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ORIGINAL PAPER

Genetic and QTL analyses of seed dormancy and preharvest sprouting resistance in the wheat germplasm CN10955

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Abstract The inheritance and genetic linkage analysis for seed dormancy and preharvest sprouting (PHS) resistance were carried out in an F_8 recombinant inbred lines (RILs) derived from the cross between "CN19055" (white-grained, PHS-resistant) with locally adapted Australian cultivar "Annuello" (white-grained, PHS-susceptible). Seed dormancy was assessed as germination index (GI7) while assessment for preharvest sprouting resistance was based on whole head assay (sprouting index, SI) and visibly sprouted seeds (VI). Segregation analysis of the F_2, F_3 data from the glasshouse and the RIL population in 2004 and 2005 field data sets indicated that seed dormancy and PHS resistance in CN19055 is controlled by at least two genes. Heritabilities for GI7 and VI were high and moderate for SI. The most accurate method for assessing PHS resistance was achieved using VI and GI7 while SI exhibited large genotype by environment interaction. Two quantitative trait loci (QTLs) *QPhs.dpivic.4A.1* and *QPhs.dpivic.4A.2* were identified. On pooled data across four environments, the major QTL, *QPhs.dpivic.4A.2*, explained 45% of phenotypic variation for GI7, 43% for VI and 20% for SI, respectively. On the other hand, *QPhs.dpivic.4A.1* which accounted for 31% of the phenotypic variation in GI7 in 2004 Horsham field trial, was not stable across environments. Physical mapping of two SSR markers, *Xgwm937* and *Xgwm894* linked to the major QTL for PHS resistance, using Chinese Spring deletions lines for chromosome 4AS and 4AL revealed that the markers were located in the deletion bins 4AL-12 and 4AL-13. The newly identified SSR markers (*Xgwm937*/*Xgwm894*) showed strong association with seed dormancy and PHS resistance in a range of wheat lines reputed to possess PHS resistance. The results suggest that *Xgwm937*/*Xgwm894* could be used in marker-assisted selection (MAS) for incorporating preharvest sprouting resistance into elite wheat cultivars susceptible to PHS.

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Introduction

Pre-harvest sprouting (PHS) is one of the most important quality issues in many wheat-producing areas especially in environments characterised by summer rainfall and high

humidity. Most of the Australian wheat varieties are white-grained, which are generally considered to be more susceptible to sprouting than red-grained wheat (Mares et al. 2005). In Australia, both durum and common wheat processors demand white wheat varieties. The northern wheat belt which consists of northern New South Wales and southern Queensland is located in a predominantly summer-rainfall area with a high probability of rain occurring during the harvest months of October–December. As such the chances of sustaining weather damage in this region are extremely likely (Derera 1982) and on average, occur every third year (Edwards et al. 1989). PHS damage often results in down-grading of premium milling quality wheat to feed quality, which results in seriously reduced pay-outs to farmers. Most recently, the cost of down-grading has been estimated at $80/tonne, and average losses due to PHS across the Australian wheat crop was estimated to be between $30 and $40 million annually (Abawi and White 2000). Resistance to PHS is therefore a highly desirable trait sought by plant breeders.

Pre-harvest sprouting is a complex trait that is affected by many environmental and genetic cues. The inheritance of components of PHS resistance including seed dormancy has been studied in wheat using classical Mendelian and molecular approaches. Mares (1996) indicated that two or more independent genes controlled dormancy in white-grained wheat. Similarly, Lawson et al. (1997) reported that resistance to sprouting in wheat assessed under artificial rain treatment was controlled by two independent genes. Results from other studies in wheat suggested that multiple genes might be involved in conferring seed dormancy and PHS resistance. This is evidenced by the reports of more than 20 QTL linked to PHS resistance in as many as 20 different chromosomes including a recently identified QTL on chromosome 1D (summarised in Flintham et al. 2002; Ogbonnaya et al. 2006) using diverse mapping populations. Anderson et al. (1993) identified eight regions related to PHS resistance in winter wheat populations. Some of the most significant QTL for PHS resistance have been located on group 3 and 4 chromosomes and were commonly observed in most of the populations (Mori et al. 2005; Mares et al. 2005; Kulwal et al. 2005; Osa et al. 2003; Groos et al. 2002; Kato et al. 2001; Bailey et al. 1999). Previous work in the 4A chromosomal region demonstrated close genetic linkage of seed dormancy with SSRs-*Xgwm269*, *Xgwm397*, *Xbarc170* (Mares et al. 2005; Tan et al. 2006). However, there is still no diagnostic marker available, which has proven fully effective in introgression of this important QTL into elite wheat cultivars because the available marker(s) are either multi-locus in nature or non-polymorphic in most wheat cultivars. This may reflect the diversity of PHS resistance mechanisms in wheat as well as the variation in the assessment of preharvest sprouting resistance. The use of co-dominant DNA markers tightly linked to various components of PHS resistance would help breeders to apply marker-assisted selection and hasten the incorporation of PHS resistance from unadapted germplasm into locally adapted elite wheat cultivars.

The objectives of our study were to: (1) utilise F_2, F_3 and RIL populations derived from a cross of "CN19055" (PHS resistant-parent) with locally adapted Australian cultivar "Annuello" (PHS-susceptible parent) to study the genetic basis of seed dormancy and PHS resistance in CN19055, (2) identify chromosomal positions of QTL controlling PHS resistance from CN19055, (3) compare QTL with those detected in previously reported studies, and (4) identify SSR markers tightly linked to components of PHS resistance that are polymorphic in diverse wheat germplasm which could be used to aid the elimination of this defect in wheat and which are also amenable to high-throughput genotyping.

Materials and methods

Plant material, field evaluations and DNA extraction

CN19055 (W98616) is a white-grained preharvest sprouting resistance wheat germplasm, which was developed at the Crop Development Centre, Department of Plant Sciences, University of Saskatchewan (Hucl and Matus-Cadiz 2002). It was derived from a single F_5 plant from the cross Aus1408/RL4137. Aus1408 (Mares 1987), a white-seeded wheat, and RL4137 (Noll et al. 1982), a red-seeded wheat, are both considered good sources of preharvest sprouting resistance. The recurrent parent "Annuello" (http://www.ipaustralia.gov.au/pdfs/plantbreed/PVJ_Vol_154.pdf.), a southern Australian commercial wheat variety, is susceptible to sprouting. Three hundred and nineteen F_2 seeds were produced from the F_1 of the cross CN19055/Annuello. These plants were grown in the glasshouse and selfed for eight generations via single-seed descent (SSD) without selection which produced the 169 $RILF_{2:8}$ derived population. The RILs including parents were grown in field trials at Horsham plant breeding centre, Victoria, southern Australia between June–December 2004 and repeated in 2005. An additional greenhouse trial was conducted in Horsham between June and December 2005. A further trial was established at Wongan Hills, Western Australia between June and December 2005. Characteristics of the testing environments including average precipitation to the year 2004 for 45 years are as described in Ogbonnaya et al. (2007a). All the experiments were sown as an alpha-lattice design with two replicates in plots of six rows, 4 m in length, with 0.15 m row spacing. The seeding rate was

15 g/plot. Plots were sprayed twice with Folicur 430 SC (430 g/L tebuconazole) at the rate of 29 mL/L to control foliar diseases such as the rusts and supplied with irrigation as needed by gravity irrigation. Leaf samples were collected from each plot in the field, frozen in liquid nitrogen and stored at −80°C. Genomic DNA was extracted according to Ogbonnaya et al. (2001).

Assessment of seed dormancy and preharvest sprouting

Three measures of PHS were used in the evaluation of lines—seed dormancy (GI7), sprouting index (whole head assay-SI) and visibly sprouted seeds (VI), the latter two following artificial weathering. Intact spikes of the RILs were hand harvested at physiological maturity by cutting the peduncle about 10 cm below the base of the spike. Loss of green colour from the spikes was used as an estimator of physiological maturity. This has been reported to precede maximum kernel dry weight by about 1 day (Paterson et al. 1989). Spikes were air-dried at room temperature for 5 days, 10 spikes/line were kept aside for artificial weathering and the rest hand threshed. Both air-dried spikes and hand threshed seed were placed in storage at −20°C to preserve dormancy (Mares 1983).

Germination tests were carried out 2 months post-harvest in a 1.2 mL micro titre polypropylene sealed tube (QSP, Quality Scientific plastics, Quantum Scientific Pty LTD, Brisbane, Queensland) filled with 0.577 gm of sand (GPR™, BDH) and 170 μL of sterile distilled water per tube. Three replicates of 24 seeds each were germinated. These were grown in an illuminated refrigerated incubator (Model, TLMRIL 396-1-SD, Thermoline Scientific, Northgate, Queensland) with 12 h of light/darkness at 20°C for 14 days. Seeds that displayed pericarp rupture were recorded daily on individual vials for 14 or 28 days. After 14 days of imbibition, ungerminated seeds were induced to germinate with 10 mM gibberellic acid (GA). Seeds that failed to germinate 1 week after treatment with GA are considered non-viable and eliminated from data analysis. Levels of seed dormancy in the lines were analysed using a weighted germination index (GI7, Walker-Simmons 1988). This index gives maximum weight to grains that germinate rapidly and is calculated from the following formula:

$$\mathrm{GI} = (7x_{\mathrm{n1}} + 6x_{\mathrm{n2}} + \cdots + 1x_{\mathrm{n7}})/\text{total days of test} \times \text{number of grains in test},$$

where $n1$, $n2$, $n3$,…,$n7$ are the number of grains that had germinated on Day1, Day2,…,Day7. The maximum index is 1.0 if all grains germinate by Day1, whilst lower indices are indicative of increasing levels of grains dormancy or reduced germinability (Mares and Marva 2001).

Whole wheat heads from the freezer were subjected to artificial weathering for 48 h using over-head misting in a controlled environment chamber (Conviron, model G30, Controlled Environment LTD, Canada). Misting was set for 30 min duration every 6 h in the rain simulator set on 16 h days at 20°C/15°C day/night temperatures with 100% humidity. After 2 days of wetting, the spikes were left in the chamber for an additional 5 days to induce maximum sprouting. Thereafter, spikes were removed and a sprouting index (SI, average number of visibly sprouting seeds/ear) for each line was determined.

Assessment of SI was based on a modified rating score of 1–5 developed by McMaster and Derera (1976) where 1 indicates no visible sprouting over the entire spike and 5, indicates extensive sprouting where roots covered at least 75% of the spike. The heads from each line were then dried for 48 h to approximately 14% moisture and threshed and the number of visibly sprouted seed (VI)/200 seeds for each line was recorded.

Microsatellites (SSRs) markers screening

One hundred and fourteen SSR markers located on chromosomes 3A, 3B, 3D and 4A including those reportedly linked to QTLs for seed dormancy and preharvest sprouting resistance (Kulwal et al. 2005; Mares et al. 2005; Mori et al. 2005) were screened for polymorphism between the parents of the mapping population—CN19055 and Annuello. Some of the SSR primers included proprietary SSRs from TraitGenetics (http://www.traitgenetics.de/ie/index) and other publicly available SSRs, *Xgwm* (Röder et al. 1998), *Xbarc* (Song et al. 2005) and *Xwmc* from USDA/ARS GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml) were used for genotyping. Primer sequences for proprietary SSRs are available upon request from TraitGenetics (Am Schwabeplan 1b, 06466 Gatersleben, Germany).

Standard PCR cycling conditions with 32–40 cycles were employed. The PCR mix contained 1.65 mM $MgCl_2$, 250 μM of each deoxynucleotide, 1× *Taq* buffer, 0.2 U *Taq* polymerase (Invitrogen, Carlsbad, CA), 100 ng template DNA and 200 nM of each fluorescently labelled primer in a total volume of 20 μL. Fluorescent primers were labelled with 6-FAM, NED or HEX. After PCR amplification, an aliquot of the PCR products was added to a mix of formamide and internal size standard and denatured. These products were then run on an automated sequencer ABI3100 (Applied Biosystems). The results were analysed with GenScan 3.1.2 software (Applied Biosystems) and scored with the Genotyper 2.5 software (Applied Biosystems) for both the detection of the different amplification peaks for each microsatellite marker and

sample and for the determination of allelic size in base pairs.

Bulk segregant analysis and physical mapping of SSR

Based on the results from GI7, SI and VI from the 2004 trials, the RIL population was divided into two classes, PHS resistant (R), and susceptible (S). Resistant and susceptible "DNA bulks" were formed by pooling 1 μg of DNA from each of the 10 most resistant and 10 most susceptible lines, respectively and used for bulk segregant analysis (BSA). The resistant and susceptible DNA bulks were screened with 44 SSR, which were polymorphic between the PHS resistant and susceptible parents.

The physical map position of the two SSR markers (*Xgwm937* and *Xgwm894)* that showed close linkage with preharvest sprouting resistance was determined using Chinese Spring deletions lines for chromosome 4AS and 4AL (kindly supplied by Dr Evans Lagudah, CSIRO Division, Canberra, Australia). Genomic DNA from deletion lines, 4AS-1, 4AS-2, 4AS-3, 4AS-4 and 4AL-1, 4AL-2, 4AL-3, 4AL-4, 4AL-5, 4AL-6, 4AL-7, 4AL-9, 4AL-10, 4AL-12, 4AL-13 and 4AL-14, euploid Chinese Spring, CN19055, Aus1408, Syn36, Syn37 and Annuello were used to perform PCR reactions with the SSR markers (*Xgwm937* and *Xgwm894*).

Statistical and genetic analysis

For fitting genetic inheritance models, individuals (lines) were classified into resistant and susceptible groups depending on whether trait values were significantly different from the resistant parent, CN19055. The *t* test was used in the F_2 generation, while the least significant difference (LSD) was used for the F_3 and RIL populations. The χ^2 test was used to test the goodness of fit of the observed segregation patterns to the theoretical segregation ratios expected under various genetic models such as one-, two-, and three-gene models.

ANOVA was conducted for each testing environment separately and across environments. Inbred-line based heritability was estimated according to Holland and Cervantes-Martinez (2003). The genetic correlation between trait performances measured at different environments was estimated according to Burdon (1977).

Linkage mapping and quantitative trait locus analysis

Forty-four polymorphic markers were used to genotype the RILs and create the genetic map. MULTIPOINT (version 2.5, http://www.multiqtl.com; Mester et al. 2003a, b, 2004) was used to determine the marker order and map distances. The map distances in the genetic maps were computed using the Haldane mapping function (Lincoln et al. 1992).

The composite interval mapping (CIM) was used for QTL mapping. Analysis was performed based on the standardized phenotypic data using the Windows QTL Cartographer, version 2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm, Wang et al. 2006). The walking speed chosen for all QTL analysis was 2 cM. A forward–backward step-wise multiple linear regression with a probability into and out of 0.05 and a window size of 10 cM was used to select the co-factor for controlling background effect. Significant thresholds for declaring the presence of a QTL were estimated from 1,000 permutations of the data (Churchill and Doerge 1994; Doerge and Churchill 1996) and a genome-wide error rate of 0.05 (suggestive) and 0.01 (significant). QTL maps were drawn with MapChart (Voorrips 2002).

Results

PHS resistance amongst parents and the RILs

For all the three traits (GI7, SI and VI), the resistant parent CN19055 was superior to Annuello (Table 1). The average performance of CN19055 across environments and years was 0.21 for GI7, 1.25 for SI and 0.50% for VI. Annuello, the susceptible parent was 0.58 for GI7, 4.5 for SI and 91% for VI. A wide range of variation among the RILs for all traits, which ranged from very dormant to non-dormant was observed in all the testing. Transgressive segregation although apparent in GI7 and VI, was not observed in SI (Table 1).

Heritabilities were generally high for GI7 and VI but moderate for SI at all testing environments (Table 1, 2). The genetic variance was greater than environmental variance for all the traits except for SI. The genotype-by-environment interaction variance for SI was about twice that of the genotypic variance while it was similar to and much smaller than the genotypic variance for GI7 and VI (Table 2). The genetic correlations between the same traits measured at different environments were high for VI, low to moderate for GI7 and low for SI, consistent with the relative contribution of GE variance to the total phenotypic variance (Table 2).

Genetic structure of PHS resistance

In the F_2, out of 319 seeds, which were counted in a period of 28 days, 188 seeds did not germinate without GA treatment (dormant D), while 131 germinated (non-dormant ND). This segregation fitted a 9:7 ratio for a two

Table 1 Phenotypic values of parental lines, means and heritability (h^2) of preharvest sprouting resistance traits investigated in RIL of CN19055/Annuello in four environments

Traits/location/year	Parents		RIL		Population		h^2 (error)
	CN19055	Annuello	Min.	Max.	Mean	SD	
GI7-2004 HF	0.24	0.69	0	0.83	0.5	0.19	0.80 (0.035)
GI7-2005 HF	0.35	0.69	0.18	0.85	0.67	0.12	0.66 (−0.057)
GI7-2005HGH	0.19	0.53	0	0.77	0.2	0.19	0.86 (−0.027)
GI7-2005 WA	0.06	0.42	0	0.69	0.26	0.14	0.72 (−0.049)
Average	0.21	0.58	–	–	–	–	
VI (%)-2004 HF	0.63	92	0	99	53.33	28.84	0.94 (−0.011)
VI (%)-2005 HF	0.36	89	0	95	38.44	25.73	0.81 (−0.034)
Average	0.50	91	–	–	–	–	
SI-2004 HF	1.5	5	1	5	2.26	1.34	0.67 (−0.056)
SI-2005 HF	1	4	1	5	1.74	0.97	0.65 (−0.058)
Average	1.25	4.5	–	–	–	–	

GI7 germination index at 7 days; *SI* sprouting index and *VI* (%) visibly sprouted; *HF* Horsham field; *HGH* Horsham glasshouse; *WA* Western Australia field

Table 2 Variance components and heritability estimated using multi-environment data and genetic correlation between testing environments (figure in bracket is estimation error)

Parameter	GI7	VI	SI
Vg	0.009 (0.0013)	476.22(63.59)	0.302 (0.089)
Vge	0.011 (0.0009)	195.83/25.58	0.594 (0.091)
Vge/Vg	1.25	0.4	1.97
Ve	0.006(0.0002)	85.09 (6.28)	0.484 (0.036)
h^2	0.673 (0.055)	0.8 (0.036)	0.419 (0.082)
r_{gb}		0.76	0.413
	↓		
Genetic correlation			
	GI7-2004HF	GI7-2005HF	GI7-2005HGH
GI7-2005HF	0.512	–	–
GI7-2005HGH	0.597	0.463	–
GI7-2005WA	0.554	0.488	0.477

GI7 Germination index at 7 days; *SI* sprouting index and *VI* (%) visibly sprouted seeds following artificial weathering; *Vg* genetic variance; *Ve* environmental variance; r_{gb} cross-site correlation; 2004*HF* = 2004 Horsham field; 2005*HF* 2005 Horsham field; 2005*HGH* 2005 Horsham glasshouse; 2005*WA* 2005 Western Australia field

complementary dominant gene model (Table 3). For GI7 at the F_3 generation, 135 and 69 lines were resistant and susceptible respectively, which fitted an 11:5 ratio expected under the two-gene model (Table 3).

The segregation in the RIL population for GI7 recorded for the field in the two locations was 45R:149S and 56R:125S in Horsham and Wongan Hills, respectively, both of which fitted a 1:3 ratio expected under the model of two complementary genes (Table 3). Under glasshouse conditions the observed segregation ratio of 124:39 is close to 3:1, which fitted a model of two independent non-interacting genes ($\chi^2 = 0.05$; $P > 0.80$). Similarly, for SI, the segregation was 138:47 in 2004 and 132:46 in 2005, which also fitted closely to the expected 3:1 ratio of the two independent non-interacting genes model (Table 3).

Quantitative trait analysis

Genetic mapping and bulk segregant analysis

Of the 114 SSR screened, 44 (39%) were polymorphic between the parents, CN19055 and Annuello. The polymorphic SSR were then used to screen the resistant (R) and susceptible (S) bulks. In the BSA, 6 SSR on chromosome 4A (*Xgwm4026*, *Xgwm4949*, *Xgwm1091*, *Xgwm894*, *Xgwm937*, *Xgwm637*) and 3 on chromosome 3B (*Xgwm4145*, *Xgwm566* and *Xgwm1616*) were polymorphic between the resistant and susceptible bulks. The 44 SSR polymorphic between the two parents were tested on the RIL population and results from amplified loci used to construct linkage groups. Three linkage groups were obtained, which spanned a total of 765 cM (data not shown). The marker orders in the three linkage groups were identical to the wheat consensus map (Somers et al. 2004) and the high density unpublished genetic map of wheat based on the ITMI population (TraitGenetics GmbH, Gatersleben, Germany). The linkage map for chromosome 4A is presented in Fig. 1 with a marker density of 15 cM/marker. No recombination was recorded between marker *Xgwm937* and *Xgwm894*, which placed them at the same map location.

Table 3 Segregation of F_2, F_3 and recombinant inbred (RI) populations, and chi-square (χ^2) fit to genetic models for PHS resistance assessed through seed dormancy (GI7) and whole head assay (SI) under artificial rain simulation and field conditions

Generation	Trait/year/ location	Total plants tested	Resistant (similar to CN19055)	Susceptible (different from CN19055)	Tested ratio (R:S)	χ^2	Probability
F_2[a]	GS-	319	188	131	9:7	0.92	0.40
F_3	GI7-2003HGH	204	135	69	11:5	0.24	0.60
$RILF_8$	GI7-2004HGH	163	124	39	3:1	0.05	0.80
$RILF_8$	GI7-2004HF	194	45	149	1:3	0.33	0.60
$RILF_8$	GI7-2005WA	181	56	125	1:3	3.4	0.08
$RILF_8$	SI-2004HF	185	138	47	3:1	0.02	0.85
$RILF_8$	SI-2005HF	178	132	46	3:1	0.07	0.75

[a] Data were recorded for germination status (GS) within 28 days with germinated as susceptible and non-germinated as resistant

2003*HGH* 2003 Horsham glasshouse; 2004*HF* 2004 Horsham field; 2005*HF* 2005 Horsham field; 2005*HGH* 2005 Horsham glasshouse; 2005*WA* 2005 Western Australia field

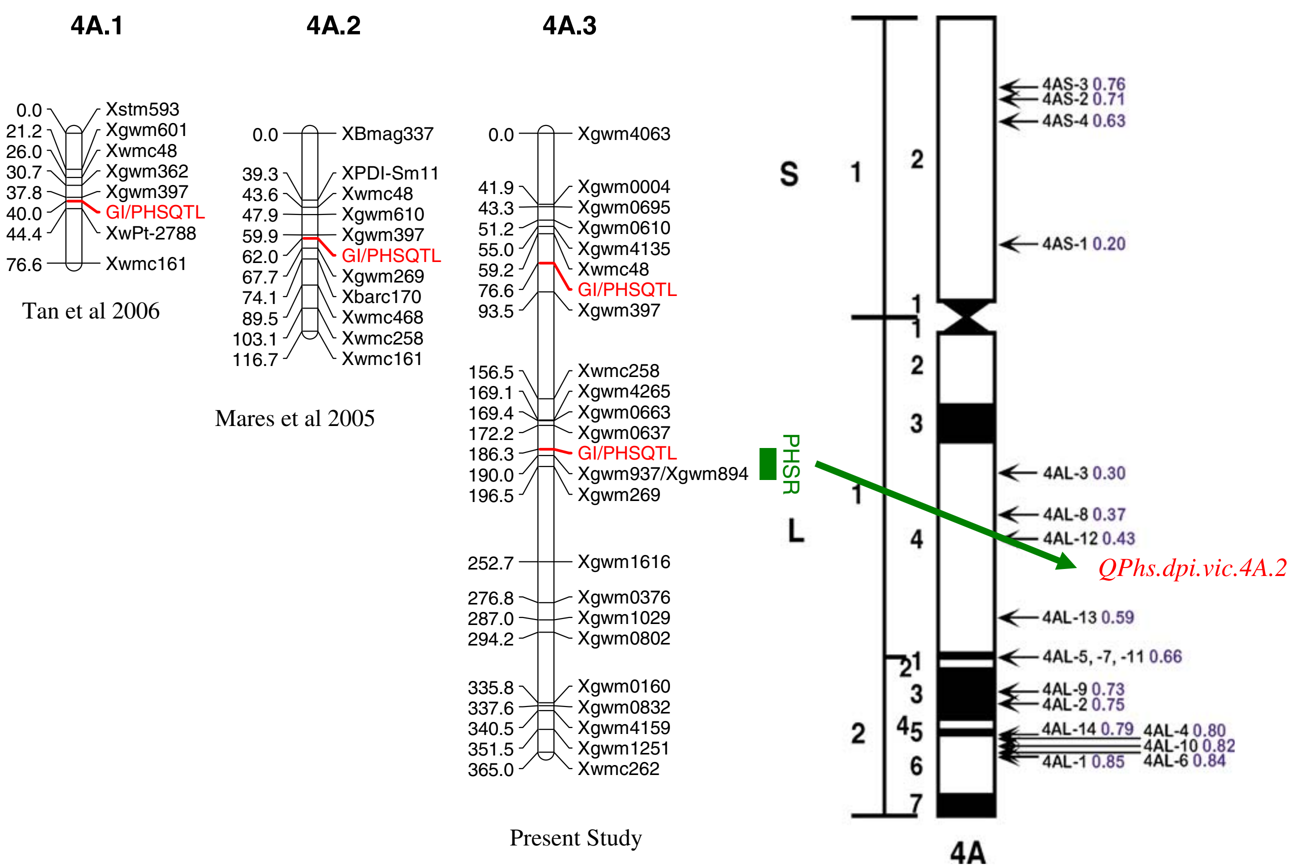

Fig. 1 Linkage map of PHS resistance gene clusters on chromosome 4A in the cross CN19055/Annuello showing the major PHS resistance locus *QPhs.dpi.vic.4A.2* and its alignment with two previously published maps (Mares et al. 2005; Tan et al. 2006). Genetic map is shown on the *left* and the Chinese Spring deletion bin assignment for *Xgwm937* and *Xgwm894* are shown on the *right*

Table 4 summarises the results of CIM analysis for preharvest sprouting resistance related traits (GI7, SI and VI) in four environments. Two significant QTL linked to components of preharvest sprouting resistance-GI7, VI and SI was identified on chromosome 4A only. One QTL designated *QPhs.dpivic-4A.1* with the closest marker *Xwmc048* lies at the proximal end of 4AS close to the centromere. The phenotypic variation (R^2) explained by *QPhs.dpivic-4A.1* was 35.44% for VI-2004HF and 30.61% for VI-2004 pooled data (Table 4). *QPhs.dpivic-4A.1* was not detected for GI7 and SI in 2004 or GI7, SI and VI in the three environments in 2005 (Table 4). The second and

Table 4 Putative QTLs for seed dormancy and preharvest sprouting resistance in four trials detected by composite interval mapping, their LOD scores, phenotypic variation explained (R^2) on chromosome 4A

Trait/year/location	QTL	Flanking markers	LOD value	R^2	EstAdd
GI7-2004HF	*QPhs.dpi.vic.4A.2*	*Xgwm637-Xgwm937*	12.10	38.68	−0.6277
SI-2004HF	*QPhs.dpi.vic.4A.2*	*Xgwm637-Xgwm937*	5.92	17.35	−0.4259
VI-2004HF	*QPhs.dpi.vic.4A.1*	*Xwmc48-Xgwm397*	3.47	35.44	−0.6203
	QPhs.dpi.vic.4A.2	*Xgwm637-Xgwm937*	16.56	40.79	−0.6549
GI7-2005HF	*QPhs.dpi.vic.4A.2*	*Xgwm637-Xgwm937*	8.48	27.75	−0.5349
SI-2005HF	*QPhs.dpi.vic.4A.2*	*Xgwm637-Xgwm937*	2.95	9.25	−0.3118
VI-2005HF	*QPhs.dpi.vic.4A.2*	*Xgwm637-Xgwm937*	12.44	32.71	−0.585
GI7-2005HGH	*QPhs.dpi.vic.4A.2*	*Xgwm637-Xgwm937*	12.55	39.84	−0.6338
GI7-2005WA	*QPhs.dpi.vic.4A.2*	*Xgwm637-Xgwm937*	4.47	14.29	−0.3828
GI7comb	*QPhs.dpi.vic.4A.2*	*Xgwm637-Xgwm937*	16.74	45.10	−0.5265
SIcomb	*QPhs.dpi.vic.4A.2*	*Xgwm637-Xgwm937*	6.87	19.43	−0.3691
VIcomb	*QPhs.dpi.vic.4A.1*	*Xwmc48-Xgwm397*	1.20	30.61	−0.5229
	QPhs.dpi.vic.4A.2	*Xgwm637-Xgwm937*	19.05	42.76	−0.6161

SI Sprouting index; *VI* visual sprouting; *GI7* germination index at 7 days; 2004*HF* 2004 Horsham field; 2005*HF* 2005 Horsham field; 2005*HGH* 2005 Horsham glasshouse; 2005*WA* 2005 Western Australia field; *GI7 comb* combined across location/year germination index data at day 7; *SI comb* combined across year data for sprouting index while *Vic comb* combined across year data for VI visual sprouting

major QTL for preharvest sprouting resistance designated *QPhs.dpivic-4A.2* was identified on chromosome 4AL in the region flanked by the marker interval *Xgwm637* and *Xgwm937/Xgwm894* (Fig. 1). The QTL was identified in all environments using CIM. The phenotypic variation explained by *QPhs.dpivic-4A.2* ranged from 9% for SI-2004HF to 40% for VI-2004HF. The same QTL was also identified based on pooled data across environments in 2005 and accounted for 45, 43, and 20% of the phenotypic variance for GI7, VI and SI, respectively (Table 4).

Physical mapping of SSR markers linked to the major QTL: QPhs.dpivic-4A.2

The physical mapping of the SSR markers associated with the major QTL for PHS resistance *QPhs.dpivic-4A.2* confirmed the presence of this locus on the long arm of chromosome 4AL. The SSR markers, *Xgwm937* and *Xgwm894*, which were tightly linked to the major QTL, amplified products in chromosome 4A Chinese Spring deletions lines: 4AS-1, 4AS-2, 4AS-3, 4AS-4, 4AL-1, 4AL-2, 4AL-3, 4AL-4, 4AL-5, 4AL-6, 4AL-7, 4AL-9, 4AL-10, and 4AL-14, PHS resistant wheat germplasm including CN19055, Aus1408, Altar84, Syn36, Syn37 and susceptible cultivar “Chinese Spring”. Both SSR markers—*Xgwm937* and *Xgwm894* failed to amplify any PCR fragment in Chinese Spring deletions lines 4AL-12 and 4AL-13 (Fig. 2). Therefore, *Xgwm937* and *Xgwm*894 were physically mapped to chromosome 4A Chinese Spring deletion bins-4AL-12 and 4AL-13.

Association of Xgwm937 and Xgwm894 markers with preharvest sprouting resistance in wheat germplasm

A collection of 64 bread wheat cultivars including current Australian bread wheat cultivars, several Canadian and New Zealand cultivars including breeding lines and some tetraploid and synthetic hexaploid wheat accessions were surveyed (Table 5). PCR products of approximately 205 and 197 bp amplified by the markers *Xgwm937* and *Xgwm894*, respectively were found only in CN19055 and lines derived from the same source of resistance (AC Domain, AC Majestic, Kanata, Snowbird) (Table 5). All the susceptible wheat cultivars showed different PCR fragments from that found in CN19055. Similar observations were found with the other sources of PHS resistance—Aus1408, Syn36 and Syn37. Therefore, within the germplasm surveyed, the CN19055-like and Aus1408-like and Syn36 and Syn37 alleles of the PCR markers *Xgwm937* and *Xgwm894* were strongly associated with grain dormancy and preharvest sprouting resistance.

Discussion

The objectives of this study were to characterise and map PHS resistance in CN19055 using an F_8 RIL population developed from a cross with a susceptible wheat cultivar, Annuello. Knowledge of the genetic basis of PHS resistance in this germplasm is essential because it will facilitate its incorporation in locally adapted elite wheat

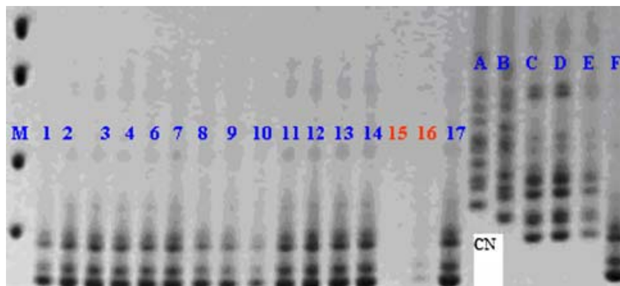

Fig. 2 Amplification products from Chinese Spring deletion lines for chromosome 4A using SSR marker—*Xgwm937*. Lanes M-pUC19 DNA/*MspI* ladder, 1-4AS-1, 2-4AS-2, 3-4AS-3, 4-4AS-A, 6-4AL-1, 7-4AL-2, 8-4AL-3, 9-4AL-4, 10-4AL-5, 11-4AL-6, 12-4AL7, 13-4AL-9, 14-4AL-10, 15-4AL-12, 16-4AL-13 and 17-4AL-14. Lanes A-CN19055, B-Aus1408, C-Altar84, D-Syn36, E-Syn37 and F-Chinese Spring

cultivars. Results from the evaluations of F_2, F_3 and RIL populations for PHS resistance either as grain dormancy or whole spike assessment following wetting indicated that PHS resistance in CN19055 was controlled by two genes. A two-gene model has previously been proposed for PHS resistance measured using grain dormancy as a measure of preharvest sprouting resistance by other authors (Mares et al. 2005; Tan et al. 2006; Paterson et al. 1989). Mares (1993) found dormancy in both red and white-grained wheat lines to be controlled by two recessive genes. Similarly, Lawson et al. (1997) using two RIL populations reported that PHS resistance assessed under artificial rain treatment was controlled by two genes that acted in additive fashion. However, a one-gene model was found in a segregating F_2 synthetic hexaploid wheat based on sprouting resistance derived from *Aegilops tauschii* (Jin et al. 1997). Furthermore, the shift in the number of resistant to susceptible lines (3:1–1:3 ratio) in this study could be attributed to the better expression of seed dormancy in glasshouse condition than under field environments. Seed dormancy is influenced by environmental factors such as light and temperature during seed development. The temperature at grain ripening was higher under field conditions than the controlled environment conditions of the glasshouse. Other authors have reported marked effect of temperature on seed dormancy. For example, Kottearachchi et al. (2006) observed that higher temperature under field conditions resulted in higher germination rate of the RILs when compared to the same RIL population grown under glasshouse conditions.

The highest heritability estimates were for VI ($h^2 = 0.94$ and 0.81 in 2004 and 2005) and GI7 ($h^2 = 0.80$ and 0.66 in Horsham field trials in 2004 and 2005). SI scored in the rain simulator displayed the lowest heritability among the three indices ($h^2 = 0.67$ and 0.65 in 2004 and 2005). The high heritability for VI and GI7 indicates that both PHS resistance indices possess a high degree of genetic control while the moderately low heritability for SI suggest that it may be more sensitive to environmental influence than VI or GI7. Thus, VI provides a more accurate way of assessing PHS resistance than either GI7 or SI. Another possible reason for the greater heritability of VI is that it provides a thorough assessment, which is based on both visual assessments of the spikes and dissecting out the grain to count germinated grains. Although SI takes into account all physical and chemical factors associated with the spike (Mares 1989; King and Richards 1984), it is difficult to take into account the germinated grains that are hidden in the glume. Given the low heritability estimated for SI, germination tests (GI7) appear to offer a more reliable alternative in the absence of facilities to carry out assessment using VI. Further, the genetic correlation between years for VI was high, moderate for GI7 and low for SI. The three measurements of PHS resistance mapped to one coincident location, *QPhs.dpivic-4A.2* on 4AL.

In the current study, two QTLs for seed dormancy and PHS resistance designated "*QPhs.dpivic-4A.1*" and "*QPhs.dpivic-4A.2*" were identified in the PHS-resistant wheat germplasm, CN19055. The QTL *QPhs.dpivic-4A.2* was consistently detected at all four individual experimental locations and overall combined analysis for PHS resistance indices—GI7, SI and VI indicating the robustness of PHS resistance at this locus and providing validation and its potential value in marker aided selection. The newly identified markers *Xgwm894* and *Xgwm937*, linked to *QPhs.dpivic-4A.2*, were physically mapped to Chinese Spring deletion bins-4AL-12 and 4AL-13 on chromosome 4A. Thus, the major QTL *QPhs.dpivic-4A.2* lies between the deletion bins 4AL-12 and 4AL-13. The SSR marker, *Xwmc48* closest to the minor QTL "*QPhs.dpivic-4A.1*" lies at the proximal end of 4AS close to the centromere based on published genetic maps from other studies. The distinct positions of both QTLs, one at the terminal region of 4AL and the other proximal to the centromere suggests that at least two different QTL were involved in controlling grain dormancy/PHS resistance on chromosome 4A in the Annuello/CN19055 RIL population. Moreover, CN19055 is a selection from Aus1408 × RL4137 cross, both considered good sources of PHS resistance, however, Ogbonnaya et al. (2007b) suggested that CN19055 is more related to RL4137 at chromosome 4A than Aus1408. Previous studies suggested that all the 21 wheat chromosomes carried seed dormancy genes (summarised in Flintham et al. 2002; Ogbonnaya et al. 2006,2007b). Of these, the QTL on wheat chromosome 4A with effect on seed dormancy have consistently been detected (Kato et al. 2001; Mares et al. 2005; Mori et al. 2005; Torada et al. 2005; Tan et al. 2006). Noda et al. (2002) used monosomic lines of Chinese Spring and

Table 5 Association of SSR markers—*Xgwm937* and *Xgwm894* with preharvest sprouting resistance in wheat cultivars

Genotype	Pedigree	Country	Wheat type	Sprouting status	*Xgwm937**	*Xgwm894**
AC Domain	Bw83/ND585	Canada	Hexaploid wheat	R	205	197
AC Majestic	Columbus*2//Saric 70/Neepawa/3/ Columbus*5//S 70/Neepawa	Canada	Hexaploid wheat	R	205	197
Kanata	RL4137*6//Thatcher/Poso48/3/AC Domain	Canada	Hexaploid wheat	R	205	197
Snowbird	RL4137*6//Thatcher/Poso48/3/AC Domain	Canada	Hexaploid wheat	R	205	197
CN19055	Aus1408/RL4137	Canada	Hexaploid wheat	R	205	197
RL 4137	RL2520//Tc*6/Kenya Farmer	Canada	Hexaploid wheat	R	205	197
Aus1408	landrace	South Africa	Hexaploid wheat	R	197	188
NZPN96-27	RL4555/1445.04	New Zealand	Hexaploid wheat	R	197	188
QT7475	Aus1408/3*Janz//Cunningham	Australia	Hexaploid wheat	R	197	188
SC8021-V2	K321.BT.1.B.1/Peck	Canada	Hexaploid wheat	R	197	188
SUN325	Hartog/Vasco//AUS1408/3/Hartog	Australia	Hexaploid wheat	R	197	188
Kenya321	Australia 45C5//Marquis/ Aguilera8	Kenya	Hexaploid wheat	R	197	188
D-105991	Not traced	Australia	Durum	R	185	176
D-105995	Not traced	Australia	Durum	R	185	–
Syn36	Altar/Aus18905	Australia	Synthetic hexaploid	R	185	176
Syn37	Altar/Aus18836	Australia	Synthetic hexaploid	R	185	176
6236.7.2	5021.16/Torfrida//Monad	New Zealand	Hexaploid wheat	R	205	197
Annuello	Pavon'S'/TM56//Janz	Australia	Hexaploid wheat	S	191	182
Arrino 85A2	Not traced	Australia	Hexaploid wheat	S	191	–
EGA Hume	Pelsart/2*Batavia DH	Australia	Hexaploid wheat	S	191	160
Sunvale	Cook*2//VPM1/Cook*3	Australia	Hexaploid wheat	S	191	160
Ventura	Sunvale/Rowan	Australia	Hexaploid wheat	S	191	160
Correll	RAC875/Yitpi	Australia	Hexaploid wheat	S	160	115
WI 24068	Not traced	Australia	Hexaploid wheat	S	160	115
WI 24072	Not traced	Australia	Hexaploid wheat	S	160	115
RAC 1192	Not traced	Australia	Hexaploid wheat	S	160	115
Gladius	Not traced	Australia	Hexaploid wheat	S	160	115
RAC 1294	Not traced	Australia	Hexaploid wheat	S	160	–
Hartog	Vicam-S-71//Ciano-F-67/Siete-Cerros/3/ Kalyansona/Bluebird	Australia	Hexaploid wheat	S	191	182
Janz	3Ag3/4*Condor//Cook	Australia	Hexaploid wheat	S	191	182
Yitpi	Champlain*8156/Mengavi*Siete Cerros/ Champlain*8156*Heron/ Mengavi *Siete Cerros/Frame	Australia	Hexaploid wheat	S	191	182
CFR00.666.28	1364.06/19ESWYT17	New Zealand	Hexaploid wheat	S	182	191
CFR00.683.7	19ESWYT17/Rubric	New Zealand	Hexaploid wheat	S	182	191
CFR00.734.32.6	3424.11.04/1632.10	New Zealand	Hexaploid wheat	S	182	191
Kohika	Otane*2/79WL8	New Zealand	Hexaploid wheat	S	182	191
Otane	Tob's'/Npo//No66/Era/3/Bd/Gallo	Mexico	Hexaploid wheat	S	182	191
Tribute	B2262/3424.11.04	New Zealand	Hexaploid wheat	S	182	191
00STHAF462	Not traced	South Africa	Hexaploid wheat	S	146	157

Table 5 continued

Genotype	Pedigree	Country	Wheat type	Sprouting status	*Xgwm937**	*Xgwm894**
CFR00.529.12	1632.10/4800.13	New Zealand	Hexaploid wheat	S	182	191
CFR00.529.18	1632.10/4800.13	New Zealand	Hexaploid wheat	S	182	191
CFR00.537.8	96WFHB5568/2*Otane	New Zealand	Hexaploid wheat	S	188	189
CFR00.713.16	Dollarbird/4800.13	New Zealand	Hexaploid wheat	S	182	191
CFR01.61	Weaver/4827.13	New Zealand	Hexaploid wheat	S	184	193
CFR02.17	Not traced	South Africa	Hexaploid wheat	S	182	191
CFR02.40.5	96WFHB5568/2*Kohika	New Zealand	Hexaploid wheat	S	182	191
5635.92	4032.22/Torlesse	New Zealand	Hexaploid wheat	S	146	157
00SWS1891-23-1	Not traced	Canada	Hexaploid wheat	S	146	213
CFR00.521.16	1445.04/1373.24	New Zealand	Hexaploid wheat	S	144	191
CFR00.687.55	98SWOBS186/19ESWYT17	New Zealand	Hexaploid wheat	S	202	213
CFR00.703.14.1	Janz/1632.9	New Zealand	Hexaploid wheat	S	144	191
CFR00.779.37.6	Janz//1632.12/Snowbird	New Zealand	Hexaploid wheat	S	182	191
Claire	Not traced	New Zealand	Hexaploid wheat	S	146	157
Domino	Not traced	New Zealand	Hexaploid wheat	S	182	191
Regency	Pastiche/C6891	New Zealand	Hexaploid wheat	S	146	157
CFR00.682.17.1	19ESWYT17/Snowbird	New Zealand	Hexaploid wheat	Intermediate	205	197
CFR00.673.6	1632.9/AC2000	New Zealand	Hexaploid wheat	Intermediate	188	197
CFR00.676.31.3	1632.12/AC2000	New Zealand	Hexaploid wheat	Intermediate	182	191
CFR00.682.17.1	19ESWYT17/Snowbird	New Zealand	Hexaploid wheat	Intermediate	196	205
CFR00.682.32.6	19ESWYT17/Snowbird	New Zealand	Hexaploid wheat	Intermediate	146	157
CFR00.869.9.5.2	WAXY/9905-3W//SWS214	New Zealand	Hexaploid wheat	Intermediate	171	181
CFR00.877.39.6.3	5027.37/Snowbird//1632.10.5	New Zealand	Hexaploid wheat	Intermediate	146	157
CFR00.878.41.1.4	5201.6/1632.10.5//Aquilla/ Snowbird	New Zealand	Hexaploid wheat	Intermediate	202	213
Tiritea	Raven/1966 ISWRN430	New Zealand	Hexaploid wheat	Intermediate	182	191
Torlesse	Not traced	New Zealand	Hexaploid wheat	Intermediate	146	157

* Allele sizes for *Xgwm937* and *Xgwm894* associated with pre-harvest sprouting resistance on chromosome 4AL

deletion lines of Chinese Spring chromosome to putatively identify 4A deletion lines associated with genotypic variation for seed dormancy. In that study based on germination index, deletion lines 4AL-2, 4AL-12 and ditelo 4AS exhibited lower grain dormancy than other regions on chromosome 4A.

One of the major objectives of QTL mapping is to identify markers linked to QTL for important agronomic traits, in particular for traits that are difficult and/or expensive to phenotype and also to put the QTL into application in breeding programs by MAS. The feasibility of using MAS in breeding programs is dependent on the reproducibility of marker-QTL associations across different crosses, generations, populations, environments and the cost effectiveness of the marker(s) (Dudley 1993; Eagles et al. 2001; Yousef and Juvik 2002). The consistent identification and co-location of the major QTL (*QPhs.dpivic-4A.2*) for the three PSH resistance indices (VI, GI7 and SI) within the marker interval *Xgwm637-Xgwm937/Xgwm894* on chromosome 4AL across different environments and years suggested that this QTL had the potential to be exploited in breeding for PHS resistance in wheat. There are minor discrepancies between the marker order obtained in our study tightly linked to PHS resistance and those published by Mares at al. (2005) and Tan et al. (2006). The differences could be attributed to density of markers, population sizes and population types used in the different studies. The marker order in the current study is consistent with the high density SSR map by Somers et al. (2004). The potential application and wider use of the two newly identified but tightly linked markers in MAS for PHS resistance was tested by genotyping a collection of 64 genetically diverse wheat cultivars. Both markers co-segregated with the reputed PHS resistance status of the wheat genotypes including Australian, Canadian and New Zealand cultivars and breeding lines. Furthermore, the presence of PHS resistant and PHS susceptible specific SSR haplotypes associated with *Xgwm973* and *Xgwm894* found in this study could not be attributed to founder effect because Ogbonnaya

et al. (2007b) reported dissimilar SSR haplotypes with unlinked markers in a set of wheat germplasm reputed to possess PHS resistance with susceptible checks including some of the lines surveyed in the current study. Therefore, they appeared to be more diagnostic than the previously identified markers such as *Xgwm397* and *Xgwm269* (Mares et al. 2005; Tan et al. 2006), which were not fully used in MAS due to their low diagnostic capability across various wheat germplasm. Amongst the 64 genotypes surveyed, the NZ genotype "6236.7.2" carries the CN19055-type *Xgwm937* and *Xgwm894* SSR haplotypes. These may have been derived from either 5021.16 or Torfrida, parents of 6236.7.2 (Table 5) but not from cv. Monad which possessed different haplotype from CN19055 (Ogbonnaya et al. 2007b). However, this warrant further investigation because we were unable to identify the pedigree of 5021.16.

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