CONTROLLED INTROGRESSION OF ALIEN CHROMATIN INTO WHEAT

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Abstract

Attempts to transfer alien genetic material to wheat have in the past involved addition or substitution of whole chromosomes or chromosome arms from an alien related species to the genome of wheat. Generally, such lines have suffered from loss of yield or quality compared to the normal wheat parent. The amount of alien chromatin present in these lines can be reduced by induction of meiotic recombination between alien and wheat chromosomes through suppression or deletion of the *Ph1* gene on the long arm of wheat chromosome 5B. Although induced allosyndetic pairing has been frequently observed, few successful alien gene transfers have been achieved in this way. The possible mechanisms of action of *Ph1*, the experience with alien introgression and the known effects of alien chromatin on wheat are reviewed.

The work reported in this thesis demonstrates for the first time that the chromosomes of wheat and cereal rye can be recombined by induction of homoeologous pairing by means of nullisomy for chromosome 5B and by the utilization of the *ph1b* mutant. The frequency of recombination is low, but by developing rapid and reliable techniques using established biochemical and other markers, it was possible to screen for these recombinants relatively easily.

Two different wheat-rye translocation lines were used as starting points for the induction of allosyndetic recombination. The translocation chromosome involving the short arm of rye chromosome 1R carries a useful gene for resistance to stem rust, but lines with this rye segment translocated to the long arms of either 1D or 1B are characterised by a dough quality defect. By testcrossing a homozygous *ph1b* plant heterozygous for the 1DL-1RS translocation, one recombinant involving 1RS and 1DS was isolated out of 394 progeny, while progeny derived by self-fertilisation of nullisomic 5B plants heterozygous for the same translocation produced a further three wheat-rye recombinants in 531 progeny. The rye segment present in the 1BL-1RS translocation has also been recombined with wheat. The recombinants were selected on the basis of their

endosperm storage protein phenotype (two independent loci on 1DS, one on 1RS and on 1BS) and their reaction to stem rust. Recombinant lines were further characterised by analysis of phenotype for two isozymes, which have structural genes on the short arms of the homoeologous group 1 chromosomes. Twelve independent plants with an altered chromosome 1DS were also obtained; eleven of these possessed the proximal but not the distal endosperm storage protein locus, while one possessed the distal without the proximal locus. These lines will prove useful in the elucidation of the contribution of the gene products of these two protein loci to dough quality.

The long arm of rye chromosome 1R, which is marked both by a heterochromatic telomere and at the *Glu-R1* locus, closely linked to the centromere, was induced to pair with wheat in a *ph1bph1b* background and 17 recombined chromosomes were recovered among 731 progeny derived by self-fertilisation. Due to self-sterility of some plants, some suspected recombinants could not be subjected to a progeny test for verification and an estimate of the total number of recombinants obtained was made, giving a gametic recombination frequency of 1.4%. Control populations, where homoeologous pairing was suppressed, did not produce any confirmed recombinants.

Chromosome 1U from *Aegilops umbellulata* was also used in a study of wheat-alien recombination. It was expected that the allosyndetic recombination frequency would be higher in this case than in rye, given that *Aegilops* and wheat are more closely related than wheat and rye. This chromosome was chosen as it possesses three easily scorable marker loci. Over a segment of the short arm of the *Aegilops* chromosome between the prolamin locus *Gli-U1* and a structural gene for the isozyme *Gpi-U1*, a gametic recombination frequency of 8.0% was estimated within a population derived from a *ph1bph1b* parent, a third of the value for homologous recombination within wheat. Some double homoeologous cross-overs in the interval *Glu-U1* - *Gli-U1* were also recovered. When both the alien chromosome and a wheat homoeologue were present as monosomes, the rate of recombination was approximately double that recorded in

populations derived from a monosomic addition of chromosome 1U. No cross-overs were found in a control population derived from a *Ph1bph1b* parent.

Since codominant genetic markers allow the classification of both gametes in a single progeny, F2 populations were employed in most of this work, rather than the more conventional backcross techniques used in previous work with alien introgression. These populations are both simple to produce and are more efficient than test-cross populations as two gametes are screened simultaneously in a single individual. A comparison of the efficiency of induction of allosyndetic recombination showed that 5B nullisomy was at least as effective as the *ph1b* mutant. The availability of a urea soluble endosperm protein controlled by a gene on chromosome 5BL made selection of 5B deficient plants simpler than those homozygous for *ph1b*, which required time-consuming cytological analysis and a progeny test to verify the identification.