

Article

The Potential of *Lr19* and *Bdv2* Translocations to Improve Yield and Disease Resistance in the High Rainfall Wheat Zones of Australia

Garry Rosewarne ^{1,2,*}, David Bonnett ^{1,3}, Greg Rebetzke ¹, Paul Lonergan ¹ and Philip J. Larkin ¹

¹ CSIRO Agriculture Flagship, Clunies Ross St, Black Mountain, Canberra 2601, Australia; E-Mails: d.bonnett@cgiar.org (D.B.); greg.rebetzke@csiro.au (G.R.); paul.lonergan@csiro.au (P.J.L.)

² Department of Environment and Primary Industries, 110 Natimuk Rd, Horsham Vic 3400, Australia

³ International Maize and Wheat Improvement Centre, Apdo. 06600 Mexico Distrito Federal, Postal 6-6-41, Mexico

* Author to whom correspondence should be addressed; E-Mail: Garry.rosewarne@depi.vic.gov.au; Tel.: +61-477-616-759.

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Abstract: Chromosomal translocations in wheat derived from alien species are a valuable source of genetic diversity that have provided increases in resistance to various diseases and improved tolerance to abiotic stresses in wheat. These alien genomic segments can also affect multiple traits, with a concomitant ability to alter yield potential in either a positive or negative fashion. The aim of this work was to characterize the effects on yield of two types of translocations, namely T4-derived translocations from *Thinopyrum ponticum*, carrying the leaf rust resistance gene *Lr19*, and the TC14 translocation from *Th. intermedium*, carrying the barley yellow dwarf virus resistance gene *Bdv2*, in Australian adapted genetic backgrounds and under Australian conditions. A large range of germplasm was developed by crossing donor sources of the translocations into 24 Australian adapted varieties producing 340 genotypes. Yield trials were conducted in 14 environments to identify effects on yield and yield components. The T4 translocations had a positive effect on yield in one high yielding environment, but negatively affected yield in low-yielding environments. The TC14 translocation was generally benign, however, it was associated with a negative impact on yield and reduced height in two genetic backgrounds.

The translocation was also associated with a delayed maturity in several backgrounds. The T4 translocations results were consistent with previously published data, whilst this is the first time that such an investigation has been undertaken on the TC14 translocation. Our data suggests a limited role for each of these translocations in Australia. The T4 translocations may be useful in high yielding environments, such as under irrigation in NSW and in the more productive high rainfall regions of south-eastern Australia. Traits associated with the TC14 translocation, such as BYDV resistance and delayed maturity, would make this translocation useful in BYDV-prone areas that experience a less pronounced terminal drought (e.g., south-eastern Australia).

Keywords: translocations; wheat; *Lr19*; *Bdv2*; *Thinopyrum ponticum*; *Thinopyrum intermedium*

1. Introduction

Hexaploid wheat (*Triticum aestivum* L.) has recently been estimated to have formed between 230,000 and 430,000 years ago through rare hybridization events between diploid and tetraploid progenitors [1] with domestication occurring approximately 10,000 years ago. Consequently, modern wheats developed from this original bottleneck [2]. An effective method to increase genetic diversity is to introgress chromosomal segments into the wheat genome from wild relatives. These chromosomal translocations have been used to enhance resistance to various wheat diseases. However, the incomplete homology, referred to as “homoeology”, between the donor and recipient [1] chromosomes, usually prevents recombination. Any deleterious genes that are introgressed along with the useful trait can be difficult to remove. One specific translocation on the 7DL chromosome of wheat, called variously 7Ag.7DL, Agatha or T4 [3–5], from *Thinopyrum ponticum* (tall wheatgrass), contained a number of characterized genes including *Lr19* for leaf rust resistance, *Sr25* for stem rust resistance, *Sd1*, a segregation distortion gene, and a linked gene deleterious for yellow pigment endosperm colour. This T4 translocation has also been associated with higher grain yield, anthesis biomass and delayed maturity in a number of genetic backgrounds [6–8]. Interestingly, the higher yield potential is only expressed under non-moisture stress conditions where yields exceed 2.5 tonnes per hectare [8].

The average yield in the Australian wheat belt is around 2 t/ha largely due to limited water conditions. Under these conditions, the T4 translocation is likely to be unproductive. Indeed, although there is a strong emphasis in maintaining stem rust resistance in Australia, and that *Sr25* is one of the few valuable and effective resistance genes available, no Australian cultivars carry this gene. However, changing agricultural markets have seen an interest in cropping in the higher rainfall areas of Australia (400–800 mm annual rainfall). Under these more favourable conditions, it is more likely that the T4 translocation may be able to provide a yield boost. The use of this translocation is further limited by the yellow pigment locus, conferring a trait that is detrimental to wheat marketing in Australia. Knott [9] used EMS mutagenesis to produce lines with reduced yellow pigment in the flour, yet retain *Lr19* effectiveness. Two of the mutant lines were further evaluated agronomically, with Agatha 28-4 maintaining both *Lr19* and *Sr25* resistance, whilst Agatha 235-6 lost the *Sr25* resistance [3].

Another major yield constraint in parts of the higher rainfall zones of Australia is Barley Yellow Dwarf Virus (BYDV). Banks, *et al.* [10] created a series of translocations from *Th. intermedium* to introduce the BYDV resistance locus, *Bdv2*, onto the 7DL chromosome of bread wheat. These translocations were known as the “TC” series, with TC14 containing the smallest alien DNA segment. Subsequently, this translocation was crossed into various backgrounds but there has not been any definitive study on how this translocation affects yield components of wheat.

In this study, we investigated the effects of the T4 and TC14 translocations on yield in Australian genetic backgrounds under Australian conditions. Potential uses in the high rainfall zones of Australia are discussed.

2. Results

Two sets of germplasm were developed to investigate the effects of the T4 translocation series, which consisted of the original yellow floured T4 translocation and two white floured mutants, Agatha 28-4 and Agatha 235-6 translocations [3], hereafter termed T4m1 and T4m2. In the trials run from 2000 and 2001, single lines from 15 genetic backgrounds were selected from multiple backcrossed material (two to six backcrosses) that contained either the T4m1 or T4m2 translocations. The molecular marker STS-Lr19130 [11] was employed to identify translocation status. The 2006–2008 trials used a different set of T4 translocation material. Multiple selections were made from the pedigree, Seri 82/Superseri, that segregated for the original T4 translocation. Similarly, selections that segregated for the T4m1 translocation were made from a six backcrossed derived series from Condor. Several other elite CIMMYT lines either with or without the T4 translocation were also included in these trials. The TC14 translocation was bred into 12 backgrounds with three to four backcrosses prior to doubled-haploid production from randomly sampled lines. Translocation status was determined with the STS molecular marker BYAgi [12,13].

In total, there were 14 yield trials conducted over five years with average yields ranging from 0.64–6.7 t/ha. There was a large amount of data collected with 1128 plots harvested in relation to the T4 translocations and 1325 plots harvested in relation to the TC14 translocation. All trials were inspected regularly (minimum of three times) throughout the growing seasons and there were no obvious disease in any of the experiments. All germplasm used in each of the trials are listed in Supplementary Table 1 and average yields, number of plots and number of genetic backgrounds in each of the trials is listed in Table 1.

There was a large range of average yields per site due to a wide range of environments and growth conditions. There were also significant treatment effects, genetic background effects and translocation effects for both the T4 translocations and the TC14 translocation. The T4, T4m1 and T4m2 translocations had a negative impact on yield when compared to non-translocation containing lines in two of four, seven of 11, and seven of seven environments, respectively. Yield reductions as a percentage of yield for the T4 and T4m1 translocations were more pronounced in the more water stressed environments and trended towards the positive in sites with higher water availability. The T4m2 translocation had negative effects in all environments tested, whether they were under low or high water stress (Figure 1).

Table 1. Trial sites, numbers of plots and number of genetic backgrounds used in trials.

Year	Site	Average Site Yield	T4 Translocation		TC14 Translocation	
			No. Plots	Genetic Backgrounds	No. Plots	Genetic Backgrounds
2000	Ginninderra ^a	4.71	192	12	-	-
2000	Gundibindyal	6.70	120	9	-	-
2000	Moombooldool	4.27	160	11	-	-
2001	Condobolin	1.57	80	10	-	-
2001	Ginninderra ^a	6.24	80	11	-	-
2001	Gundibindyal	4.26	96	12	-	-
2001	Moombooldool	3.09	96	11	-	-
2006	Griffith ^a	4.66	23	7	177	13
2006	Gundibindyal	n.d.	6	4	90	11
2007	Gundibindyal	1.55	63	8	225	9
2007	Yanco ^a	3.44	29	8	211	9
2008	Temora	1.91	63	9	225	9
2008	Yanco	0.74	59	9	213	9
2008	Yanco ^a	3.97	61	9	211	9
Total	14		1128		1325	

^a Signifies an irrigated trial.

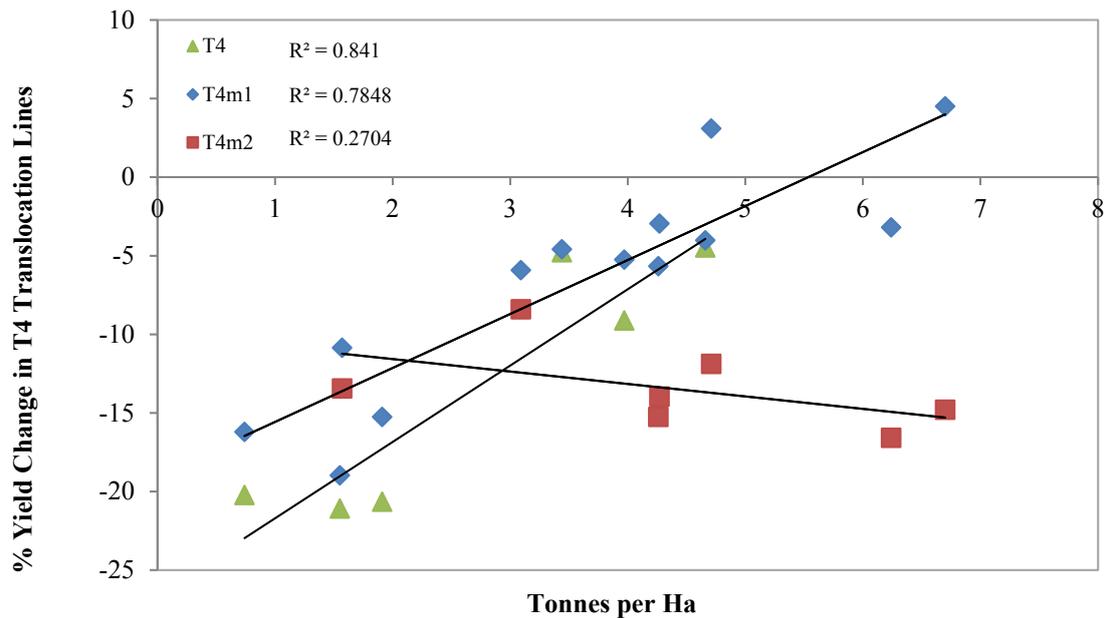


Figure 1. Effects of translocations on yield. Linear trends showing the percentage effects of the different T4 translocations against non-T4 containing lines plotted against the average site yield.

Yield components were most commonly measured for the T4m1 and T4m2 translocations (Table 2). The effects of T4 and T4m1 on yield in the different sites were similar (Figure 1). The T4 or T4m1 translocations had a significant effect on grains·m⁻² at three of the five sites where this was measured

(Table 2) and the percentage effect of the translocation in comparison to non-T4 lines increased as site yield increased (Figure 2a). Thousand kernel weight (TKW) was significantly reduced in three of the five sites tested with T4m1 and the percentage effect of this change in T4 lines was proportionally smaller in higher yielding environments. Harvest index (HI) and height were also significantly different in approximately half of the sites where these traits were investigated for the T4m1 translocation. This compares to the T4m2 translocation which was significantly different from the recurrent parents in most environments for virtually all traits tested. T4m2 was associated with a lower level of grains·m⁻² in two of two environments, lower TKW in one of two environments, lower HI in all five environments, lower biomass in four of five environments, later maturity in three of six environments and were significantly taller in four of six environments.

The T4m2 lines were consistently taller than the recurrent parents by an average of 7% while T4m1 were 2% shorter. Some of the translocations also affected maturity. The T4m2 containing lines were on average 3.4 days later in maturity than the recurrent parents while the other T4 translocations were no different.

In 2008, the Seri 82/Superseri segregants indicated that the T4 translocation had a highly significant effect on yield in rainfed trials where moisture limited yield. The irrigated trial did not show such an effect, neither did the T4m1 translocation in the Condor background (Table 3).

The TC14 translocation had a significant negative effect on yield in two of the six locations tested, namely Temora 2008 and the irrigated site at Yanco in 2008, both being moderately yielding sites. Maturity was significantly delayed in the TC14 lines in all four environments tested, and ranged between 1.8 and 4.0 days (Table 4). The TC14 translocation had a significant negative effect on grains·m⁻² in one environment and in this environment, it also was associated with a significant yield reduction. TKW was unaffected by the TC14 translocation, and hectolitre and height were significantly different in one environment each.

Background genotypes had an effect on whether TC14 was associated with reduced yield. The lower yields in the irrigated site at Yanco in 2008 were most prevalent in the H45 background ($p > 0.01$), followed by Janz ($p > 0.05$) and to a lesser degree in the Westonia/Sunbrook backgrounds ($p > 0.1$). The H45 background also showed a highly significant correlation of yield reduction in the TC14 lines at Temora 2008 ($p > 0.01$) along with Camm and Westonia ($p > 0.05$) (Table 5). Maturity and height were also investigated where records were taken. At the irrigated Yanco 2008 site, the TC14 containing lines in H45 and Janz backgrounds were significantly shorter than their null counterparts ($p > 0.05$) (Table 5). This data was not collected for the Temora 2008 trial.

Table 2. Effects of T4 translocations on grain yield, yield components, maturity and height.

Environment	Germplasm ^a	Yield (t/ha)	KGrain·m ⁻²	TKW (mg)	HI	Total Biomass (t/ha)	Maturity ^b	Height (cm)
Ginninderra 2000 ^{c,d}	Recurrent Parents	4.80					61.5	
	T4m1	5.24					58.3	
	$H^2 = 0.81$	T4m2	4.46 **				58.3 **	
Gundibindyal 2000 ^d	Recurrent Parents	6.64					58.4	101.3
	T4m1	6.93					61.2	100.7
	$H^2 = 0.89$	T4m2	5.32 **				58.6 *	116.1 *
Moombooldool 2000 ^d	Recurrent Parents	4.35			0.429	10.48	61.5	79.8
	T4m1	4.31			0.438	9.74	63.0	77.5 ***
	$H^2 = 0.74$	T4m2	3.81 **		0.415 *	9.09 *	61.6 ns	87.1 ***
Condobolin 2001 ^d	Recurrent Parents	1.67	6017	28.1	0.357	4.74	67.2	63.4
	T4m1	1.56 ***	5786	26.6	0.341	4.50	68.7	61.5 *
	$H^2 = 0.83$	T4m2	1.39 ***	5420 *	26.7	0.321 ***	4.42 *	65.0 ns
Ginninderra 2001 ^{c,d}	Recurrent Parents	6.57	17981	36.9	0.430	15.28	62.3	91.0
	T4m1	6.28	18517	34.1 ***	0.406 **	15.53	61.4	90.6
	$H^2 = 0.86$	T4m2	5.49 ***	17150 *	32.3 ***	0.371 ***	14.75 ns	59.2 **
Gundibindyal 2001 ^c	Recurrent Parents	4.57			0.393	11.64		89.2
	T4m1	4.23 **			0.380 *	11.18		85.4 ***
	$H^2 = 0.68$	T4m2	3.82 ***			0.344 ***	11.10 *	90.8
Moombooldool 2001 ^c	Recurrent Parents	3.33			0.384	8.81	58.8	78.9
	T4m1	3.06 **			0.375 *	8.10	58.9	74.6 **
	$H^2 = 0.73$	T4m2	2.98 ***			0.359 ***	8.33 **	57.6

Table 2. Cont.

Environment	Germplasm ^a	Yield (t/ha)	KGrain·m ⁻²	TKW (mg)	HI	Total Biomass (t/ha)	Maturity ^b	Height (cm)
Gundibindyal 2007 ^c	T4 negative lines	1.92	5527	34.9	0.366	5.05	59.5	63.8
	T4	1.70 ***	5163 **	33.5	0.343 *	4.88	63.5 ns	64.3
	$H^2 = 0.66$ T4m1	1.59 **	5152	31.4 **	0.371	4.22 *	62.0 ns	62.6 *
Temora 2008 ^c	T4 negative lines	2.22	6006	35.5				
	T4	1.85 ***	5150	34.9				
	$H^2 = 0.73$ T4m1	1.74 ***	4844 **	35.8 *				
Yanco 2008 ^{c,e}	T4 negative lines	4.00	13194	30.5			69.4	82.4
	T4	3.80	13626	27.9 *			69.9	86.3 **
	$H^2 = 0.81$ T4m1	3.70 *	12975 *	28.7			68.9 *	83.7
Yanco 2008 ^c	T4 negative lines	0.71						
	T4	0.69						
	$H^2 = 0.47$ T4m1	0.56 *						

* Represents significant at $p > 0.1$, ** at $p > 0.05$ and *** at $p > 0.01$ between the recurrent parents/T4 negative lines and either the T4, T4m1 or T4m2 lines; ^a Recurrent parents refers to the backcross parents that did not contain the translocation, T4 negative lines to a range of elite germplasm and null lines from the two T4 doubled haploid series, T4 to lines containing the original translocation, T4m1 and T4m2 to the mutated translocations as described in materials and methods [9]; ^b Maturity scores according to Zadoks *et al.* [14]; ^c irrigated trials; ^d Trials compared single lines from highly backcrossed selections that contained the translocation to the recurrent parents; ^e Trials compared a number of elite CIMMYT lines (with and without the translocation) combined with two sets of sister lines that segregated for either the T4 or T4m1 translocations; ns = not significant.

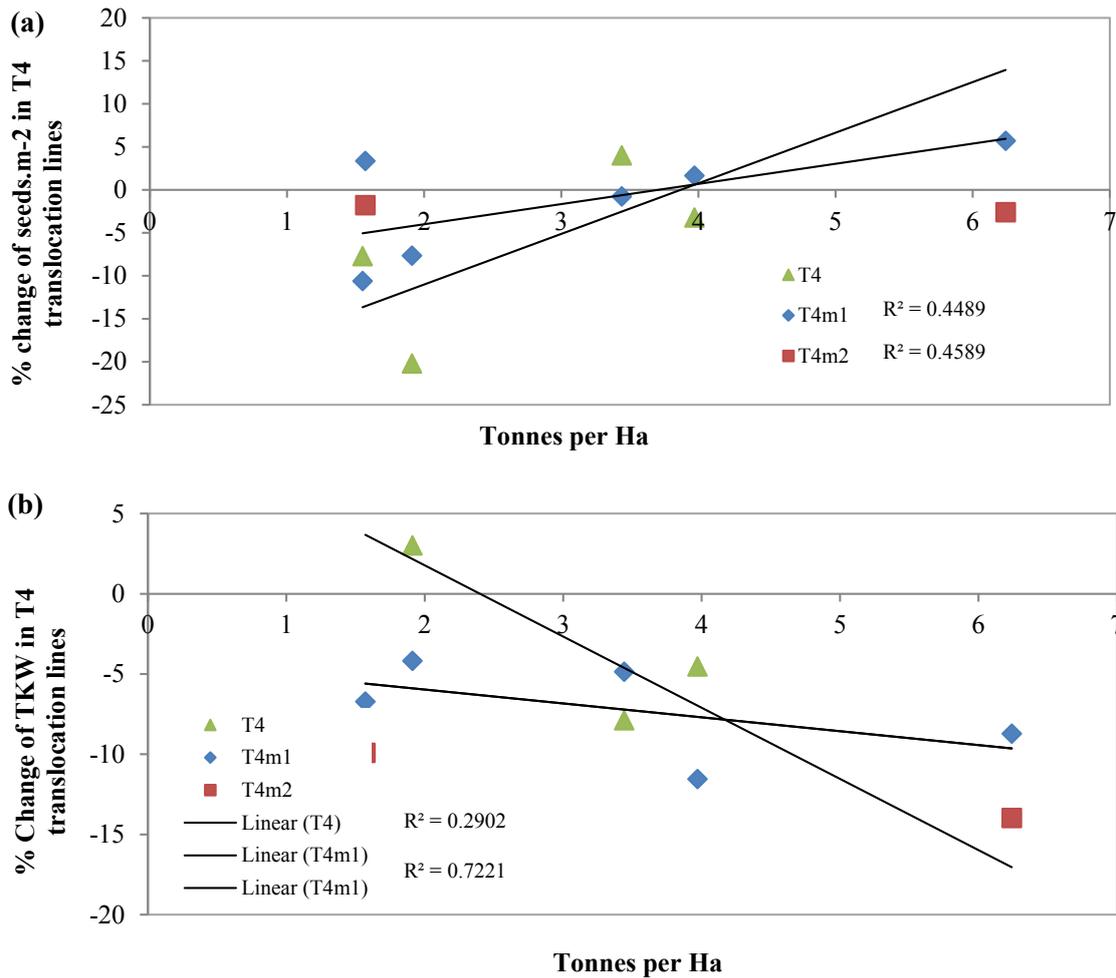


Figure 2. The percentage change attributed to the T4 translocations for (a) seeds·m⁻² and (b) TKW. T4m2 only had two data-points.

Table 3. Effects of the T4 and T4m1 translocations on yield (t·ha⁻¹), grains·m⁻², thousand kernel weight (TKW) and maturity. Comparisons were made from two sets of sister lines that segregated for the original T4 translocation in the Seri 82/Superseri cross, and for the T4m1 translocation in a highly backcrossed Condor background.

Site	Germplasm	No. of lines	t·ha ⁻¹	Grains·m ⁻²	TKW	Maturity ^a
Temora 2008	Seri 82/Superseri null	15	2.43	7387	32.8	
	Seri 82/Superseri T4	6	1.74 ***	5432 ***	32.0	
	Condor null	13	2.14	6389	32.9	
	Condor T4m1	10	1.93	6055	31.7	
Yanco 2008	Seri 82/Superseri null	13	0.81			
	Seri 82/Superseri T4	6	0.50 ***			
	Condor null	10	0.58			
	Condor T4m1	10	0.54			
Yanco 2008 ^b	Seri 82/Superseri null	15	3.98	13484	29.53	69.13
	Seri 82/Superseri T4	6	3.58	13355	26.73 ***	68.67
	Condor null	10	3.77	13745	27.59	69.50
	Condor T4m1	10	3.38	13634	24.87 ***	69.90

*** Represents significance at $p > 0.01$, significance was tested at $p > 0.1$, $p > 0.05$ and $p > 0.01$.^a Maturity scores according to Zadoks *et al.* [14];^b irrigated trial.

Table 4. Effects of the TC14 (*Bdv2*) translocation on yield, yield components, maturity and height.

Environment	Germplasm ^a	Grain Yield (t/ha)	Grain Number (no·m ⁻²)	TKW (mg)	Maturity ^b	Hectolitre Weight	Height (cm)
Griffith 2006 ^c	Parents	4.87			57.4		87.6
	Null	4.55			59.1		85.7
	$H^2 = 0.72$ TC14	4.62			55.1 ***		86.3
Gundibindyal 2007	Parents	1.43	4,012	36.2	60.0	80.0	57.3
	Null	1.55	4,642	34.5	60.8	79.3	60.4
	$H^2 = 0.59$ TC14	1.48	4,398	34.8	59.0 *	80.0 **	60.2
Yanco 2007 ^c	Parents	3.82	11,362	33.9	63.7	79.7	76.3
	Null	3.75	11,572	32.7	63.8	80.0	75.6
	$H^2 = 0.60$ TC14	3.63	11,093	33.0	61.9 *	80.1	73.0 *
Temora 2008	Parents	2.34	6,757	33.6			80.4
	Null	2.28	6,611	33.5			79.7
	$H^2 = 0.46$ TC14	2.10 **	6,238	33.0			79.8
Yanco 2008	Parents	0.69					
	Null	0.67					
	$H^2 = 0.49$ TC14	0.65					
Yanco 2008 ^c	Parents	4.19	13,494	31.4	66.4	79.6	83.3
	Null	4.11	14,170	29.2	68.0	78.5	81.9
	$H^2 = 0.71$ TC14	3.95 ***	13,763 *	29.2	65.6 **	78.9	80.2

* Represents significance at $p > 0.1$, ** at $p > 0.05$ and *** at $p > 0.01$ between the null and TC14 containing lines; ^a Parents represents the backcross parents used to generate the null and TC14 lines, null lines are progeny that do not contain TC14, and TC14 are progeny lines with the translocation; ^b Maturity scores according to Zadoks *et al.*[14]; ^c irrigated trials.

Table 5. Association of the TC14 lines with changes in yield, maturity [14] and height in different genetic backgrounds.

Germplasm	No. of Lines ^a	2006 Griffith ^b			2007 Gundibindyal			2007 Yanco ^b			2008 Yanco ^b			2008 Temora	2008 Yanco
		Yield	Maturity	Height	Yield	Maturity	Height	Yield	Maturity	Height	Yield	Maturity	Height	Yield	Yield
Thelin/4*Camm Null	12	-	-	-	1.81	49	63	3.66	63	77	3.76	70	77	2.68	0.62
Thelin/4*Camm TC14	12	-	-	-	1.70	48	63	3.54	62	75	3.71	69	78	2.39 *	0.56
Thelin/4*Chara Null	12	-	-	-	1.26	47	61	4.14	57	75	4.52	67	85	2.33	0.45
Thelin/4*Chara TC14	12	-	-	-	1.32	44	63	3.86	55	70 *	4.33	64	82	2.28	0.46
Thelin/4*Drysdale Null	(15) 11	4.87	57	87	2.17	48	63	4.23	60	75	4.25	70	83	1.46	0.55
Thelin/4*Drysdale TC14	(4) 7	5.85	52	96 *	2.09	46 *	61	4.08	58 *	72	4.10	67 *	76	1.60	0.53
Thelin/4*H45 Null	(17) 12	4.99	67	85	1.53	52	67	3.85	66	73	4.70	70	85	2.86	0.76
Thelin/4*H45 TC14	(8) 12	4.85	64 **	85	1.53	52	67	3.66	66	73	4.33 **	70	81 **	2.66 **	0.76
Thelin/4*Janz Null	(5) 12	4.65	51	79	1.14	48	56	3.83	60	73	4.23	69	81	2.40	0.69
Thelin/4*Janz TC14	(3) 12	4.61	49	76	1.13	46 **	51 *	3.85	56 *	72	3.87 *	68	78 *	2.13	0.64
Thelin/3*Drysdale//Sunbrook Null	(19) 14	4.19	56	88	0.95	45	53	3.71	53	78	4.14	60	83	2.53	0.65
Thelin/3*Drysdale//Sunbrook TC14	(10) 13	4.29	52	86	1.15	45	56	3.43	51	70 *	4.04	60	81	2.39	0.78
Thelin/3*Westonia//Sunbrook Null	(14) 14	4.54	60	84	1.75	48	62	3.42	59	75	4.40	65	79	2.21	0.75
Thelin/3*Westonia//Sunbrook TC14	(10) 17	4.35	54	91	1.58	47	61	3.74	57	78	4.09	59	83	2.10	0.69
Thelin/4*Westonia Null	(10) 12	4.69	63	79	1.15	54	50	4.01	66	70	4.37	70	77	2.24	0.64
Thelin/4*Westonia TC14	(9) 12	4.86	59	79	1.16	52	52	4.27	65	74	4.38	69	80	1.80 *	0.54
Thelin/3*H45//Darter Null	(5)	3.72	63	91	-	-	-	-	-	-	-	-	-	-	-
Thelin/3*H45//Darter TC14	(2)	3.89	64	95	-	-	-	-	-	-	-	-	-	-	-
Chara/Ohm/Sunbrook Null	(2)	4.25	65	74	-	-	-	-	-	-	-	-	-	-	-
Chara/Ohm/Sunbrook TC14	(2)	4.94	41 **	95	-	-	-	-	-	-	-	-	-	-	-

* Represents significance at $p > 0.05$ and ** at $p > 0.01$ between the null and TC14 containing lines; ^a The number of lines in each trial are shown for Griffith 2006 as a parenthesis, and as normal text for the remainder of the trials; ^b Indicates trials that had irrigation.

3. Discussion

Extensive genetic material was developed and thoroughly tested in yield trials in Australia. Previous research with the T4 translocation indicated that it drove a significant yield boost under high-moisture conditions, but had a negative impact on yield when water limited production to under $2 \text{ t}\cdot\text{ha}^{-1}$ [6–8]. This study identified similar phenomenon with the white floured T4m1 translocation, but not with the T4m2 translocation (Figure 1). In our trials, the original T4 translocation was only tested in relatively low yielding environments, and although it did not show a yield boost, its relative yield effect in relation to average site yield trended in a similar fashion to that of T4m1. Both T4m1 and T4m2 were developed through an EMS mutagenesis screen with selection based upon white flour colour [9]. Zhang and Dubcovsky [15] surmised that the different mutations in the original translocation were responsible for the white flour colour in these two lines but it was not clear which regions within the translocation were affected. These mutations did not affect leaf rust resistance however T4m1 retained the effective *Sr25* locus whilst T4m2 did not [3], indicating this latter mutation line may have an abnormal makeup following the mutagenic treatment. Physical mapping placed *Sr25* and the yellow flour colour locus (*PSY-E1*) together at the telomeric end of the translocation [16–18]. This large T4 translocation obviously contains many loci affecting different traits in wheat, the data presented in Figure 1 suggests that the T4m2 translocation not only lost yellow pigmentation and *Sr25* resistance, but appears to no longer provide a yield increase under non-water stress conditions.

Simplistically, grain yield can be determined from the two major yield components of $\text{grains}\cdot\text{m}^{-2}$ and grain weight [19]. The correlations between yield and $\text{grains}\cdot\text{m}^{-2}$ with both the T4 and T4m1 translocations indicate that $\text{grains}\cdot\text{m}^{-2}$ impacted on yield, and this impact overrode a negative correlation between yield and kernel weight (Figure 2). Interestingly, the T4m2 lines had neither correlation, further supporting the lack of a genetic effect of this mutated translocation on yield. Previous work has shown that under high yielding conditions, the increased yield potential conferred by the T4 translocation was mainly due to an increase in $\text{grains}\cdot\text{m}^{-2}$, grains per spike and harvest biomass [6,7]. Singh, Huerta-Espino, Rajaram and Crossa [6] also observed a higher kernel weight but a lower harvest index in the T4 material. Harvest index appeared altered most significantly in the T4m2 containing lines, and is likely to be related to a significant change in maturity.

Significant drivers of wheat yield are optimal height and maturity for any given environment. The evidence presented herein indicates that T4 and T4m1 had no effect on maturity but did reduce height, while T4m2 lines were taller and later. Previous work provided conflicting results with Singh, Huerta-Espino, Rajaram and Crossa [6] indicating that T4 lines were significantly later in maturity and taller, whereas Reynolds, Calderini, Condon and Rajaram [7] indicated that there was no difference in maturity conferred by the presence of T4. Knott [3] developed the original white floured mutants of T4m1 and T4m2 and found that they conferred a mild delay in maturity, averaging one day, although significance tests were not presented. Knott's study also indicated that there was no difference in height. The average delay in flowering of the T4m2 lines of 3.4 days could have a significant impact on yield under the conditions in which we tested them. The trial sites in southern NSW generally have a tight finish brought on by hot, dry weather conditions. Later flowering would result in grain-filling under less than optimal conditions and is evidenced by the consistently lower harvest index. The effect of T4m2 on TKW was only investigated in one environment, and

showed a highly significant reduction, and this could also be attributed to grain-filling under sub-optimal conditions.

The genetic background may have affected the expression of the yield reduction associated with the T4 translocation. The highly significant lower yield in the T4 containing lines in moisture-stressed environments was only observed in the Seri 82/Superseri series. It seems unlikely that the Condor background was better adapted to these environments as the null lines yielded lower than the corresponding Seri 82/Superseri null lines. It would be interesting to trial these two series in higher yielding environments. This potential background genetic interaction needs to be considered when deploying such material in a breeding program.

A negative effect associated with the TC14 translocation on yield appeared to be both environment and genotype dependent. Moderately yielding 2008 trials were the only ones to show this negative effect. September and October were drier and hotter than long term averages in 2008 (http://www.bom.gov.au/climate/annual_sum/2008/index.shtml), conditions which may have affected yields in the later maturing TC14 retaining lines. However, the background genotypes with the greatest yield reductions associated with TC14 were H45 and Camm. There was no evidence that TC14 was associated with changed maturity in these particular backgrounds. Furthermore, in the Drysdale background, maturity was consistently delayed in the TC14 containing lines, yet there was no significant yield difference. This evidence would suggest that later maturity did not drive yield reductions associated with the TC14 translocation. On the other hand, a height reduction was significantly associated with both a yield reduction and the presence of TC14 in some genetic backgrounds. The impact of height on yield in the 2008 season is unknown although if the shorter plants also had smaller root systems, the hot, dry spring of that year may have enabled longer rooted plants to better extract water from the soil profile, however no direct evidence of this was collected.

The resistance loci that are present on these translocations are of undoubted use in Australia. However, their deployment would need to be done judiciously. The T4m1 translocation is preferred in Australia over T4 or T4m2 due to loss of yellow flour pigmentation and the maintenance of both *Sr25* and *Lr19*. The effects on yield would limit the use of T4m1 to the more productive region where the yield effect could be advantageous. Due to the lack of use in Australia, *Lr19* is still effective against all known leaf rust pathotypes in this country, and could potentially be a useful adjunct to breeders. However, there is a caveat to this in that *Lr19* has been overcome in Mexico [20] and the Indian sub-continent [21]. This would be unlikely to happen in Australia unless *Lr19* were to become common in varieties across the wheat growing regions. It is more probable that the yield effects of this translocation would limit its use to the more productive higher rainfall zones in south-eastern Australia.

The stem rust resistance gene, *Sr25*, also has potential for Australian deployment as it is effective against a potentially devastating family of stem rust races currently evolving in western Africa, the Ug99 stem rust family. Stem rust can be of major concern in highly productive regions and this gene would not only protect varieties from endemic stem rust races, but also against the Ug99 family should an incursion into Australia occur.

The TC14 translocation also contains a useful resistance locus in the *Bdv2* gene. Barley yellow dwarf virus is a disease that has the potential to cost the Australian wheat industry \$15 million annually [22] and is also associated with the more productive Australian wheat growing regions. Ayala-Navarrete, Mechanicos, Gibson, Singh, Bariana, Fletcher, Shorter and Larkin [18] recombined T4 translocations

with TC14 translocations to develop the Pontin translocation series, some of which contain combinations of *Bdv2* and *Lr19* on a much shorter recombinant translocation than the T4 translocations in this current study. Other Pontin lines have *Lr19* and *Sr25* on a short translocation, or *Sr25* alone on a shorter translocation. Some Pontin recombinants have retained *Bdv2* and *Lr19* while losing the PSY-E1 marker associated with yellow flour. It is hoped that some of these will have useful combinations of genes for use in breeding, and it will be important to assess the impact they have on yield potential in different environments.

4. Experimental Section

4.1. Development of Germplasm

Germplasm was developed through a combination of traditional backcrossing and doubled-haploid production. Most of the T4-containing lines were developed by the National Cereal Rust Control Program (Cobbitty, Australia) and comprised 15 recurrent parents (B2806, Batavia, Banks, Bayonet, Condor, Cook, H45, Kiata, Lark, Lowan, Matong, Oxley, R38549H, R38568C and Vulcan) using two white-floured EMS generated T4 donor mutants Agatha 28-4 and Agatha 235-6 [9]. Herein, we have termed these donor lines as T4m1 and T4m2, respectively, maintaining consistency with the original T4 translocation. Both of these variants maintained their *Lr19* status but only the former retained *Sr25* conferred resistance. Other sets of sister lines were developed through doubled haploid methodology from Seri 82/Superseri which segregated for the original yellow floured T4 translocation and from Condor/CondorT4m1 which segregated for T4m1. The TC14 lines used Oasis 86//TC14/2*Spear as the translocation donor and either three or four backcrosses made to either elite parents or from crosses between elite parents. The recurrent parents of these lines were Brookton, Camm, Chara, Drysdale, H45, Janz, Westonia, H45/Darter, Janz/Sunbri, Drysdale/Sunbrook, Westonia/Sunbrook and Chara/Ohm//Sunbrook. All lines and pedigrees are listed in Supplementary Table 1. These doubled haploids were generated using the maize cross method under contract through the South Australian Research and Development Institute, Adelaide.

4.2. Yield Trials

Yield trials were run in two stages. Most of the T4 material was used in experiments conducted at multiple sites in the year 2000 (Ginninderra, ACT, Stockinbingal, NSW and Moombooldool, NSW), and in 2001 (Ginninderra, Gundibindyal, NSW and Condobolin, NSW). A second set of experiments contained up to 19 Condor-derived lines and up to 23 Seri/Super Seri derived lines that had differential statuses for the T4m1 and the T4 translocations, respectively. These experiments were also used for the TC14 translocation. The second set of experiments were conducted between 2006 and 2008 at Griffith, Gundibindyal, Yanco and Temora (all in NSW). Details of trial sites are listed in Table 1. The trials conducted in 2000 and 2001 were alpha-lattice designs, the 2006, 2007 and 2008 experiments were partially replicated, row-column designs.

All trials were sown in 10-row plots with 18 cm spacing and a 50 cm gap between plots. Plots were 8 m in length. Sowing rate was 100 kg/ha with 105 kg/ha Starter 15[®] fertilizer applied at sowing while irrigated experiments were broadcast with an additional 40–80 kg/ha urea at late booting. Where trials

were irrigated, seed was sown on a full soil-water profile with an additional irrigation at late booting. Dryland sites were sown under the prevailing weather conditions. Sites were monitored for foliar diseases, but as none were observed, no fungicide treatments were required. Maturity scores were taken according to Zadoks, Chang and Konzak [14] when most of the lines were at mid ear emergence (Growth Stage 55). Heights were also scored on plots. Thousand kernel weight was determined from 250 random grains and hectoliter weight calculated on a chondrometer designed in CSIRO that weighs the volume of 0.5 L of grain.

4.3. Statistical Analysis

Data were analyzed statistically after first checking for normality and error variance heterogeneity across environments. Hereafter, a two-stage, mixed model approach was utilized [23] using ASReml [24]. Each experiment was analyzed separately with the best spatial models being determined after first fitting the experimental design and then modelling the residual variation with autoregressive row and column terms. Significant spatial effects were then identified and residuals assessed before determinations made to the need for fitting of other (e.g., linear) effects [25]. For each experiment, the best linear unbiased estimates (BLUEs) and their weights [26] were used as inputs in the subsequent across-experiment analyses. Individual entry and translocation group means were obtained for each experiment and SED and LSDs obtained for pairwise comparison. Broad-sense heritability (H^2) was calculated on a single-plot and genotype-mean basis with standard errors estimated after Holland, *et al.* [27].

5. Conclusions

In conclusion, the T4 and T4m1 translocation results confirmed previous reports that indicate they provide a yield boost under high yielding conditions, yet are detrimental to yield under low yielding conditions. However, the T4m2 translocation would appear to be of little use as the yellow flour mutation also rendered *Sr25* and a yield locus ineffective. The TC14 translocation appeared to have a specific interaction with reduced height in two genetic backgrounds that were correlated with lower yields. Overall, the TC14 translocation appeared to delay flowering, but more detailed analysis showed this was also dependent on genetic background and did not appear to be associated with a yield penalty. BYDV resistance conferred by TC14 should make it useful in higher yielding environments where this disease is prevalent.

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Author Contributions

Garry Rosewarne conducted trials in 2006–2008, collated and interpreted all data and wrote manuscript. David Bonnett conducted all trials in 2000–2001. Greg Rebetzke conducted trial design and statistical analysis, Paul Lonergan oversaw field trial operations, Philip J. Larkin originally conceived experiments and developed TC14 genetic material.

Conflicts of Interest

The authors declare no conflict of interest.

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