromosomes (Hart 1975). From these findings it can be concluded that the *A. longissima* chromosome arm involved in the present substitution line is homoeologous to the short arms of group 3 chromosomes of common wheat.

The *A. longissima* mildew resistance introduced into wheat does not provide absolute/strong resistance to all isolates used. The susceptible reaction (infection type 4) after inoculation with most of the mildew isolates may imply that no valuable resistance was transferred. However, a comparison with wheat cultivar 'Maris Huntsman' also showing infection type 4 with two isolates leads to another conclusion. 'Maris Huntsman' reacted highly resistant in the field in 1984 although some mildew isolates selected in the field the same year were virulent in greenhouse tests. The same is true for the substitution line reacting highly resistant in the field despite its susceptibility in the greenhouse. Therefore, additional traits must be used to improve the characterization of resistance. First, the infection grade is applied demonstrating partial resistance of the CS – *A. longissima* lines. The infection grades of the substitution line are uniformly reduced in relation to CS. In contrast, other resistances react in a specific way: e.g. resistance gene *Pm3* a gives absolute resistance inoculated with 9 mildew isolates, but reacts highly susceptible when inoculated with KA-10.

For a more epidemiological characterization (Parlevliet 1979; Kranz 1983; Leonard and Mundt 1984) the latent period as well as the spore production have been measured. In these experiments no differences in latent period were observed. However, the CS – *A. longissima* substitution line only produced approximately 10% of the spores formed on CS. This type of resistance has been detected for the wheat – mildew system earlier (Shaner 1973; Martin and Ellingboe 1976; Rouse et al. 1980; Royer et al. 1984) and is called 'partial', 'rate-reducing' or 'slow-mildewing' (for review see Bennett 1984).

Summarizing these data, it is evident that partial resistance is present in the above mentioned substitution line. Transferring genes from related species to cultivated crops is one possibility for broadening the genetic variability. Partial resistance may possess high breeding value (Röbbelen 1978; Parlevliet 1983). However, the usefulness of new resistance genes may be limited by subsequent adaptation of the pathogen. Since durability cannot be predicted (Johnson 1983, 1984) this new source of resistance should be used within an integrated concept of disease control (Wolfe 1981).

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