Chromosome re-assembly for wheat improvement

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ABSTRACT

Undomesticated species from wheat's tertiary gene pool have been the source of numerous beneficial traits for wheat breeding when introduced as chromosomal translocations. However, the presence of deleterious traits, have constrained their extensive use in food production. Using the *ph1b* mutant system, a precise crossing program, and a strong selection with molecular markers and bioassays, we demonstrated that it is possible to recombine two different alien translocations and recover stable trigenomic chromosomes with useful combinations of genes. Recombinant alien translocations were selected with new combinations of genes for disease resistance derived from Thinopyrum intermedium and Th. ponticum onto wheat chromosome arm 7DL. The integrity and stability of the newly assembled chimeric chromosomes were confirmed in F2 and F3 derived populations segregating for Leaf rust (Lr19) and BYDV (Bdv2) resistance. In a similar way we are attempting to obtain useful translocations from another BYDV resistance (Bdv4) from Th. intermedium chromosome 2Ai-2.

INTRODUCTION

Species from wheat tertiary gene pool are an important source of beneficial traits for wheat improvement. However, their use has been prevented by deleterious genes dragged along when transferred into wheat. In addition, the lack of recombination between the foreign translocations (Knott 1980) and wheat or between translocations belonging to different genomes makes it virtually impossible to combine desirable genes eliminating the undesirable ones. Such is the case of two important translocations one carrying the Lr19 and the Sr25 genes for rust resistance derived from Thinopyrum ponticum and the other one carrying the Bdv2 gene for resistance to BYDV derived from Th. intermedium. Both translocations are mutually exclusive because both are located on the long arm of chromosome 7D of wheat. The translocation from Th. ponticum in addition to the beneficial rust resistance genes carries undesirable genes for yellow pigment, and genes producing segregation distortion. Efforts to shorten the size of the Th. ponticum fragment, has caused its self elimination from the wheat genome (Prins et al. 1997, Prins and Marais 1998).

One crucial characteristic of wheat, a polyploid specie, is that it behaves as a diploid during cell division. This attribute is mainly controlled by a single gene, the Ph1 gene that strongly favours the pairing of homologous chromosomes while preventing the pairing of homoeologous chromosomes. When the activity of Ph1 gene is eliminated (e.g. by the ph1b deletion mutant), homoeologous pairing happens giving the opportunity for recombination between homoeologous chromosome segments.

In our work we have employed the ph1b mutant system to produce recombination between Th. intermedium and Th. ponticum translocations in wheat. Homozygosity for ph1b and the relatively close phylogenetic distance between the two relevant Thinopyrum genomes allowed the two alien translocations to recombine yielding a variety of inter-genomic recombinants. Detailed molecular screening of the foreign genomes and the development of genome specific markers provided the tools for screening and selection for desirable genotypes with useful gene combinations.

MATERIALS AND METHODS

Crosses were made between cv. 'Batavia' with T4m (*Lr19*) (Agatha mutant source Australian Cereal Rust Control Program, PBI, Cobbitty); and cv.'Hartog' with TC5 or TC14 (*Bdv2*). To induce homoeologous pairing the translocations were crossed into the cultivar 'Angas' with the *ph1b* mutation. 'Angas *ph1b*' was obtained from Dr Ian Dundas, University of Adelaide. Self progeny with both translocations, and homozygous for *ph1b*, were selected with STSLr19130 (Prins et al. 2001) for T4m, with BYAgi (Stoutjesdijk et al. 2001) for TC5 or TC14 and with ABC920 (Wang et al. 2002) for *ph1b*.

Plants with the genotype T4m / TC5 (or TC14), *ph1b/ph1b* were selfed allowing one meiosis to happen, then F2 half seeds were screened with molecular markers to select for individuals with both translocations that were supplied for double haploid production with the maize pollen method to SARDI, Adelaide, Australia.

Molecular markers specific for *Th. intermedium* and *Th. ponticum* translocations were identified by mainly two methods: screening SSRs and STSs for chromosome 7DL, and by designing primers for wheat EST sequences mapped in the target region. Standard PCR conditions were employed with four µl of 10 ng/µl DNA was amplified using the HotStartTaq® DNA polymerase and Master Mix buffer from QIAGEN following the manufacturer's guidelines. Amplification products were separated on 1, 2 or 3% agarose. The selected primer

pairs once screened on the parental lines, were tested on nullitetrasomic lines for group 7 and deletion lines for chromosome arm 7DL.

To identify recombinants, F2 and F3 populations derived from the hemizygote for both translocations and homozygote for the *ph1b* mutation (T4m / TC5, *ph1b/ph1b*), were genotyped with newly developed molecular markers and bioassayed with the two pathogens.

BYDV resistance was tested at CSIRO Plant Industry at Black Mountain. Two leaf seedlings were infested with viruliferous *Ropalosiphum padi* carrying BYDV-PAV. Fifteen days after inoculation the virus concentration was measured by ELISA. Leaf rust resistance was tested in the following generation at the Australian Cereal Rust Control Program of the Plant Breeding Institute, Cobbitty.

Y4212 is a substitution line 2Ai-2 (2D) derived from the 2Ai-2 addition line Z6 and obtained from Dr Xin Zhiyong (CAAS, Beijing). The short arm of 2Ai-2 carries the BYDV resistance Bdv4. Attempts were made to produce translocations from the substitution line using the *ph1b* effect.

RESULTS AND DISCUSSION

Identification of recombinant translocations

Recombination was recorded when only one or two of three expected *ponticum* markers, or one of two expected *intermedium* markers was present in an individual. Heterozygotes, where apparently no recombination had occurred were considered those individuals showing the presence of all *intermedium* and *ponticum* markers of dominant and co-dominant nature. The presence of the translocation was verified by the absence of wheat dominant markers in the region.

Testcrossing the individuals with recombinant translocations restored the wild type *Ph1* function and the translocation was stabilized. F2 and F3 populations of the testcrosses were screened with specific molecular markers. Following the segregation of each specific marker dominant and co-dominant for *Th. intermedium*, *Th. ponticum* and wheat and the segregation of the whole set of markers in each individual, we confirmed that the new recombinant translocation inherited all genes and markers together as a block in Mendelian proportions.. Production of doubled haploids was very valuable in confirming the composition of the recombinant translocations. The work with TC5 leading to these recombinants was recently reported (Ayala-Navarrete et al, 2007) and here we name specific useful recombinants as Pontin 1, 2 and 3 (Fig 1). The presence or absence of *Sr25* on these recombinants is still being confirmed.

Obtaining smaller recombinant translocations

The recombinant translocations produced above had the same size as the largest translocation used in the hybrid. The recombinant therefore had the size of T4m comprising most of the long arm of chromosome 7D. To produce smaller recombinant translocations and minimize the amount of foreign chromatin brought into wheat, we followed the same steps as described in Materials and Methods but using the smaller *Bdv2*-carrying translocation TC14 instead of TC5 and this time selected for recombinants with 7D markers rather than *ponticum* markers in the proximal region.

As before recombination was identified with molecular markers specific for each genome and recombinant translocations test-crossed to wheat. Stable recombinant translocations were recovered which appeared to be the size of TC14 comprising 1/3 of 7DL. Bioassays to confirm the presence of the three genes *Bdv2*, *Lr19* and *Sr25*, are underway with all the different recombinants recovered (Fig 2).

Identification of recombinant individuals between 2D and 2Ai-2.

A similar method to induce homoeologous recombination, using the *ph1b* mutant system, was attempted between chromosomes 2D and 2Ai-2. Chromosome 2Ai-2 carries a gene with stronger BYDV resistance than *Bdv2* (Larkin et al 1995; Lin et al 2005; Zhang et al 2001). Heterozygote individuals 2D/2Ai derived from the substitution line Yi4212 and with *ph1b* in homozygosity were selfed and screened with molecular markers specific for wheat and for 2Ai-2. After analysis of several selfing and testcross generations, very low levels of recombination were detected but not recombinant individuals could be recovered. It appears that compensation was not occurring between 2D and 2Ai-2. Once the 2Ai-2 chromosome was broken self elimination was occurring as happened before with the *Th. ponticum* translocation carrying the *Lr19* gene (Prins et al. 1997; Prins and Marais 1998).

To avoid self elimination and to recover regions of 2Ai-2 to test for the presence of the BYDV resistance, we are now attempting to employ other “bridge” translocations from a number of donor genomes. using stable translocations onto chromosome 2D carrying useful genes from other genomes.

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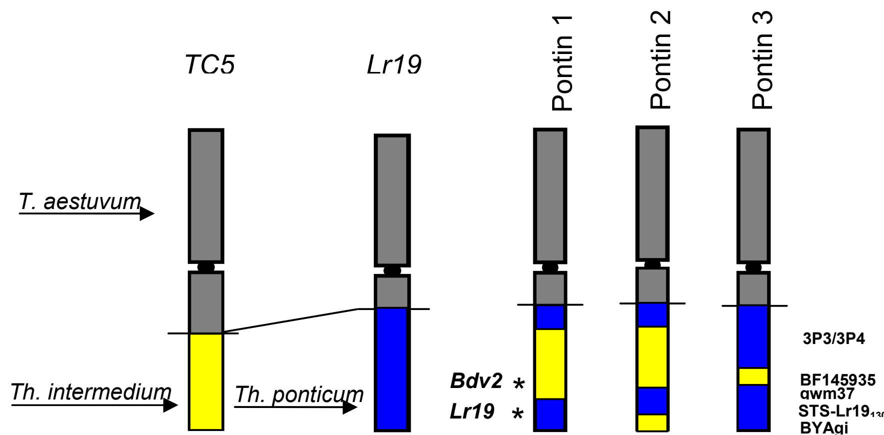

Fig 1.- Combination of *Thinopyrum intermedium* and *Th. ponticum* translocations in wheat chromosome 7DL. The predicted size and genomic composition of recombinant translocations is shown schematically.

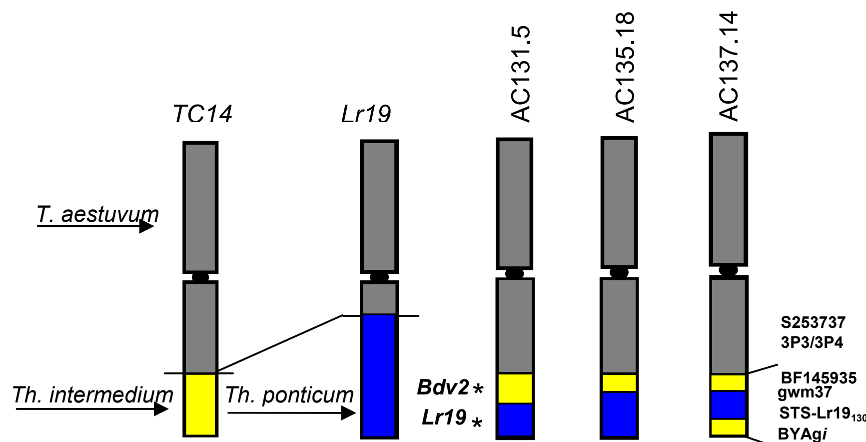

Fig 2.- Recombinant translocation assembled from the small TC14 and the T4m translocations. Smaller translocations recovered through molecular marker selection. The predicted position of *Bdv2* and *Lr19* genes to be confirmed with bioassays.