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The introduction into bread wheat of a major gene for resistance to powdery mildew from wild emmer wheat

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Summary

A new source of resistance to wheat powdery mildew caused by *Erysiphe graminis* has been transferred to hexaploid bread wheat, *Triticum aestivum*, from the wild tetraploid wheat, *Triticum dicoccoides*. The donor was crossed to bread wheat and the pentaploid progeny was then self-pollinated. Plants having a near stable hexaploid chromosome complement were selected in the F_3 progeny and topcrossing and backcrossing of these to a second wheat cultivar to improve the phenotype was undertaken. Monosomic analysis of early backcross lines showed the transferred gene to be located on chromosome 4A. The gene has been designated *Pm16*.

Introduction

Powdery mildew of wheat caused by the pathogen *Erysiphe graminis* f.sp. *tritici* is one of the major diseases of wheat; in the U.K. it has been estimated that it can inflict as much as five per cent loss in yield nationally per annum (Cook & King, 1984; Priestley & Bayles, 1988). However, infection by this pathogen can affect other characters besides yield, and an estimated eventual loss in cash value of up to fifty per cent is thought possible (Priestley & Bayles, 1988). To combat these losses, plant breeders have incorporated specific genes into commercial cultivars which offer in-built resistance to disease. These create an alternative to fungicide application and subsequently a reduction of inputs into agricultural wheat production whilst also benefiting the environment. However, in recent years most of the established genes for resistance have become ineffective (Bennett, 1984; Brown et al., 1990) and there is therefore, a continual need for new resistance genes. Most of these have been obtained from within wheat itself (Bennett, 1984) and also from its close relatives (Miller et al., 1988; Ceoloni et al., 1988).

In the course of work to introduce other characters from wild emmer wheat, *Triticum dicoccoides*, into macaroni wheat, *T. durum*, resistance to the powdery mildew pathogen was observed in derived plants. A screening test indicated that the *T. dicoccoides* parent was the source of the resistance and the transfer of the resistance to hexaploid wheat was commenced.

Materials and methods

The following plant stocks were employed:
Triticum aestivum cultivars Maris Nimrod and Norman (2n = 6x = 42, AABBDD). Both carry the gene *Pm2* (Wolfe & Wright, 1972; McIntosh, 1988) conferring resistance to wheat powdery mildew.

This resistance has now been overcome by the pathogen (Bennett, 1984; Brown et al., 1990).
Triticum dicoccoides (2n = 4x = 28, AABB) accession CL1060025. This was collected in the Samarian mountains by the Hebrew University Expedition of 1977.
T. aestivum cv. Chinese Spring monosomic series (2n = 41), (Sears, 1954).

The bread wheat cv. Maris Nimrod was pollinated by the *T. dicoccoides* accession CL1060025. The resulting pentaploid plants were self-pollinated. Both mitotic and meiotic chromosome behaviour was monitored in these and the progenies of sucessive generations. Following one more self-pollination, progeny testing of the F_3 generation revealed individuals that were homozygous resistant. Within these, plants having 19 bivalents and 2 univalents at meiotic metaphase 1 were observed, indicating that they possessed a full complement of 21 chromosomes from the hexaploid wheat and were suitable for topcrossing with the more modern cultivar, Norman. In the progeny of the topcross, plants having 21 bivalent chromosomes during meiosis were observed and subsequent backcrossing was performed with these.

Resistance to wheat powdery mildew was assessed by screening in a small self-contained glasshouse. Isolates with virulence corresponding to all known resistance genes and gene combinations currently employed in U.K. cultivars (Van Kints, 1987) (including *Pm2* carried by the *T. aestivum* parents) were maintained as an epiphytotic on a series of differential cultivars within the glasshouse. Seedling progenies of all parents, F_3, F_4, the topcross and all backcross generations were exposed to the epiphytotic. As the glasshouse had only basic frost protection, the local weather conditions dictated the growth of plants and development of the pathogen. Therefore a range of growth stages was scored for resistance to the pathogen; from 2 or 3 leaves on the first tiller through to 1 or 2 leaves on the second tiller. Symptoms of disease fell into distinct classes upon assessment, and although they do not strictly match the Infection Type (I.T.) system of Moseman et al. (1965), they do approximate to it in some cases. In all progenies except those of the monosomic analysis F_1 and F_2, only three segregation classes were apparent. Resistant plants were easy to select being virtually free from symptoms, with a few individuals exhibiting a very slight chlorotic flecking on the distal half of the first leaf (I.T. 0). Progeny testing of this type showed them to be homozygotes. Similarily, susceptible plants were also easily detected with large pustules on most leaves, abundant sporulation and no necrosis evident (I.T. 4). Between these two were the heterozygous resistant plants which exhibited slight symptoms on their leaves, with scattered colonies beginning to necrose before much sporulation had occurred. By the second tiller stage, symptoms of infection had progressed to a characteristic chlorosis spreading in from the tip of the oldest leaves (I.T. 1–2). This became a clear indicator of heterozygous resistant plants in the backcross generations. Only the homozygous and heterozygous resistant classes described above were used for successive generations.

In the monosomic analysis, a fourth segregant class was apparent. Resistant (presumed homozygous) plants had a characteristic limited necrotic flecking on leaves with occasional tip senescence of the first leaf (I.T. 0–1). The second class, also regarded as resistant, had scattered necrosing colonies on leaves with tip senescence on first and sometimes second leaves (I.T. 1). In the third class, plants displayed more obvious symptoms of susceptibility with moderate, sometimes heavy infection on first and second leaves, some sporulation, infection beginning on the underside of the leaf and no apparent necrosis of colonies (I.T. 3–4). The fourth class was also definitive with one to three leaves covered with massive sporulating pustules on both upper and lower leaf surfaces and no evident necrosis (I.T. 4). These were presumed to be homozygous susceptible.

Monosomic analysis was performed by crossing resistant plants from the second backcross progeny as pollen parents on to each of the Chinese Spring monosomics. Feulgen stained root-tip squashes of the F_1 progeny were observed and the somatic chromosome number was determined. Plants having 41 chromosomes were selected, but because the resistant parent was a backcross plant and thus heterozygous, screening for resistance to the path-

ogen was necessary; only resistant monosomics were grown on for the F_2 generation. The chromosome behaviour of these plants at meiotic metaphase 1 was checked.

F_2 seedling progenies were screened for resistance to the pathogen to determine the location of the gene(s) responsible.

Results and discussion

Indication that a single gene was responsible for the powdery mildew resistance came from the segregation of F_1 progenies of early backcrosses which gave ratios of approximately one resistant to one susceptible plant. Similarily, the F_1 progenies of all 21 monosomic families in the monosomic analysis gave an overall ratio of sixty-two resistant to fifty-six susceptible plants. This result is not significantly different from the expected ratio of 1 : 1, again consistent with the hypothesis of a single gene for resistance. Further proof came from the progenies of resistant 42-chromosome F_1 plants derived from three different monosomic lines of the analysis referred to above. These were self-pollinated and the F_2 progenies screened for resistance (Table 1) giving a ratio not significantly different from the expected three resistant plants to one susceptible plant.

Table 2 shows the results of screening the F_2 progenies of the monosomic hybrids for resistance. Two monosomic families deviate significantly from the expected ratio of three resistant plants to one susceptible plant.

The chromosome 4B monosomic line[1] was significantly different, but this occurence in 20 lines could be explained as a chance event. However, the 4A line had a clear excess of resistant plants making it the likely carrier of the resistance gene. The single susceptible plant in the 4A line was grown on and the behaviour of meiotic chromosomes studied at metaphase 1. The plant had 20 bivalents and a telosomic univalent, and it was assumed that it was susceptible because the resistance gene was carried on the missing chromosome arm.

[1] Chromosome nomenclature follows the recommendation of the 7th International Wheat Genetics International Symposium, 1988 where 4A became 4B and vice versa.

Table 1. Segregation of resistant and susceptible plants in F_2 progenies of 42-chromosome plants from three cv. Chinese Spring monosomics × second backcross selections

Monosomic family	Resistant plants	Susceptible plants	x^2
3B	17	3	1.067[a]
4B	13	3	0.333[a]
6A	14	6	0.267[a]
Total	44	12	0.381[a]

[a] = observed ratios of resistant plants to susceptible plants not significantly different from expected ratio of 3 : 1.

Table 2. Segregation of resistant and susceptible plants in the F_2 progenies of Chinese Spring monosomics × second backcross selections

Monosomic line	Resistant plants	Susceptible plants	x^2
1A	18	2	2.400
1B	18	2	2.400
1D	16	4	0.267
2A	2	2	*
2B	13	7	1.067
2D	17	3	1.067
3A	31	8	0.429
3B	15	5	0.000
3D	16	4	0.267
4A	39	1	10.800^{++}
4B	40	5	4.629^{+}
4D	13	6	0.428
5A	16	4	0.267
5B	15	1	3.000
5D	17	3	1.067
6A	4	2	*
6B	24	14	2.842
6D	2	0	*
7A	17	3	1.067
7B	15	5	0.000
7D	15	5	0.000

* = Calculation of X^2 not valid due to low population size. Level of significance of differences between observed and expected ratio of 3 resistant to 1 susceptible plants is denoted by: $^{+}$ = $p < 0.05$; $^{++}$ = $P < 0.005$.

The primary hybridization between the hexaploid *T. aestivum* cv. Maris Nimrod and tetraploid *T. dicoccoides* produced F_1 plants that had brittle, speltoid spikes; both characters derived from the tetraploid parent. Pollen mother cells of some F_1 plants were observed at meiotic Metaphase 1 and were seen to contain 11 bivalents, a quadrivalent and 9 univalents, or 14 bivalents and 7 univalents. By the F_3 generation, the chromosome complement had risen to nearer the hexaploid level and chromosome pairing had stabilized, thus reducing the impact on fertility suffered by previous generations. But it was not possible to select individuals that were non-brittle and square-headed until the first backcross generation with cv. Norman. By the fourth backcross generation, the phenotype of plants closely resembled that of their topcross parent, *T. aestivum* cv. Norman, whilst still retaining the mildew resistance.

When seedlings heterozygous for the transferred resistance are exposed to the pathogen, they exhibit only slight symptoms of infection. The first and sometimes second leaves have limited necrosing colonies and a characteristic chlorosis which spreads from the tip inwards along the whole leaf. As the plants grow on, the odd necrotic colony can be found on the base of the culms of some individuals. This is thus an example of near-complete dominance of the resistance gene with full dominance being reached as the plant develops. The chlorosis of early leaves was typical of the heterozygous condition and was also seen during transfer of the gene to other cultivars. It could thus provide an easy method of detecting the gene when used in breeding programmes.

Conclusions

A single gene for resistance to wheat powdery mildew caused by *Erisyphe graminis* f.sp. *tritici* was located in wild *T. dicoccoides*. It has been transferred to the bread wheat *T. aestivum* cv. Norman. The gene has been designated *Pml6* and has been located on chromosome 4A.

When exposed to high levels of the pathogen in the glasshouse, plants homozygous for *Pml6* are virtually free from symptoms. However, heterozygotes exhibit a characteristic chlorosis on early leaves when similarly exposed, thus facilitating selection for the resistance in a breeding programme. Thus the gene is a major new source of resistance to wheat powdery mildew.

This demonstrates further the value of the wild relatives of wheat as a source of useful genetic variation.

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