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The production and characterization of recombination between chromosome 3N of *Aegilops uniaristata* and chromosome 3A of wheat

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Six wheat lines with recombination between *Aegilops uniaristata* chromosome 3N and wheat chromosome 3A were produced. These were characterized in terms of exchange points by RFLP analysis. Chromosome 3N carries an undesirable brittle rachis gene and three of the recombinant lines had lost this character. The results also support previously published evidence of a pericentric inversion in chromosome 3N relative to the wheat homoeologous group 3 chromosomes.

Keywords: *Aegilops uniaristata*, pericentric inversion, recombination, RFLP, wheat.

Introduction

Genetic variation in cultivated wheat has diminished due to the artificial human selection of pure line varieties. To counter this a new gene pool of desirable characters is required and the wild relatives of wheat are proving to be a very important source of useful genes. A number of agronomically important genes have been transferred to wheat from related species, such as stem rust resistance (Kibirige-Sebunya & Knott, 1983), barley yellow dwarf virus resistance (Sharma *et al*., 1995) and mildew resistance (Miller *et al*., 1988; Ceoloni *et al*., 1992). The wild grass *Aegilops uniaristata* Vis. has been reported as a source of aluminium tolerance genes for wheat (Berzonsky & Kimber, 1986); and Miller *et al*. (1995) confirmed the presence of an aluminium tolerance gene(s) on chromosome 3N of that species by screening wheat–*Ae*. *uniaristata* addition lines in a hydroponics system. They also reported a brittle rachis gene on the same chromosome.

The suppression of alien genes transferred into wheat has been observed. As an example Aniol & Gustafson (1984) reported that addition and substitution lines of rye chromosomes into wheat were less tolerant to high aluminium concentrations than the rye itself. However, when tolerant N genome amphiploids were combined with sensitive AB and AG genome tetraploids, no suppression was observed in aluminium tolerance (Berzonsky & Kimber, 1989), suggesting that the aluminium tolerance of *Ae*. *uniaristata* may be transferred to wheat without significant suppression of the expression of the gene(s).

Alien gene transfers in wheat have been achieved by manipulating the genetic control of homoeologous chromosome pairing. Maan *et al*. (1978) reported that pairing between the N and D genomes was inhibited because of the *Ph1* gene. The effect of the *Ph1* gene can be removed by using plants nullisomic for chromosome 5B or by using *ph1* deletion mutants in the hybridization programmes. Initially, chromosome pairing at meiosis was studied to confirm the absence of the *Ph1* locus, but later isoelectric focusing (IEF) of the iodine binding factor (IBF) was used to select plants nullisomic for chromosome 5B (Liu & Gale, 1989) and hence lacking the *Ph1* locus. More recently, identification of deletion at the *Ph1* locus has employed RFLP and PCR-based markers allied to chromosome pairing studies. Gill *et al*. (1993) identified RFLP markers located within the *Ph1* deletion region and later two PCR-based markers were reported to confirm the absence of the *Ph1* locus during cytogenetic manipulations (Gill & Gill, 1996; Segal *et al*., 1997). Now a rapid and simple PCR-based method has been devised that uses fresh plant tissue as a template for the amplification of sequences from the *Ph1* region deleted in the wheat *ph1b* mutant by using specifically designed primers (Qu *et al*., 1998).

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The presence of alien chromosomes in successive generations of crosses can be confirmed by simple root tip cytology if the alien chromosome has a distinct morphology or biochemical and/or molecular markers can be used for this purpose (Koebner & Shepherd, 1985; Yang *et al.*, 1996).

The objective of the present study was to determine whether homoeologous recombination could be produced between chromosome 3N and its wheat homoeologues without transferring the brittle rachis character present on the same chromosome.

Materials and methods

Materials

The following plant materials were used in this study. Three wheat, *Triticum aestivum* L. 'Chinese Spring', lines in which *Aegilops uniaristata* chromosome 3N was substituted for wheat chromosome 3A, 3B or 3D — CS(3A)3N, CS(3B)3N and CS(3D)3N (Miller *et al.*, 1995); 'Chinese Spring' nullisomic–tetrasomic lines N3AT3B, N3BT3A, N3DT3A and N5BT5D (Sears, 1966) along with the 'Chinese Spring' *ph1b* mutant (Sears, 1975). The 'Chinese Spring' line monosomic for chromosome 3A was also utilized to produce a hemizygous condition of the recombinant chromosome.

PCR primers specific for both the *Ph1* deletion region and for a positive reaction control, as described by Qu *et al.* (1998), were used to select plants homozygous for the *ph1b* deletion.

Eight homoeologous group 3 RFLP probes (PSR series) developed at the John Innes Centre (five for the long arm, PSR56, PSR156, PSR170, PSR394 and PSR483; and three for the short arm, PSR547, PSR598 and PSR1196) were used. These probes, which exhibit polymorphism between wheat and *Ae. uniaristata* and are distributed over the full length of the group 3 chromosome map, were employed for the characterization of the recombinants. Chromosome 3N of *Ae. uniaristata* carries a pericentric inversion relative to its wheat homoeologues (Iqbal *et al.*, 1999), and Fig. 1 shows the relative order of the eight RFLP probe loci on the two chromosome types.

Methods

To produce recombinants, substitution lines carrying chromosome 3N were crossed and backcrossed with plants lacking the *Ph1* locus. Plants carrying chromosome 3N were selected in each generation. After the second crossing step, plants were also screened for the absence of the *Ph1* locus. Unfortunately, only lines derived from the (3A)3N substitution produced enough

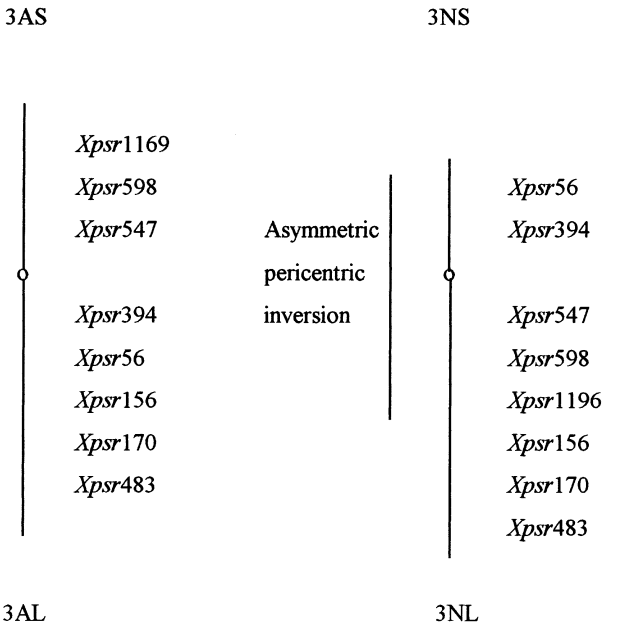

Fig. 1 The order of the eight RFLP probe loci on wheat homoeologous group 3 chromosomes (including 3A) and *Aegilops uniaristata* chromosome (3N).

seeds and plants that lacked the *Ph1* locus and carried chromosome 3N. These were selected and crossed as male parents to 'Chinese Spring' monosomic for chromosome 3A. Chromosome number determination was carried out on the progenies of these crosses and 41-chromosome plants were selected for RFLP analysis.

Chromosome 3N is a heterobrachial chromosome with a subterminal centromere (Miller *et al.*, 1995). Selection for its presence was based on recognition of this distinct morphology in root tip cytological preparations and on the occurrence of brittle rachis at maturity. Esterase isozyme polymorphism (Iqbal, 1998) was also used to identify the presence of this chromosome. A nondissociating discontinuous buffer system was used to separate the grain esterases. Acrylamide gel preparation and buffers were as described by Laemmli (1970), except that SDS was omitted from the buffers and vertical slab gels (8.5% acrylamide) were used to separate the different enzyme forms. Gels were stained in a solution containing α-naphthyl acetate (50 mg) and Fast Blue RR salt (100 mg) dissolved in 5 mL acetone and made up to 100 mL with 0.05 M phosphate buffer pH 7.5 (Ainsworth *et al.*, 1984).

Absence of *Ph1* was established by studying chromosome pairing at meiosis, and also confirmed by the PCR-based method. For PCR analysis, samples were prepared as described by Klimyuk *et al.* (1993). Immature anthers were collected in a 1.5-mL microfuge tube containing 40 μL of 0.25 M NaOH. These were

incubated in a boiling-water bath for 30 s and neutralized by adding 40 μL of 0.25 M HCl and 20 μL of 0.5 M Tris-HCl buffer pH 8.0 with 0.25% Nonidet P-40 (Sigma). Samples were again incubated in the boiling-water bath for 2 min and the entire anther was used in the PCR reaction. The PCR reaction was performed in a total volume of 50 μL with treated anthers (as described above) as the DNA template, and 75 ng each of the primers specific to the *ph1b* deletion region (Qu *et al*., 1998) were employed. PCR reactions were performed for 36 cycles with a 94°C denaturation step for 30 s, an annealing step for 30 s (as described below) and an extension step at 72°C for 1 minute. The annealing temperature in the first cycle was 65°C, subsequently reduced in each cycle by 0.7°C for the next 12 cycles, and was continued at 56°C for the remaining 23 cycles (Vos *et al*., 1995).

For RFLP analysis the methods described by Davis *et al*. (1986) were followed.

Results and discussion

Following the crossing sequence designed to create recombinants, 185 plants with 41 chromosomes (hemizygous for a potential 3A/3N recombinant chromosome) were selected. Forty plants, appearing to have a nonbrittle rachis, were selected for RFLP analysis. Screening with the eight probes showed the presence, or absence, of a complete chromosome 3N in most of the tested plants. In the end, only six plants out of 185 were found that carried segments from both chromosome 3N and chromosome 3A.

Recombinants 1 and 2 showed the same RFLP pattern with only one *Ae. uniaristata* locus (*Xpsr547*) present, while all other signals were wheat specific. The occurrence of two crossovers, one on each side of locus *Xpsr547*, and the exchange of segments between the short arm of chromosome 3A and the long arm of chromosome 3N would result in recombinants that carry the exchanged RFLP locus from *Ae. uniaristata* present at its original position on the wheat group 3 map (Fig. 2, REC 1 & 2). The physical distance between loci *Xpsr547* and *Xpsr598* in the wheat group 3 chromosome short arms is not known, and more detailed mapping is required to locate the exact position of the crossovers in these recombinants. The exchange points may therefore be different for the two recombinants and consequently they have been treated separately. Both these recombinant plants showed absence of brittle rachis at maturity.

Two *Ae. uniaristata* specific loci, *Xpsr547* and *Xpsr483*, were revealed in recombinant 3, indicating the transfer of two segments from chromosome 3N to chromosome 3A of wheat. Whilst the *Xpsr483* locus from *Ae. uniaristata* was present in this recombinant, the other long-arm-specific loci were absent. The wheat specific signals for these homoeoloci did not show increased dosage indicating a duplication, thus there has not been a simple exchange process involved in the transfer of the *Ae. uniaristata* fragment carrying the *Xpsr483* locus. In an exchange recombinant only one locus should be present, whereas in this case loci from both chromosomes were identified. The possibilities are that a small segment, containing locus *Xpsr483*, was translocated from chromosome 3N to the recombinant chromosome, or that a distal break in chromosome 3N has resulted in an intrachromosomal terminal duplication. It is also possible that another group 3 wheat chromosome was involved in a separate recombination event with chromosome 3N and also carries this fragment. The possible arrangement of these loci on the recombinant chromosome is presented in Fig. 2 (REC 3). The plant did not show brittle rachis at maturity.

Only two *Ae. uniaristata* specific loci, *Xpsr598* and *Xpsr1196*, were detected in recombinant 4, whereas all the other 3N specific loci were absent. As locus *Xpsr547* is wheat specific, the exchange point was therefore present between loci *Xpsr547* and *Xpsr598*. The *Ae. uniaristata* loci distal to *Xpsr1196* were absent, indicating a second chiasma formation and exchange of segments. Possible exchange points and the resulting recombinant are presented in Fig. 2 (REC 4). The locus *Xpsr1196* is present at the terminal end of the short arm of chromosome 3A and an exchange point beyond this locus is difficult to detect due to the lack of available markers. The presence of the small terminal 3A segment could not therefore be established in this study. The other possibility is that this fragment may have been lost during the crossover at this point. However, it seems that the recombinant chromosome has all the RFLP loci present in the same order as on chromosome 3A, except that the loci *Xpsr598* and *Xpsr1196* were replaced by homoeoloci from *Ae. uniaristata*. The plant had a brittle rachis.

In recombinant 5, *Ae. uniaristata* specific loci were detected with probes PSR598, PSR1196, PSR156 and PSR170. This indicates that a large part of chromosome 3N has been transferred to chromosome 3A. The terminal 3N specific *Xpsr483* locus is absent and may be deleted due to crossover at this point. RFLP analysis showed the absence of only two short arm *Ae. uniaristata* loci, *Xpsr394* and *Xpsr56*, whereas loci *Xpsr156* and *Xpsr170* were present from both chromosomes. This can be explained by the occurrence of one chiasma between *Xpsr547* and *Xpsr598* during the pairing of the 3A short arm with the 3N long arm. As these loci are present on the long arm of chromosome 3N due to inversion (Iqbal, 1998; Iqbal *et al*., 1999), along with the loci *Xpsr156* and

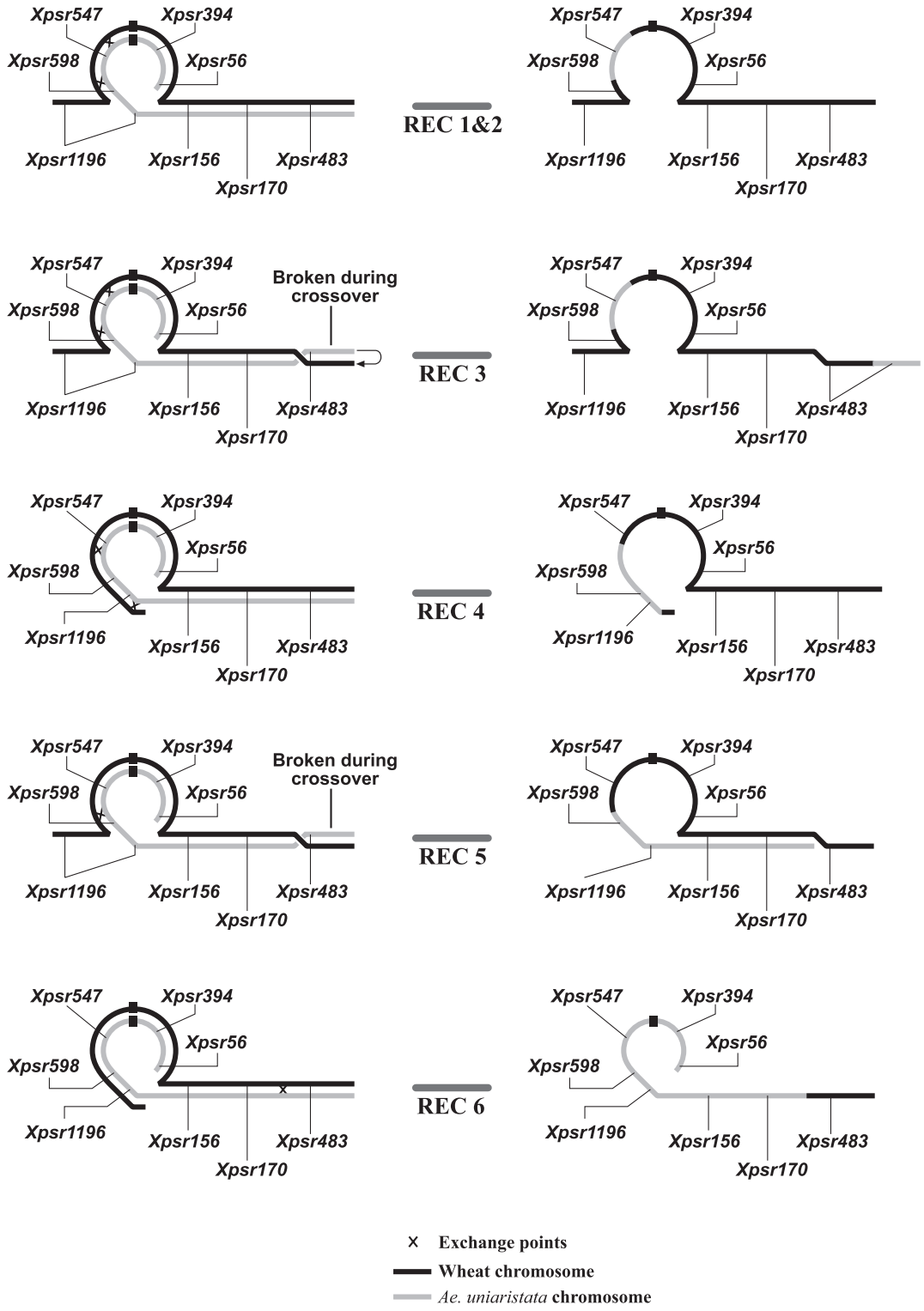

Fig. 2 Diagrammatic representation of the six recombinant chromosomes (right) derived from chromosomes 3N and 3A (left).

Xpsr170, the whole fragment carrying all these loci has been transferred to the 3A short arm. There probably was a cross-over at the telomeric ends of the chromosomes resulting in the deletion of the *Ae. uniaristata Xpsr483* locus as in recombinant 3. The resulting chromosome has a complete 3A long arm but the short

arm now carries one locus (*Xpsr547*) from wheat and four loci (*Xpsr598*, *Xpsr1196*, *Xpsr156* and *Xpsr170*) from *Ae. uniaristata*. Figure 2 (REC 5) shows the exchange and crossover points between the pairing chromosomes along with the resulting recombinant. The plant showed the brittle rachis character at maturity.

Recombinant 6 is different from all the other recombinants as it showed the presence of only one wheat-specific locus, i.e. *Xpsr483*, whereas all other loci were from *Ae. uniaristata*. It is therefore evident from these results that this plant carried an almost complete chromosome 3N from *Ae. uniaristata* resulting from a recombinational event, where only terminal segments carrying the specific locus have been exchanged between chromosomes 3A and 3N. The resulting recombinant is presented in Fig. 2 (REC 6). The plant also showed brittle rachis at maturity.

The presence of both short arm and long arm RFLP loci from *Ae. uniaristata* in one recombinant and duplication of other loci supports the presence of a pericentric inversion in chromosome 3N.

Although only six recombinants were isolated, three exhibited brittle rachis (recombinants 4, 5 and 6) and three were nonbrittle (recombinants 1, 2 and 3). It was found that only 3N specific loci *Xpsr598* and *Xpsr1196* were common to those recombinants showing brittle rachis at the time of maturity. Three homoeologous group 3 short arm loci (*Xpsr547*, *Xpsr598* and *Xpsr1196*) were transferred to the long arm during the asymmetric pericentric inversion which gave rise to chromosome 3N (Iqbal *et al*., 1999). In two of the recombinants locus *Xpsr547* was absent but no recombinants were isolated where loci *Xpsr598* or *Xpsr1196* were present separately. It is therefore suggested that the brittle rachis gene is located very close to *Xpsr598* or between the loci *Xpsr598* and *Xpsr1196*. As these loci were transferred from the short arm to the long arm of chromosome 3N, this particular character therefore was originally present on the short arm but was transferred to the long arm during the chromosomal rearrangement. Yang *et al*. (1996) also reported a brittle rachis gene on the short arm of chromosome $3S^{v}$ of *Ae. variabilis*.

This study has shown that it is possible to induce recombination between chromosome 3N of *Ae. uniaristata* and a wheat homoeologue, and in so doing exclude the undesirable brittle rachis gene. It should therefore be possible by the same procedure to transfer the aluminium tolerance from chromosome 3N to a homoeologous wheat chromosome.

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