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ORIGINAL PAPER

The *Pontin* series of recombinant alien translocations in bread wheat: single translocations integrating combinations of *Bdv2*, *Lr19* and *Sr25* disease-resistance genes from *Thinopyrum intermedium* and *Th. ponticum*

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Abstract Two bread wheat lines each with a translocation on chromosome 7DL from either *Thinopyrum intermedium* (TC5 and TC14) or *Thinopyrum ponticum* (T4m), were hybridized in a *ph1b* mutant background to enhance recombination between the two translocated chromosomal segments. The frequency of recombinants was high in lines derived from the larger and similar-sized translocations (TC5/T4m), but much lower when derived from different-sized translocations (TC14/T4m). Recombinant translocations contained combinations of resistance genes *Bdv2*, *Lr19* and *Sr25* conferring resistance to *Barley yellow dwarf virus* (BYDV), leaf rust and stem rust, respectively. Their genetic composition was identified using bioassays and molecular markers specific for the two progenitor *Thinopyrum* species. This set of 7DL *Th. ponticum/intermedium* recombinant translocations was termed the Pontin series. In addition to *Thinopyrum* markers, the size of the translocation was estimated with the aid of wheat markers mapped on each of the 7DL deletion bins. Bioassays for BYDV, leaf rust and stem rust were performed under greenhouse and field conditions. Once separated from *ph1b* background, the Pontin recombinant translocations were stable and showed normal inheritance in successive backcrosses. The reported Pontin translocations integrate important resistance genes in a single linkage block which will allow simultaneous selection of disease resistance. Combinations of *Bdv2* + *Lr19* or *Lr19* + *Sr25* in both long and short translocations, are available to date. The smaller Pontins, comprising only 20 % of the distal portion of 7DL, will be most attractive to breeders.

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Introduction

There is an unrelenting need to improve both yield potential and yield stability of wheat (*Triticum aestivum* L., $2n = 42$, genome AABBDD). Traits not found, or scarce, in the primary gene pool of wheat, can and have been sourced from other Triticeae species.

BYDV is one of the main viral diseases in wheat for which resistance has been transferred from *Th. intermedium* (Brettell et al. 1988; Banks et al. 1995; Sharma et al. 1995; Larkin et al. 1995; Xin et al. 2001; Wang et al. 2010). Breeding lines carrying the *Bdv2* allele have been available since the mid-1990s (Larkin et al. 2002) and was subsequently deployed in Australian varieties Mackellar (Davidson and Kleven 2002) and Glover (Larkin and Banks 2005), and Chinese lines (Zhang et al. 2009). BYDV resistance is

difficult to select in segregating populations. The pathogens causing the disease are a group of viruses belonging to different families (Luteovirus and Polerovirus) with different specificities for host and insect vectors. These viruses are not transmitted mechanically but in a persistent manner by several species of aphids. Hence, testing for resistance needs special facilities, the availability and management of insect vectors, living sources of virus strains and means to assay for the presence of the virus by ELISA or RT-PCR (Balaji et al. 2003) in infected plant material.

Rust diseases on the other hand are caused by fungal pathogens which are highly variable. The evolution of virulence in rust pathogens makes breeding for resistance a continuous effort. Resistance to leaf rust, stem rust and stripe rust are valued traits and new sources are constantly being sought in diverse wheat germplasm including its wild relatives. At present >50 resistance genes against each one of the rust pathogens are formally named and about half of them are derived from wild relatives (McIntosh et al. 2008) and this proportion is increasing. Because of the dynamic nature of these pathogens, a single major resistance gene can often succumb to new pathotypes within a short period of its deployment and therefore deployment of combinations of two or more genes is desirable (Bariana et al. 2007).

In 1999 an unexpectedly high level of stem rust infection was observed in Uganda on wheat germplasm carrying stem rust resistance gene *Sr31,* derived from *Secale cereale*, and the pathotype was named as Ug99 (Pretorius et al. 2000). This pathotype, commonly referred to as TTKSK according to the North American nomenclature, has since spread to Kenya, Ethiopia, South Africa, Tanzania, Sudan, Yemen and Iran (Singh et al. 2011). Global efforts have shed light on existing and newly defined resistance genes which are effective against the various strains within Ug99 lineage (Jin et al. 2007; Rouse et al. 2011). Importantly, combinations of genes are recommended as the best strategy to diminish losses and contain the spread and evolution of this new family of stem rust races. One of the genes in recommended combinations is *Sr25* (Yu et al. 2010). *Sr25* and the linked leaf rust resistance gene *Lr19* are carried in a translocation from *Th. ponticum* on wheat chromosome 7DL. Although a rust virulence for *Sr25* has been reported in India (Jain et al. 2009), this gene remains effective in all other wheat growing areas of the world (Njau et al. 2010) and remains effective against the Ug99 family.

Wheat lines with translocations either from *Th. intermedium* or *Th. ponticum* have been available for a number of years, however, they have not been fully exploited because of genetic drag. The chromosome segments in both cases are derived from genomes that are only homoeologous to the wheat genomes and do not recombine with wheat chromosomes (Knott 1980). Furthermore, in the absence of recombination, it is not feasible to eliminate detrimental genes, or to exploit other useful wheat alleles that reside in the region corresponding to the translocation.

With the use of the *ph1b* mutant system, induction of homoeologous recombination happens at different frequencies depending on the phylogenetic relationship of the participating genomes. We showed that the *ph1b*-mediated homoeologous recombination was very effective in inducing recombination between the large translocations, TC5 from *Th. intermedium* and T4m from *Th. ponticum* (Ayala-Navarrete et al. 2007). Recombinant individuals were identified and followed through subsequent generations using molecular markers. The digenomic translocations showed Mendelian inheritance and no deleterious effects were observed on yield or plant morphology. In a number of these recombinant translocations resistance genes *Bdv2* and *Lr19* had been combined. The recombinant translocations were named *Pontins* (Ayala-Navarrete et al. 2007). In the present study, individual fixed recombinants are given unique numbers in the series (Pontin 1, Pontin 2, etc.). The earlier reported recombinant translocations replaced almost the whole chromosome arm 7DL, as did the T4m translocation itself. Reduced-sized Pontin translocations are desirable to reduce potential genetic drag, and reduce constraints on exploiting other allelic diversity on 7DL in breeding programmes. Furthermore, the threat of the Ug99 group of stem rust races has increased the value of *Sr25*.

This investigation reports the characterization of wheat lines, with chromosome segments from *Thinopyrum intermedium* ($2n = 42$ genome JJJ^SJ^SSS) and *Th. ponticum* ($2n = 70$ genome $JJJJJJ^SJ^SJ^SJ^S$) recombined with each other and present as single heritable units carrying combinations of disease-resistance genes *Bdv2*, *Lr19* and *Sr25* on the long arm of wheat chromosome 7D.

Materials and methods

Plant material

The genotypes utilized in this study included wheat lines *Triticum aestivum* L. ($2n = 42$ genome AABBDD) with and without translocations on chromosome 7DL. Translocation line T4m carries *Lr19* and *Sr25* genes for resistance to leaf rust and stem rust, respectively, on a chromosomal translocation in which almost the whole arm of 7DL is replaced by chromatin from a 7L chromosome arm of *Th. ponticum* ($2n = 70$, genome $JJJJJJ^SJ^SJ^SJ^S$). This line was derived from an EMS mutant of translocation line T4 selected for low yellow flour pigment (Sharma and Knott 1966; McIntosh et al. 1995). A backcross derivative of T4m in the Australian wheat cultivar Cook was used in this study.

Line TC5 has almost the whole arm of 7DL replaced by *Th. intermedium* ($2n = 42$ genome JJJ^SJ^SSS) and line

TC14 has just 20 % of the distal portion of 7DL replaced by a chromosome fragment from *Th. intermedium* (Banks et al. 1995; Hohmann et al. 1996; Ayala-Navarrete et al. 2009). Both TC5 and TC14 carry BYDV resistance gene *Bdv2*. A *ph1b* carrying derivative of cv. Angas was obtained from Dr. Ian Dundas, University of Adelaide. Australian wheat varieties Hartog and Westonia were the background genotypes used for the newly formed translocations.

Homoeologous recombination was induced between two different translocations by selecting plants having the two target translocations each in the hemizygous state, one from *Th. ponticum* and one from *Th. intermedium*, in the same individual, and containing the *ph1b* mutant gene in homozygous state. To achieve this, the two translocation lines were individually crossed to the *ph1b/ph1b* source; the two types of F1 were intercrossed and progeny screened with molecular markers to find individuals that were for example, TC14/T4m and *ph1b/ph1b* (Fig. 1). Once the target germplasm was obtained, individuals with large translocations (TC5 and T4m) from *Th. intermedium* and *Th. ponticum*, showed recombinant progeny after a single meiosis (under the influence of *ph1b/ph1b*). Doubled haploid populations were developed from a number of these individuals using the wheat–maize method at the facilities of the South Australian Research and Development Institute (SARDI). Doubled haploid populations were bioassayed and tested with molecular markers to detect and define fixed recombinants.

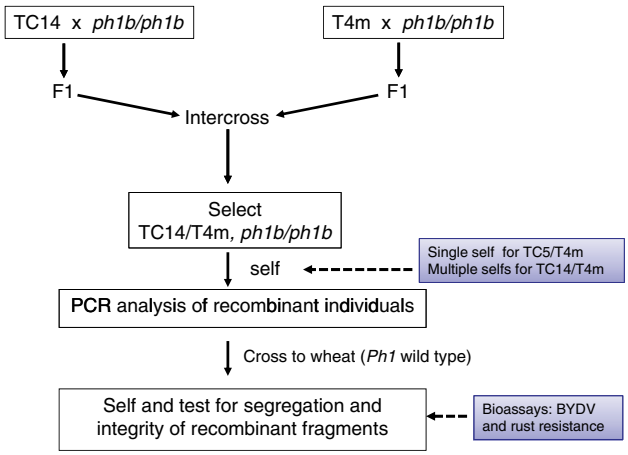

Fig. 1 An outline of the procedure to induce recombination between two translocation fragments located on wheat chromosome arm 7DL. The small *Th. intermedium* translocation, TC14, is used to illustrate the general scheme. For the larger *Th. intermedium* translocation, TC5, some doubled haploids were also generated as described in the "Materials and methods". Once the two alien translocations are together, each in hemizygous state, and in the presence of homozygous *ph1b,* the following meiosis proved to be critical (permissive) for the induction of recombination between the two alien fragments

The stability of the lines carrying desired recombinant chromosomes required the removal of the *ph1b* mutation which was achieved by crossing to cv. Hartog. Genotypes carrying the *Ph1* wild-type gene were identified with the dominant marker ABC920 (Wang et al. 2002) among F2 or backcross populations.

In order to achieve smaller recombinant translocations, the above process was repeated but beginning with individuals with the short *Th. intermedium* translocation TC14 and long T4m from *Th. ponticum.* In this case up to three meiotic cycles were allowed under the influence of *ph1b/ph1b* to obtain recombinants, each cycle selecting for TC14/T4m individuals. Also in this case the rarer putative recombinants were crossed and backcrossed to the wheat cv. Westonia and tested for the presence of the *Ph1* gene. F2 populations of these testcrosses were used for further molecular marker genotyping.

Molecular markers

The characterization of *Thinopyrum* and wheat genome segments was carried out with previously mapped molecular markers (Ayala-Navarrete et al. 2007, 2009) for *Thinopyrum* and *Triticum* genomes. Populations derived from the identified putative recombinants were tested with all relevant markers. Data of the segregating progeny, with and without translocations, were tabulated using the Chi-square (χ^2) test to determine the goodness of fit between the expected ratio for a single gene and the observed phenotypic segregation.

Half seed DNA was extracted in 96-deep-well plates as described in Ayala-Navarrete et al. (2007). An aliquot of 4 μl of the re-suspended DNA was included in a 10-μl PCR reaction and fractionated in 1–3 % agarose gels according to the expected fragment size. Visualization and scoring was done using ethidium bromide under UV light.

Six molecular markers were used to identify individual recombinants at the distal region of 7DL. One dominant *Th. intermedium* (BYAgi), one co-dominant wheat/*Th intermedium* (*gwm37*), three dominant *Th. ponticum* ($Lr19_{130}$, PSY-E1, 3P3/3P4) and one co-dominant *Th. intermedium*/*Th. ponticum*/wheat (BF145935) markers were used. Five more markers were utilized to determine the composition of the proximal region on the recombinant 7DL. These included three co-dominant wheat/*Thinopyrum* (wmc221, BF239181, cfd68) and two dominant wheat (S253737, gwm437) markers (Fig. 1). The order of the markers used is as determined in Ayala-Navarrete et al. (2007).

Bioassays

Virus and rust assays were performed to confirm recombinant lines with and without *Bdv2, Lr19* or *Sr25*. Resistance

to BYDV was evaluated in a controlled environment facility in the CSIRO Canberra laboratories. Five viruliferous aphids were deposited at the base of each of five or more 7-day-old seedlings, covered with transparent vented tubes, and allowed an inoculation period of 3 days at 18 °C. Then seedlings were uncovered and sprayed with insecticide followed by incubation at 18 °C. At 12–15 days post-inoculation the new youngest leaf of each seedling was sampled to perform ELISA as described in Ayala et al. (2001). OD values from each inoculated individual (*I*) were divided by the mean OD of un-inoculated healthy controls (*H*). Typical *I*/*H* values for susceptible controls were above 10.

BYDV assays were also independently conducted in New Zealand Plant and Food Research, Lincoln. Similarly five viruliferous aphids were deposited in clip cages at the base of each of five 7-day-old seedlings, and allowed an inoculation period of 3 days at 22 °C. Then seedlings were uncovered and sprayed with insecticide followed by incubation in a glasshouse at 18–20 °C. At 56 days post-inoculation a mid-leaf of each plant was sampled to perform TAS-ELISA coating with in-house developed anti-BYDV 045 γ globulin and probing with PAV and MAV monoclonal antibodies (Neogen Europe Ltd.) according to the manufacturer's instructions. Similarly, the OD values from each inoculated individual (*I*) were divided by the mean OD of un-inoculated healthy controls (*H*). Typical *I*/*H* values for susceptible controls were above 2.

Resistance to leaf rust and stem rust at the seedling stage, were determined under greenhouse conditions at the University of Sydney, Plant Breeding Institute, Cobbitty. Seedlings of recombinant individuals in homozygote and heterozygote stage were tested for the presence/absence of *Lr19* and *Sr25* according to Bariana and McIntosh (1993). *Puccinia triticina* pathotype 104-1,(2),3,(6),(7),11,13 (culture 547) and *Puccinia graminis* f. sp. *tritici* pathotype 34-1,2,3,6,7,8,9 (culture 205) were used to detect the presence of *Lr19* and *Sr25*, respectively.

Because the Ug99 group of stem rust pathotypes could not be imported and used in Australia, resistance to stem rust under field conditions were carried out in Njoro, Kenya, in 2008, 2009 and 2010 with Ug99 (TTKSK) and Ug99 + Sr24 (TTKST). The dominant strain TTKST overcomes the other resistances in the background wheats, but not *Sr25*. Rust response data were collected on two occasions in each of the three experiments and a final consensus is presented. High disease pressure was achieved in each of the 3 years. The scores in Table 3 from the Kenya experiments use a number to refer to the % leaf area showing infection, and the letters R, MR, M, MS, and S for the host response (where R = no uredinia present; MR = small uredinia with slight sporulation; M = small to medium size uredinia with moderate sporulation; MS = medium size uredinia with moderate to heavy sporulation; S = large uredinia with abundant sporulation). Controls always included the background wheats and the T4m source of *Sr25*.

Results

Identification of recombinants using molecular markers

Recombinant individuals were classified based on the combination of *Th. ponticum* and *Th. intermedium*-specific markers present. Using these markers six recombinant classes were defined for those derived from the two long translocations TC5 and T4m. The recombinants which had undistorted Mendelian inheritance in testcross F2, F3 or backcross populations were selected for further characterization. These recombinant translocations were named Pontin 1 to Pontin 8 (Table 1). Similarly, four recombinant classes were defined which were derived from the TC14 (small *Th. intermedium* translocation) and T4m. Following crosses to control cv. Westonia to stabilize these and separate them from the *ph1b* mutation, they were named Pontin 9–Pontin 14 (Table 1). Pontins 4, 5 and 7 were indistinguishable by this set of markers even though independently derived; Table 1 therefore shows only one of these. The frequency of recovery of recombinants of any type between the two large translocations using the available molecular markers was about 40 %; the frequency of recombination between *Lr19* and *Bdv2* for the large translocations based on a set of doubled haploids was about 16 % (10 out of 61). Recombination between the small TC14 translocation and T4m was much rarer, but difficult to quantify because families were sampled after one to three critical meioses. After the second selfing meiosis, four individuals were of the correct TC14/T4m, *ph1b/ph1b* genotype; these were selfed again and 80–90 progeny were tested from each. Two of the four populations did not show the presence of recombinant individuals. The other two had a high segregation distortion, and around 36 % of putative recombinants. From those recombinants six in one population and four in the other had the desired small alien fragment. Taken together this lineage produced about 3 % recombinants.

The primers derived from the dispersed repetitive sequence, 3P3/3P4, amplified two bands in the *Th. ponticum* parental translocation (T4m) and one similar size band in the *Th. intermedium* parental translocations (TC5 and TC14), but gave no amplification in wheat. Lines derived from TC14 and containing *Th. intermedium* chromatin, amplified one band with the 3P3/3P4 primers and showed the presence of wheat-specific marker S253737 in bin 3; whereas the lines derived from TC5 showed the presence of one 3P3/3P4 band and absence of S253737 marker. Bin4 is immediately proximal relative to bin3 (Fig. 2); when bin

Table 1 The molecular composition of 7DL in Pontin lines

Molecular markers			Parents				Pontin lines (long)						Pontin lines (short)				
Bin	Name	Type	T4m	TC5	TC14	wheat	1	2	3	5*	6	8	9	11	12	13	14
6	cfd68	Co-Dom wheat/*Ti*	0	0	0	0	0	0	0	0	0	0	a	0	0	0	0
1	wmc221	Co-Dom wheat/*Thinopyrum*	2	1	0	0	1	1	1	1	1	1		0	0	0	0
5	gwm437	Dom wheat	2	-	0	0	-	-	-	-	-	-		0	0	0	0
4	BF293181	Co-Dom wheat/*Thinopyrum*	2	1	0	0	1	1	1	1	1	1		0	0	0	0
3	S253737	Dom wheat	2	-	0	0	-	-	-	-	-	-		0	0	0	0
8	3P3/3P4	Dom *Tp*	2	1	1	0	2	1	2	1	1	1	2	1	1	1	1
	BF145935	Co-Dom *Ti*/*Tp*/wheat	2	1	1	0	1	1	1	2	2	2	1	2	2	1	1
	gwm37	Co-Dom *Ti*/wheat	2	1	1	0	1	2	2	2	1	2	1	1	2	2	1
	$Lr19_{130}$	Dom *Tp*	2	1	1	0	2	2	2	2	1	2	2	1	2	2	2
	PSY-E1	Dom *Tp*	2	1	1	0	1	1	1	2	2	2	1	2	2	1	1
	ByAgi	Dom *Ti*	2	1	1	0	2	1	2	2	1	1	2	1	1	1	1

Under the markers type column, the dominant (Dom) and co-dominant (Co-Dom) status is indicated with respect to *Th. intermedium* (Ti), *Th. ponticum* (Tp) and wheat chromatin. At each position the interpretation is given whether the wheat (yellow, 0), *Th. intermedium* (green, 1) or *Th. ponticum* (blue, 2) allele is present. A colourless hyphen means the identity of the allele has not been determined. The asterisk on Pontin 5 is to signify that it is indistinguishable by markers from Pontin 4 and Pontin 7, although they are independently derived; the latter are therefore not included here

[a] The proximal region of Pontin 9 was not genotyped

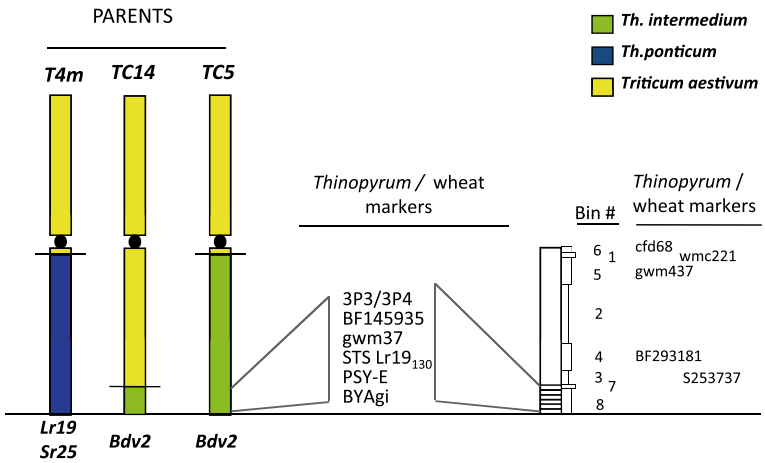

Fig. 2 Schematic representation of the translocations on wheat 7DL from *Th. intermedium* and *Th. ponticum* in different colours: *yellow*, *green* and *blue*, respectively. In the *middle* a list of the *Thinopyrum*/wheat markers used to characterize the distal 20 % region of the recombinant chromosomes. At the *extreme right* the molecular markers identifying the bins at the proximal region of 7DL (colour figure online)

4 markers specific for *Th. intermedium* were tested on TC5 and TC14-derived lines, a diagnostic band was obtained in lines derived from TC5 but not in lines derived from TC14. Therefore, we assumed that the position of 3P3/3P4 in individuals with a small translocation shows the boundary between the end of the TC14 *Th. intermedium* fragment and the beginning of wheat.

Th. intermedium/ponticum segregation

When the two complementary translocations, TC5 and T4m, of similar size but different genomes, were together in the same individual, the various *Th. ponticum* and *Th. intermedium* alleles segregated in Mendelian proportions in the progeny. In contrast, when the translocations were of substantially different sizes, TC14 and T4m, the recovery of *Th. intermedium* alleles from the small translocation was much reduced in progeny compared to *Th. ponticum* alleles. The *Th. ponticum* translocation spanned almost the whole long arm of the chromosome, whereas the TC14 *Th. intermedium* translocation covered just the distal 20 %.

Once the recombinant individuals were identified, the normality of segregation of the recombinant fragments was evaluated using either F2 families or doubled haploid families generated from F1 hybrids with restored *Ph1* gene function (Table 2). For the long recombinant translocations, there were significant distortions from that expected with Pontin 2 and Pontin 3; however, the distortion was in both directions in different families. Pontins 1, 5 and 6 were not distorted. In the case of the short recombinant translocations, Pontins 11–14, all the tested populations showed a normal Mendelian segregation (Table 2).

Table 2 Segregation of recombinant translocations in either F2 or F1-derived doubled haploid families as identified by their composition of molecular marker fingerprints

	Wheat:Het:Pontin (1:2:1 or 1:1)			Total	Chi-square	Significance
	–	het	+			
Long fragment						
Pontin 1	27		15	42	3.4	ns
Pontin 2	15	32	32	79	10.2	**
	40		21	61	5.9	*
Pontin 3	2	4	9	15	9.8	*
	21		12	34	2.4	ns
Pontin 5	13	29	9	51	1.6	ns
Pontin 6	2	4	3	9	0.3	ns
Short fragment						
Pontin 11	15	22	11	48	1.0	ns
Pontin 12	23	39	16	78	1.3	ns
Pontin 13	7	21	9	37	0.9	ns
Pontin 14	2	6	3	11	0.3	ns

Shown are the number of individuals with and without the Pontin translocations (or heterozygotes). The goodness of fit was calculated either to a 1:1 or 1:2:1 segregation as appropriate

ns not significantly different to expected ratio

Significantly different at * $P = 0.05$; ** $P = 0.01$

Bioassays

Individuals with recombinant translocations and with wild-type *Ph1* gene were subject to bioassays for each pathogen and the resistances interpreted in concert with the markers and location of the resistance genes from the linkage map published previously (Ayala-Navarrete et al. 2007).

BYDV bioassays were carried out on all the identified genotypic groups, at both Canberra, Australia, and Lincoln, New Zealand. Resistance was judged using the ratio of ELISA values of infested individuals to un-infested individuals plus an assessment of symptoms. Resistance was assigned to a Pontin class only after consistent assays in two generations or locations; otherwise it is recorded as uncertain (Table 3). The presence of *Bdv2* was determined in two long translocations, Pontins 1 and 2, and in two short translocations, Pontins 13 and 14.

Initially leaf and stem rust evaluations were done in 20 plants at the seedling stage. The set of Pontins from 1 to 8 were developed in Hartog background. Seedling tests from Cobbitty clearly demonstrated the presence or absence of *Sr25* (Table 3). Seedling stem rust results from Cobbitty were confirmed through field screening of Pontins in Kenya against highly virulent pathotypes TTKSK and TTKST. The phytoene synthase (PSY-E1) marker remained linked to *Sr25*, demonstrated by seedling and adult plant stem rust resistance, in this set of recombinants.

In the case of leaf rust, the molecular marker $Lr19_{130}$ consistently detected the presence of leaf rust resistance gene *Lr19* in all individuals tested. In summary, for the long recombinants: Pontins 1 and 2 have *Bdv2* and *Lr19*; Pontins 5 and 8 have *Lr19* and *Sr25*. In summary, for the small recombinants: Pontin 12 has *Lr19* and *Sr25*; Pontins 13 and 14 have *Bdv2* and *Lr19*. None of the Pontins have been shown to have all three resistance genes. Even though Pontin 12 does not carry *Bdv2*, it possesses *Lr19* and *Sr25* on a short translocation. Pontin 11 carries *Sr25* without Lr19 on a short translocation.

Discussion

Recombination between *Th. intermedium* (TC5, TC14) and *Th. ponticum* (T4m) distally located translocations on chromosome arm 7DL of wheat, was obtained by bringing together the translocations in a homozygous *ph1b* mutant background (Ayala-Navarrete et al. 2007; this work). Identification of trigenomic recombinant chromosomes was accomplished by the use of molecular markers. Recombinants were isolated carrying gene combinations of leaf rust (*Lr19*), stem rust (*Sr25)* and BYDV (*Bdv2*) resistances. Since the source of *Lr19* employed in this work, Tm4, was already mutated to reduce the unwanted yellow pigmentation in the flour (Sharma and Knott 1966; Knott 1980, 1984; McIntosh et al. 1995), we have not yet analysed the recombinants for flour colour. However, even in this mutated form, the presence or absence of the *Th. ponticum* phytoene synthase allele, *Psy-E1*, was determined with

Table 3 Bioassay summary for BYDV, leaf rust and stem rust and inferred presence or absence of the target genes *Bdv2*, *Lr19* and *Sr25*

Assigned names	Working names	BYDV (Aust)	BYDV (NZ)	Adult plant stem rust response (Kenya)	Status of resistance loci[a]		
					Bdv2	*Lr19*	*Sr25*
TC5-derived lines							
Pontin 1	PL(C8.4)*33	R	MR	50S	+	+	–
Pontin 2	B11.106.10	R	R	70S	+	+	–
Pontin 3	B11.164.8	R		90S	+	+	–
Pontin 5	PL(B11.15)*9	S	S	5R	–	+	+
Pontin 6	PL(C8.11)*1		S	10R	–	–	+
Pontin 8	PL87-2C		S		–	+	+
TC14-derived lines							
Pontin 9	PL37-2G		S		–	+	(–)
Pontin 11	P84-2D, 2E	S	S	5M	–	–	+
Pontin 12	P84-4D, P85-2H	S	S	5R	–	+	+
Pontin 13	P84-10E, 11F	R	R	40MS	+	+	–
Pontin 14	P86-10F	R	R		+	+	–
Controls							
T4m		S	MS	1R	–	+	+
Hartog*5/TC5		MR	R	50MS-S	+	–	–
Hartog*2/TC14		MR	R	60M	+	–	–
Westonia		S		60S	–	–	–
Hartog		S		30MS-S	–	–	–

(–) absence inferred from marker PSY-E1 and seedling stem rust susceptibility from Cobbitty results

[a] Based on BYDV bioassays (Canberra and Christchurch), field adult plant stem rust assays in Kenya, and seedling leaf rust and stem rust tests in Cobbitty

the presumed perfect marker (Zhang and Dubcovsky 2008). Therefore, none of the Pontins are likely to have the yellow flour associated with the original *Th. ponticum* translocation; furthermore, Pontins 1, 2, 3, 9, 13 and 14 all have the *Th. intermedium* allele of this marker and are unlikely to have yellow flour.

Among the recombinant individuals reported in this work, we describe individuals with *Lr19* and *Bdv2*, *Lr19* and *Sr25*; but to this point we did not find individuals with all three resistance genes. The failure to achieve this and even to achieve a simple *Sr25* and *Bdv2* combination, may be because *Bdv2* and *Sr25* are in closely corresponding regions of the *Th. ponticum* and *Th. intermedium* translocations making it rare to get recombination between them. If that is the case, we would need to do a larger sampling than the one done in this work to identify individuals with combination of three genes. A better strategy to generate recombinants with triple resistance, if it is feasible, would be to intercross Pontin 11 (*Sr25*) and Pontin 13 (*Bdv2* + *Lr19*).

Recombination between translocations from homoeologous genomes, in our case *Th. intermedium* and *Th. ponticum*, have been demonstrated to occur fairly frequently in wheat when the effect of the *Ph1* gene is removed (Ayala-Navarrete et al. 2007). It has been documented that during meiosis homologous chromosomes initially pair through their telomeres forming a structure called a bouquet, and then become intimately associated along their lengths completing their pairing at the centromeres. Colas et al. (2008) demonstrated that at the beginning of meiosis during the bouquet formation, homologous chromosomes go through synchronous conformational changes, greatly enhancing the likelihood of their pairing. The specificity of this process is controlled by the *Ph1* gene that controls synchronous remodelling between homologues to enhance recombination-competent association. The *Ph1* locus is thought to be a cluster of transcribed but defective Cdk-like Ser-Thr phosphatase genes, which suppresses Cdk activity, effecting changes in histone phosphorylation (Greer et al. 2012). In the absence of the *Ph1* gene, asynchronous remodelling occurs, which appears to remove the advantage of homologues over homoeologues in pairing and thereby enables homoeologue association and recombination. In the case of the two large *Thinopyrum* chromosome segments in *ph1b/ph1b* background, their comparable size and phylogenetic relatedness allows contact, pairing and recombination to occur. It would seem that the homology between the two alien translocations is greater than between wheat 7DL and either of the translocations, because no recombinants have

been obtained with wheat even in the absence of the *Ph1* gene.

Marker 3P3/3P4 was based on repetitive sequences (Wang and Wei 1995) and amplified several bands from the *Th. intermedium* and *Th. ponticum* translocations. This suggests that the two relevant genomes have significant similarity, contributing to the successful recombination observed. Despite the similarity, they can only be described as homoeologous genomes and require the absence of the *Ph1* gene effect to recombine. The main evidences that T4m and TC5 are from different genomes are: that the translocations fail to recombine in a normal *Ph1* background; genomic in situ hybridisation suggests the source chromosome of T4m is 7J, and of TC5 is 7S; repetitive sequence-derived markers readily indicate differences between the two. A fuller exploration of this issue is in Ayala-Navarrete et al. (2007).

In our earlier study, we induced recombination using the *ph1b* mutant between two similar-sized translocations Tm4 and TC5 (Ayala-Navarrete et al. 2007), demonstrated that the genes of interest were all in the telomeric portions of both translocations, namely those regions corresponding to wheat deletion bins 7 and 8. The smaller *Th. intermedium* translocation TC14 therefore also overlapped with the region of interest in the large *Th. ponticum* translocation. This information and the high rate of recombinants obtained between the large translocations encouraged us to use the smaller TC14 in order to reduce the amount of foreign chromatin while at the same time retaining the genes of interest. There was a substantially lower number of recombinants in the cross involving mismatched sizes of alien translocation (TC14/T4m), as demonstrated by the need to employ three meiotic cycles to find recombinants. This may simply result from the reduced length of shared adequate homoeology. The 7J chromosome contributing T4m and the 7S chromosome contributing to the TC translocations are more similar to each other than to the 7D wheat chromosome.

The 3P3/3P4 marker was a key feature for finding and characterizing recombinants. This marker amplified a single band in individuals derived from TC5 (large translocation) and a similar band in TC14 (small translocation). Although the target sequences of this marker are dispersed repetitive sequences, it appears that the telomeric 20 % of the *Th. intermedium* fragment in the translocations represented in TC14 has sufficient target sequence for this marker. The mapping undertaken in Ayala-Navarrete et al. (2007, 2009) further showed that the 3P3/3P4 marker behaves as a single locus in TC14 and maps closest to the breakpoint with the wheat chromatin compared to the other markers tested. In T4m the 3P3/3P4 primers amplified a similar size band as in the TC *Th. intermedium* lines plus a larger-sized band. We therefore selected for individuals derived from a TC14/T4m cross containing only the shared smaller 3P3/3P4 *Thinopyrum* amplicon and missing the larger *Th. ponticum*-specific amplicon; i.e. we selected for the *Th. intermedium* 3P3/3P4 allele in combination with any other of the *Th. ponticum* marker alleles; such individuals were assumed to carry a recombinant translocation which was also likely to be a small fragment translocation. This simple operational screen and assumption proved true in most of the cases as was shown by subsequent applications of markers.

Mendelian segregation was observed in most of the families carrying a recombinant fragment; however, three populations showed skewed segregation, two of them towards and the other one against the largest alien fragment. The distortion in favour of the large translocation is probably related to the presence of the segregation distortion genes found in T4m (Prins et al. 1997) and not compensated for in these recombinant families. We offer no explanation for the one case where the distortion is away from the recombinant translocation and toward wheat individuals; however, this individual was derived from a doubled haploid and perhaps that process introduced some unknown rearrangement responsible for the segregation distortion. However, most of the Pontin translocations appear to be normally inherited and should be useful for wheat improvement. Field trial studies are in progress to confirm the behaviour of these translocations in a number of elite wheat backgrounds. Smaller translocations are likely to be more attractive to breeders even in the absence of detected deleterious genes, because larger alien blocks of chromatin represent constraints on utilization of other genetic diversity in those regions.

The series of Pontin recombinant translocations is a new resource for wheat breeders, at this stage offering combinations of *Bdv2* + *Lr19*, or *Lr19* + *Sr25* in the latter case on a smaller alien fragment than has been available to date. Furthermore, these combinations are on a single alien block of chromatin as short as the distal 20 % of chromosome 7DL, which is unlikely to recombine further during normal breeding schemes.

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Conflict of interest The authors declare that they have no other conflict of interest.

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