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ORIGINAL PAPER

Association mapping for pre-harvest sprouting resistance in white winter wheat

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Abstract Association mapping identified quantitative trait loci (QTLs) and the markers linked to pre-harvest sprouting (PHS) resistance in an elite association mapping panel of white winter wheat comprising 198 genotypes. A total of 1,166 marker loci including DArT and SSR markers representing all 21 chromosomes of wheat were used in the analysis. General and mixed linear models were used to analyze PHS data collected over 4 years. Association analysis identified eight QTLs linked with 13 markers mapped on seven chromosomes. A QTL was detected on each arm of chromosome 2B and one each on chromosome arms 1BS, 2DS, 4AL, 6DL, 7BS and 7DS. All except the QTL on 7BS are located in a location similar to previous reports and, if verified, the QTL on 7BS is likely to be novel. Principal components and the kinship matrix were used to account for the presence of population structure but had only a minor effect on the results. Although, none of the QTLs was highly significant across all environments, a QTL on the long arm of chromosome 4A was detected in three different environments and also using the best linear unbiased predictions over years. Although previous reports have identified this as a major QTL, its effects were minor in our biparental mapping populations. The results of this study highlight the benefits of association mapping and the value of using elite material in association mapping for plant breeding programs.

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Introduction

Pre-harvest sprouting (PHS) is characterized by the premature germination of the seed while still attached to the mother plant and is an important quality constraint in hexaploid wheat (*Triticum aestivum* L.). PHS in wheat results from early break of seed dormancy under humid and wet weather prior to harvest. Pre-harvest sprouting has been a major problem in different parts of the world resulting in the loss of grain weight and poor end use quality. Wild relatives of wheat have extended seed dormancy that has been reduced through domestication so that harvested seed will germinate after a short after-ripening period.

There are several factors and mechanisms that affect PHS in wheat including kernel color, phytohormones such as ABA and GA and after ripening (AR). These and other factors have been adequately studied and reviewed in several articles (Gerjets et al. 2010; Kulwal et al. 2010). There have been several studies using biparental mapping

populations in the past to understand the genetic basis of PHS through QTL mapping and almost all the 21 chromosomes of common wheat have been reported to contain QTLs for PHS (reviewed in Kulwal et al. 2010). Of these 21, chromosomes 2B and 2D as well as chromosomes of groups 3 (3A, 3B and 3D), and 4A are of particular importance as they have been reported to contain major QTLs for PHS in both white and red wheat. Chromosomes 2B and 2D are important because major and consistent QTLs have been identified for PHS in several studies (Kulwal et al. 2004; Mohan et al. 2009; Munkvold et al. 2009); whereas group 3 chromosomes are important because, besides major QTLs (Kulwal et al. 2005; Mori et al. 2005; Imtiaz et al. 2008; Nakamura et al. 2011), they also contain the red kernel color gene '*R*' (Sears 1944) and the viviparous gene *TaVp1* (Bailey et al. 1999; Nakamura and Toyoma 2001). Chromosome 4A has been reported to contain a '*Phs*' gene and several studies have identified major QTLs for PHS and seed dormancy on the long arm of chromosome 4A (Anderson et al. 1993; Kato et al. 2001; Flintham et al. 2002; Lohwasser et al. 2005; Mares et al. 2005; Torada et al. 2005; Chen et al. 2008; Ogbonnaya et al. 2008; Liu et al. 2011).

Because previous QTL mapping studies for PHS in wheat were conducted using biparental mapping populations, they sampled only allelic variability present between the two parental genotypes. The limitations include low resolution for the QTL location and the effects of individual QTLs are overestimated. Use of these QTL in the marker-assisted selection program often does not give expected results, because the biparental population may not represent the breeding material or the important QTLs do not segregate in the biparental population and are not detected. Linkage disequilibrium (LD) based association mapping (AM) has a number of advantages over other mapping techniques including the potential for increased QTL resolution, and an increased sampling of genetic variation. This is because the panel of accessions used can be more informative than the offspring of biparental crosses, can be representative of breeding germplasm, and can take into account historical recombination events (Flint-Garcia et al. 2003; Gupta et al. 2005; Breseghello and Sorrells 2006a; Yu and Buckler 2006; Nordborg and Weigel 2008; Zhu et al. 2008). Therefore, AM is receiving increasing attention as a method complementary to bi-parental mapping populations to associate the genotype to phenotype based on LD by exploiting the genetic variation present in germplasm collections (Breseghello and Sorrells 2006a). Often, the AM studies are carried out using exotic germplasm, which facilitates the discovery of novel alleles, but can limit the application in practical plant breeding programs and multiple alleles reduce the power of detection. Although there have been a number of AM studies on wheat (Breseghello and Sorrells 2006b; Crossa et al. 2007; Reif et al. 2011), to date there is no genome wide association study for PHS.

In nature, developmental traits are under the control of genes, which are expressed at specific stages of development in response to the existing environmental conditions. The mapping of such genes has been referred to as functional genome-wide association mapping (fGWAS) (Wu and Lin 2006; He et al. 2010). For a trait like PHS, the period of AR is very important for the expression of the trait and maximizing the differences among genotypes. Evaluation of genotypes for PHS in response to AR periods of different duration may identify significant QTLs that could escape detection when the genotypes are tested for PHS after a single AR period.

The present study was undertaken with the objective of mapping the QTLs for PHS in an elite association mapping panel of white winter wheat genotypes using DArT and SSR markers. In addition, fGWAS was implemented by testing the spikes for PHS response at different durations of the AR period. Here we report the identification of new QTLs, validation of some of the earlier known QTLs for PHS in white winter wheat and the importance of using elite material in association mapping studies.

Materials and methods

Plant material

The association mapping panel used in the present study comprised a set of 208 elite soft white winter wheat lines including some released varieties, parental genotypes used in the breeding program at Cornell University as well as advanced breeding materials. Except for two genotypes, all genotypes were selections from breeding populations generated by the Cornell wheat breeding program. Re-examination of the kernel color of these accessions revealed ten genotypes with either red kernel color or segregation for kernel color and they were removed from the analysis leaving 198 genotypes for association mapping.

PHS experimental design

The experimental material was planted in 1 m rows in a randomized complete block design with two replications over the period of 4 years from 2008 through 2011 in the fields near Ithaca, NY, USA. Field, location means, standard deviations, and average temperatures and precipitation for a fortnight before the last harvest date of spikes are given in Table 1.

Table 1 Descriptive statistics of the 198 genotypes in the association mapping panel for pre-harvest sprouting (PHS), heading date (HD) and plant height (Pl Ht)

Trait	Field Location	Year	Mean ± SD	Range (min/max)	Temp. range (°C)	Precipitation (cm)
PHS	Snyder	2008	1.7 ± 0.80	0.00 to 5.20	26.3/13.8	5.7
PHS	Snyder	2009	1.38 ± 0.94	0.00 to 4.70	23.2/11.2	4.5
PHS	McGowan	2010	2.25 ± 1.31	0.00 to 5.20	25.9/13.7	2.2
PHS	McGowan	2011 (3 days)	2.50 ± 1.16	0.30 to 5.70	26.8/12.9	3.9
PHS	McGowan	2011 (5 days)	2.60 ± 1.13	0.20 to 5.95		
PHS	McGowan	2011 (7 days)	2.96 ± 1.12	0.55 to 5.85		
HD (days)	McGowan	2011	0.00 ± 2.11	−3.48 to 4.52		
Pl Ht (cm)	McGowan	2011	94.01 ± 10.63	68.00 to 114.00		

The HD trait is plus or minus days based on the location mean set equal to zero

SD standard deviation, *min* minimum, *max* maximum

The PHS evaluation was as described by Anderson et al. (1993). Briefly, for the 2008 through 2010 trials, five spikes were harvested from each plot when they reached physiological maturity and were dried for 5 days at ambient temperature and humidity and later placed in the mist chamber for PHS evaluation for 4 days. During 2011, a total of fifteen spikes were harvested from each plot at physiological maturity and five spikes each were transferred to the mist chamber for PHS evaluation after 3, 5 and 7 days of drying (after-ripening period). PHS data were recorded for each spike on a scale of 0 (no visible sprouting) to 9 (extensive sprouting) and averaged for the five spikes. All the genotypes were not harvested on the same day because of differences in the date of physiological maturity. Consequently, the final PHS scores were adjusted for harvest date by subtracting the mean of the PHS scores of the genotypes harvested on the same day from the PHS score of the individual genotypes and then adding the mean PHS score of all 198 genotypes.

Heading date and plant height

During the 2011 trial, heading date (HD) was measured by recording the date on which spike emergence was complete for 50 % of the plot, whereas plant height (Pl Ht) was measured to the top of the spike for the mean of the plot. The data on HD and Pl Ht were recorded to compare the results of PHS with these traits, particularly HD, for which a major QTL has been reported on chromosome 2BS in our earlier study (Munkvold et al. 2009) in the vicinity of the photoperiod gene *Ppd-B1* (Hanocq et al. 2004).

DNA isolation and genotyping

Two types of molecular markers, Diversity Array Technology (DArT) and simple sequence repeat (SSR), were used in the present study. DNA was extracted from young seedlings following Heun et al. (1991) and sent to Triticarte Pty Ltd, Australia (http://www.triticarte.com.au/) for whole-genome profiling using DArT markers. The SSR marker data were generated using direct-labeled primers as well as M-13 tailed primers and genotyped using an ABI 3730 at Cornell University Life Sciences Core Laboratory Center as described in Yu et al. (2010). Some of the SSR marker data were generated using polyacrylamide gel electrophoresis followed by silver staining. The DArT genotypic data consisted of 1,103 markers, whereas the SSR marker data comprised 62 loci. In addition, one polymerase chain reaction (PCR) based landmark unique gene (PLUG) marker (Ishikawa et al. 2007) was also used, thus making a total of 1,166 markers. The Wheat Interpolated Maps v4 (Triticarte Pty Ltd, Australia, personal communication) were used as references to locate the positions of the majority of the DArT markers. Apart from this, some DArT markers were assigned to respective chromosomes based on earlier published literature as well as on the association ($r^2 > 0.8$) with other mapped markers. The chromosome location of some of the DArT markers used in the present study was not yet known, however, the other DArT markers covered the entire genome of wheat. The SSR markers corresponded to previously reported locations of PHS QTL and were on chromosomes 2B, 2D, 4A, 6D and 7B. The total number of markers after removing markers with <10 % MAF was 841 and after removing markers with <10 % MAF and $r^2 = 1$ was 657.

Population structure

Population structure was assessed using a set of 42 unlinked DArT and SSR markers representing all the chromosomes. Software package Structure 2.3.3 (Pritchard

et al. 2000) was used with the admixture model and the number of sub-populations (K) as 1 through 20 with the variable length of burn-in period and number of iterations set at 20,000–250,000.

Statistical analysis

Simple correlations between PHS scores across the years as well as Spearman's rank correlations for PHS scores, broad sense heritability estimates, and descriptive statistics for different traits were calculated in a spreadsheet. The best linear unbiased predictors (BLUP) for the PHS data of 4 years (2008 through 2011) were computed using the statistical software package JMP (http://www.jmp.com) treating genotypes as random effects. Additive main effects and multiplicative interaction (AMMI) bi-plot was constructed using MATMODEL 3.0 (Gauch 2007).

Association analysis

Association analysis was performed using general linear model (GLM) and mixed linear model (MLM) approaches as implemented in the software package TASSEL 3.0 (Bradbury et al. 2007). Out of a total of 1,166, the markers with minor allele frequencies (MAF) less than 10 % were excluded from analysis. The markers with $r^2 = 1$ (r^2 represents the correlation between alleles at two loci and a value of 1 indicates that markers were in complete LD) were also excluded from the analysis and the one marker with the least amount of missing data was retained. To take into account the population structure, Q values were determined using the software STRUCTURE, and principal components (PCs) were obtained using TASSEL 3.0 and used as covariates in GLM and MLM analysis. For MLM analysis, marker-based kinship matrix (K) obtained using TASSEL was used along with the Q matrix to correct for both family and population structure and the phenotypic variation explained (r^2) by the marker is reported. In the case where a marker was detected only in GLM, the corresponding r^2 values are reported. The variation explained by a marker locus is calculated differently for each method (GLM or MLM) so the r^2 values are not directly comparable. The optimum levels of compression in combination with P3D for variance component estimation were used as criteria in MLM. The PHS data of individual years as well as BLUP for the PHS scores of 4 years and for the two locations [Snyder (2008 and 2009 environments) and McGowan (2010 and 2011 environments)] were analyzed separately. For calculating the BLUPs for 4 years (2008–2011), the PHS data from the 5-day after-ripening period from 2011 trial were used along with data from 2008 to 2010 trials. For functional GWAS, PHS data for the 2011 experiment from the three different durations of after ripening periods (3, 5 and 7 days) were analyzed separately as well as by taking the average of the three periods. In addition to this, analysis was also performed using all the 1,166 markers as well as after excluding markers with <10 % MAF following GLM and MLM approaches with and without the Q matrix. However, we have reported here the results obtained using the robust set of data after removing markers with <10 % MAF and $r^2 = 1$.

Significant marker-trait associations were assigned based on the false discovery rate (FDR) criteria of Benjamini and Hochberg (1995) (called stringent FDR criteria hereafter) and the results are presented in Tables. At the same time other FDR criteria of q values corresponding to the p values obtained in GLM and MLM analyses were used to assign the significant associations using the software QVALUE (Storey 2002). More markers were significant using these criteria and results are presented in supplementary Tables.

Results

Phenotypic data

The PHS data across years were normally distributed except for 2009. Therefore, 2009 data were log transformed and standardized before analysis using an option available in TASSEL. The PHS scores were higher for the McGowan location as compared to the Snyder location (Table 1). Analysis of variance (ANOVA) indicated highly significant differences ($p < 0.01$) for PHS scores across years (data not shown). The broad sense heritability (h^2) across the 4 years was 0.37, whereas by locations, it was much higher for the McGowan location (0.71) as compared to the Snyder location (0.50). For the different durations of after-ripening period, heritabilities were 0.68, 0.70 and 0.63 for the 3, 5, and 7 day AR durations, respectively.

Significant correlations as well as rank correlations were observed for the PHS scores across the years except for the PHS scores of 2008 with 2009 and 2011 (Table 2). Similarly, significant correlations and rank correlations were observed for PHS scores for the different durations of AR period in the 2011 experiment (Table 2). There were non-significant negative correlations between the PHS score and plant height and days to heading, and a significant positive correlation between plant height and days to heading (Table 2). As the values of correlations and the rank correlations were similar, only the values of the correlations are given in Table 2.

Table 2 Correlation coefficients for the pre-harvest sprouting (PHS) scores (a) across the years and (b) for different days of after-ripening period and plant height (Pl Ht) and heading date (HD) for the 2011 trial

	PHS08	PHS09	PHS10	PHS11	Mean$^{\xi}$
(a)					
PHS09	0.06				
PHS10	0.14*	0.37***			
PHS11	0.06	0.25***	0.46***		
Mean$^{\xi}$	0.40***	0.63***	0.82***	0.74***	
BLUPs	0.40***	0.63***	0.82***	0.74***	0.99***
	3 days	5 days		7 days	Pl Ht
(b)					
5 days	0.65***				
7 days	0.58***	0.65***			
Pl Ht	−0.06	−0.10		−0.08	
HD	0.01	−0.08		−0.08	0.23***

Significant at * $p < 0.05$ and *** $p < 0.001$, respectively

$^{\xi}$ Mean of 4 years PHS data from 2008 through 2011

Population structure

A set of 42 markers (one marker from the short and long arm of each chromosome) was used to estimate the underlying population structure. The logarithm of the probability of likelihood [LnP(D)] value declined after $K = 2$ and then again increased. The principal component analysis (PCA) based on all the markers identified three sub-groups (Fig. 1). The first sub-group consisted of genotypes mostly related to 'Whatford' and NY87048W-7388, while the second sub-group consisted of genotypes mostly related to 'Cayuga' and 'Caledonia'. The third sub-group consisted of genotypes related to NY91017-8080. As some of the genotypes from sub-groups 1 and 2 overlapped, it was considered to be one larger sub-group. To examine the effect of population structure, the association analysis was conducted without the population structure covariarates as well as with different numbers of sub-populations and their respective Q matrices. In addition to this, different numbers of PCs were used as covariates in the analysis. Because of the relatively small contribution of individual components using PCA (Fig. 1), two sub-groups/two components were considered to be optimal. Moreover, similar results obtained using Q matrices from STRUCTURE and principal components considering $Q = 2$ supported the same sub-groups. Only results obtained using PCs as covariates are presented in Tables. As such, there was little influence of population structure on the significant marker-trait associations. The only effect that was observed was for the marker gwm397 mapped on chromosome 4AL. This marker was not significant when population structure was not taken into account, although another linked marker, gwm610, was significant (see "Discussion").

Significant marker-trait associations for PHS

A total of eight QTLs linked with 13 markers representing seven chromosomes were detected for PHS in this study (Table 3; Supplementary Table S1). One QTL was detected on each arm of chromosome 2B and one each on chromosome arms 1BS, 2DS, 4AL, 6DL, 7BS and 7DS. The significant markers obtained in GLM and MLM were different for the Snyder (2008 and 2009 environments) and McGowan locations (2010 and 2011 environments) and none of these QTLs was significant in all the environments.

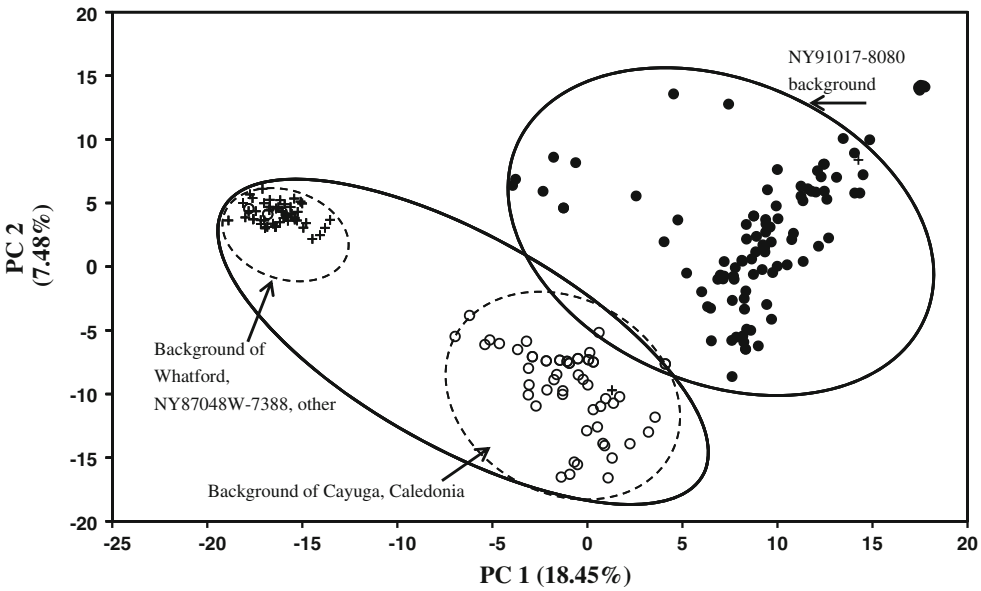

Fig. 1 Scatterplot of principal component 1 (PC1) drawn against principal component 2 (PC2). Each *circle* or *dot* or *plus sign* represents an individual genotype in different subpopulations of the association mapping panel. Subpopulations are enclosed by *ovals* in *continuous* and *dashed lines* and labeled according to the genetic background by *arrows*. Proportion of the variance explained by the principal components is given in *parenthesis*

However, a QTL on 4AL linked with marker gwm397 was significant in three out of four environments as well as for the BLUPs over environments (Table 3). This QTL was also linked with two other markers (gwm610 and wPt-730913) on the same chromosome arm and explained up to 12 % of the phenotypic variation. Although the genetic position of the marker wPt-730913 is far from the markers gwm397 and gwm610 based on two different maps (Triticarte personal communication; Somers et al. 2004), because they were on the same arm of the 4A chromosome, we counted it as a single QTL. However, it is possible that these are two separate QTLs. The QTLs on chromosomes 4AL and 7DS were prominent in the Snyder location (2008 and 2009 environment), while QTLs on chromosomes 1BS, 2BS, 2BL, 6DL and 7BS were prominent at McGowan locations (2010 and 2011 environments). All these QTLs were also detected with varying level of significance using BLUPs calculated over four environments. The QTL on 2DS was significant using BLUPs, but not in the individual environments (Table 3). As most significant marker-trait associations were not consistent across individual environments and locations, the additive main effects and multiplicative interaction (AMMI) biplot were constructed following Gauch et al. (2011). The AMMI bi-plot as seen in Fig. 2 showed that the two locations, Synder (2008 and 2009 environment) and McGowan (2010 and 2011 environments), not only differ from each other for their main effects (horizontal distribution) but also for their interaction effects (vertical distribution). Therefore, BLUP values were also calculated for individual locations across 2 years and used for analyzing the data to facilitate interpretation of the results. Using this approach, two QTLs representing chromosomes 4AL and 5B/7B were detected across both locations (Table 4). The genetic location of marker wPt-3873 is uncertain and has been mapped on chromosomes 5B and 7B in different studies (Triticarte Pty Ltd, Australia, personal communication; Crossa et al. 2007). This QTL was not significant using the data from individual environments. Contrary to this, four QTLs that were detected in individual environments (2DS, 6DL, 7BS and 7DS; from Table 3) did not pass the stringent FDR criteria using this approach, although three of them (excluding 7DS) were significant for q values (Table 4; Supplementary Table S2). All the markers that were significant in multiple environments had allelic effects in the same direction in each environment (Supplementary Table S1). The results of marker-trait association presented in Tables 3, 4 and 5 as well as Supplementary Tables show all markers that were significant for at least one location in a single year according to the stringent false discovery rate criteria and the q value criteria, respectively.

When the analysis was conducted using all 1,166 markers, as well as after excluding markers with <10 % MAF using GLM and MLM approaches with and without Q matrix, the results obtained for the significant associations were similar to those reported here (data not shown). Ignoring the stringent false discovery rate criteria, large numbers of marker-trait associations were observed for PHS across the years at $p < 0.05$ using GLM and MLM. The majority of significant associations obtained through MLM was also observed using GLM (Supplementary Table S3).

Functional GWAS

As expected, mean PHS scores were lower for the 3-day AR period and higher for the 7-day AR period. However, the greatest range for PHS scores was at the 5-day AR period reflecting a higher genetic variance (Table 1). Four DArT markers linked with three QTLs were significant for PHS for different durations of AR period. However, only two of these markers [wPt-0697 (chromosome 2BL) and wPt-666223 (chromosome unknown)] were common for all three durations of AR period (Table 5), whereas the remaining two markers [(wPt-666931 (chromosome 2BL) and wPt-8283 (chromosome 7BS)] were significant for only the 5 and 3 days of AR, respectively. All the markers significant for both the 5- and 7-day AR periods were also detected for the 3-day AR period and all were detected using the mean data calculated over the 3-, 5- and 7-day AR periods with varying degrees of significance (Table 5). There were additional markers with lower p values that were significant using q value criteria, but because they did not pass the stringent FDR criteria, they are not listed in Table 5. Details of these markers are given in Supplementary Table S4.

Significant QTLs for heading date and plant height

The data on heading date and plant height were recorded during 2011 only. For heading date, a highly significant QTL linked with the marker gwm429 mapped on chromosome 2BS was identified. For plant height, a significant QTL was linked with DArT marker wPt-730772 on chromosome 6AS (Supplementary Table S5). Neither of these two marker loci was significantly associated with PHS QTLs in the present study.

Discussion

Because of the practical limitations of QTL mapping in biparental populations, association mapping has gained wide acceptance as an efficient method for mapping QTLs in plant populations. This has been facilitated by the

Table 3 Markers showing significant association with pre-harvest sprouting in general linear model (Q) and mixed linear model ($Q + K$) by location and year

Marker	Chromosome arm[A]	Putative position (cM)[B]	Snyder location						McGowan location								
			2008			2009			2010			2011			BLUP (2008–2011)		
			p		R^2	p		R^2	p		R^2	p		R^2	p		R^2
			Q	$Q + K$		Q	$Q + K$		Q	$Q + K$		Q	$Q + K$		Q	$Q + K$	
wPt-666564	1BS	33.5[a]	–	–	–	–	–	–	**5.71E−05**	1.40E−03	0.06	3.54E−02	–	0.02	2.47E−03	1.84E−02	0.03
barc328.217	2BS	20.2[b]	–	–	–	–	–	–	**9.74E−05**	2.33E−03	0.05	2.19E−02	–	0.03	1.80E−03	3.14E−02	0.02
stm773.183	2BS	48.0[c]	–	–	–	–	–	–	**1.92E–04**	2.00E−02	0.03	8.31E−03	–	0.03	5.18E−04	4.19E−02	0.02
wPt-0697	2BL	86.6[a]	–	–	–	–	4.83E−02	0.02	2.99E−03	4.51E−02	0.02	3.70E−03	2.12E−02	0.03	**2.33E−04**	1.01E−02	0.04
wPt-666931	2BL	97.8[a]	–	–	–	1.00E−02	–	0.03	5.49E−03	–	0.04	**3.83E−05**	**4.18E−04**	0.07	**2.22E−04**	7.96E−03	0.04
wPt-6003	2DS	14.7[b]	3.23E−02	–	0.02	–	–	–	4.15E−04	3.66E−03	0.05	–	–	–	**2.43E−04**	7.59E−03	0.04
gwm610.162	4AL	12.0[d]	4.05E−03	2.87E−03	0.05	**3.63E−08**	**3.59E−06**	0.12	–	–	–	–	–	–	1.35E−03	7.29E−03	0.04
gwm610.172	4AL	12.0[d]	4.80E−03	3.02E−03	0.05	**1.01E−07**	**5.78E−06**	0.11	–	–	–	–	–	–	1.42E−03	4.39E−03	0.04
gwm397.191	4AL	18.0[d]	3.39E−02	–	0.02	**5.03E−05**	3.94E−04	0.07	1.59E−03	–	0.05	–	–	–	**3.91E−05**	1.99E−03	0.05
wPt-730913	4AL	93.1[a]	**2.32E−04**	9.27E−04	0.06	–	–	–	–	–	–	–	–	–	–	–	–
cfd37.208	6DL	60.0[d]	–	–	–	2.22E−02	–	0.03	**1.16E−04**	1.97E−02	0.03	–	–	–	3.62E−03	–	0.04
wPt-8283	7BS	46.5[a]	–	–	–	**–**	–	–	1.12E−02	3.91E−02	0.02	**2.83E−04**	2.61E−03	0.05	7.82E−04	4.12E−03	0.04
wPt-663918	7DS	00.5[a]	–	–	–	**1.85E−04**	6.01E−03	0.04	–	–	–	–	–	–	3.65E−02	–	0.02

R^2: variation explained by the marker in MLM (when marker is significant only in GLM, R^2 value corresponding to it is shown)

– Denotes $p > 0.05$; p values which passed the false discovery rate criteria are highlighted in bold and their corresponding p values in different years are given only if they were <0.05

[A] Markers on the same chromosome arm are considered to be linked with the same QTL

[B] Putative genetic position based on [a]Triticarte map (Triticarte Pty Ltd., Australia personal communication), [b]Munkvold et al. (2009), [c]Lehmensiek et al. (2005), [d]Somers et al. (2004)

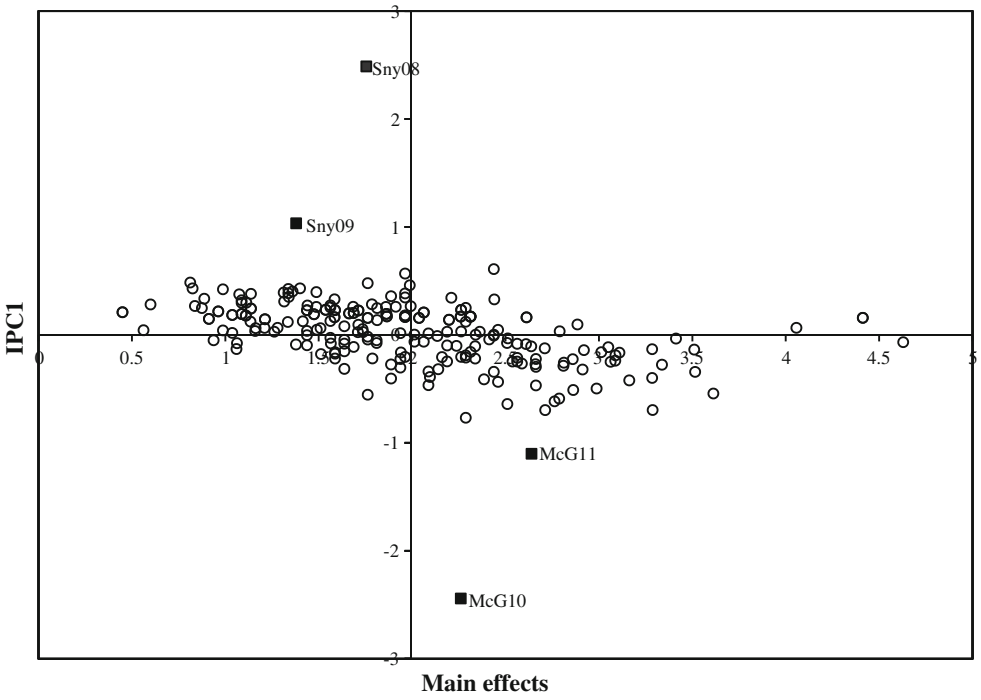

Fig. 2 The AMMI1 biplot for pre-harvest sprouting (PHS) data used in association mapping. The abscissa shows main effects, namely genotype means and environment means, and the ordinate shows interaction principal component 1 (IPC1) scores. The *vertical line* indicates the grand mean (a PHS score of 1.99) and the *horizontal line* indicates an IPC1 score of 0; *Sny08* Snyder 2008, *Sny09* Snyder 2009, *McG10* McGowan 2010 and *McG11* McGowan 2011

Table 4 Significant marker-trait associations for pre-harvest sprouting obtained through general linear model (Q) and mixed linear model ($Q + K$) using best linear unbiased predictors (BLUPs) of individual locations

Marker	chromosome arm[A]	Putative position (cM)[B]	BLUP (Snyder)			BLUP (McGowan)		
			p		R^2	p		R^2
			Q	$Q + K$		Q	$Q + K$	
wPt-666564	1BS	33.5[a]	–	–	–	**2.77E−04**	2.80E−03	0.05
barc328.217	2BS	20.2[b]	–	–	–	**1.16E−04**	9.03E−03	0.04
stm773.183	2BS	48.0[c]	–	–	–	**1.19E−04**	1.92E−02	0.03
wPt-666931	2BL	97.8[a]	–	–	–	**1.18E−04**	3.18E−03	0.05
gwm610.162	4AL	12.0[d]	**1.69E−07**	**6.75E−06**	0.11	–	–	–
gwm610.172	4AL	12.0[d]	**2.29E−07**	**6.63E−06**	0.11	–	–	–
gwm397.191	4AL	18.0[d]	**1.17E−05**	**4.10E−05**	0.09	4.57E−03	–	0.04
wPt-3873	5B/7B	27.3[a]/61.5[e]	**1.71E−04**	1.36E−03	0.06	2.72E−02	4.11E−02	0.02

R^2: variation explained by the marker in MLM (when marker is significant only in GLM, R^2 value corresponding to it is shown)

– denotes $p > 0.05$; p values which passed the false discovery rate criteria are highlighted in bold and their corresponding p values in different years are given only if they were <0.05

[A] Markers on the same chromosome arm are considered to be linked with the same QTL

[B] Putative genetic position based on [a]Triticarte map (Triticarte Pty Ltd., Australia personal communication), [b]Munkvold et al. (2009), [c]Lehmensiek et al. (2005), [d]Somers et al. (2004), [e]Crossa et al. (2007)

availability of increasing numbers of markers and advances in computational tools. As a result, several association mapping studies have been reported in wheat that mostly focused on quality traits (Breseghello and Sorrells 2006b; Emebiri et al. 2010; Reif et al. 2011), disease resistance (Crossa et al. 2007; Miedaner et al. 2011; Yu et al. 2011) and yield and related traits (Crossa et al. 2007; Yao et al. 2009; Neumann et al. 2011). In the majority of these studies, either a small set of genotypes (91 genotypes and 395 markers; Emebiri et al. 2010) or markers (96 lines and 93 markers; Breseghello and Sorrells 2006b) or both have been used to find the marker-trait associations for different traits. In this study, we used a large number of genotypes and markers for identifying the QTLs for PHS in elite wheat germplasm using both general-linear and mixed-linear models.

Table 5 Significant marker-trait associations for pre-harvest sprouting obtained through general linear model (Q) and mixed linear model ($Q + K$) for the data of 2011 for different durations of after-ripening period

Marker	chromosome arm[a]	Putative position (cM)[b]	3 days			5 days			7 days			Mean		
			p		R^2	p		R^2	p		R^2	p		R^2
			Q	$Q + K$		Q	$Q + K$		Q	$Q + K$		Q	$Q + K$	
wPt-666223	?	–	**2.22E−04**	4.01E−03	0.04	7.52E−04	1.17E−02	0.04	1.31E−02	2.85E−02	0.03	**2.27E−04**	5.22E−03	0.04
wPt-0697	2BL	86.6	5.30E−03	1.82E−02	0.03	3.70E−03	2.12E−02	0.03	**1.83E−04**	4.49E−04	0.07	**2.75E−04**	2.33E−03	0.05
wPt-666931	2BL	97.8	2.58E−02	–	0.03	**3.83E−05**	4.18E−04	0.07	–	–	–	1.46E−03	6.41E−03	0.04
wPt-8283	7BS	68.9	**1.74E−04**	4.07E−03	0.04	**2.83E−04**	2.61E−03	0.05	–	–	–	5.72E−04	7.80E−03	0.04

R^2: Variation explained by the marker in MLM (when marker is significant only in GLM, R^2 value corresponding to it is shown)

– denotes $p > 0.05$; p values which passed the false discovery rate are highlighted in bold and their corresponding p values in different years are given only if they were <0.05

[a] Markers on the same chromosome arm are considered to be linked with the same QTL

[b] Genetic position based on Triticarte map (Triticarte Pty Ltd., Australia personal communication)

Effect of population structure

The results of association mapping can be influenced by the presence of underlying population structure. However, the association mapping panel used in the present study had minimal subpopulation structure that was evident from the relatively small contribution of the individual components in PCA (Fig. 1). The only effect of population structure was on the highly significant marker gwm397.191 on chromosome 4AL. Without correction for population structure (GLM without Q matrix), this marker was not significant, whereas another marker from chromosome 4AL (gwm610) linked with the same QTL was highly significant. After taking into account the population structure using either the Q matrix from STRUCTURE or principal components (PCs) and/or using the kinship matrix (K), marker gwm397.191 was significant. Genetically, both of these markers (gwm397 and gwm610) have been mapped very close (6 cM) to each other in the wheat consensus map (Somers et al. 2004) and were linked with the same QTL in the material used in the present study. In the majority of the AM studies, the Q matrix obtained using STRUCTURE was used to account for population structure, while in some studies PCs were also used. Price et al. (2006) have advocated the use of PCs to effectively account for population stratification. In the present study, we used both (Q matrix from STRUCTURE, as well as PCs) and they produced similar results. Although little gain was achieved by accounting for population structure in this study, the use of Q and $Q + K$ matrices reduced the probability of false positives (Fig. S1). The unified mixed-model method used in the present study has been reported to reduce the Type I error in association mapping (Yu et al. 2006). Removing up to 20 % of the related genotypes based on cluster analysis as suggested by Breseghello and Sorrells (2006b) did not change the results except that slightly higher p values were obtained, suggesting minimal population structure in this population. It has also been suggested that for some traits and populations, the K-only model may be as good as or better than the $Q + K$ model (TASSEL user manual). In our study, the results obtained using both K-only and $Q + K$ were not different for the most significant markers (data not shown). Although the Q-model (GLM) was not as effective as the K-only or $Q + K$ model (MLM) for controlling population structure, inclusion of both GLM and MLM results in the present study suggests that some significant associations may not be detected using MLM because they do not pass the FDR criteria. It has also been suggested by Moncada et al. (2001) that in QTL mapping, the most conservative approach is to focus on those QTLs that are detected by more than one analysis or in multiple environments. The use of both models also facilitates comparisons with previous studies.

Importance of elite material for association mapping

In genome-wide AM studies, the results are strongly influenced by the choice of germplasm, size of the population, number and distribution of markers, and the trait under study. Often, in AM studies, exotic germplasm or diverse materials representing different geographic regions of the world are used to minimize LD and find QTLs for a variety of traits. Although the results obtained from such studies may provide some insight about the diversity, it is not usually optimal for application in a breeding program. Entries from a core collection from the germplasm bank or exotic material may be suited for AM of traits less influenced by adaptation, however, elite material is more useful for studying quantitative traits such as PHS that can be

strongly influenced by biotic and abiotic stresses and differences in maturity. For an adaptive trait like PHS, it is not feasible to accurately phenotype an association panel of diverse wheat genotypes from different regions because diseases and maturation at different times under different climatic conditions can affect seed development and confound the phenotype. Moreover, the QTLs identified using elite material can have direct application in the breeding program through marker-assisted selection because many of the same alleles will be represented in an association panel (Breseghello and Sorrells 2006a). Another important advantage of association mapping is that more QTLs can be detected than in a biparental mapping population. The present study is an excellent example of such a situation, wherein, we have identified a major QTL for PHS on chromosome 4AL (previously reported by other investigators) that was present in our breeding lines, but was a minor QTL (LOD score of 3.56 in one environment) based on the results of a biparental mapping population (Munkvold et al. 2009). In the same study, the QTL on chromosome 2BS was detected in 16 environments with high significance (Munkvold et al. 2009). In addition, this study has identified additional markers around the previously identified major QTL on 2BS that are useful for fine mapping. The availability of large numbers of markers and improved statistical tools greatly increases the efficiency of identifying QTLs and the use of MAS in breeding programs.

QTLs × environment interaction

In QTL mapping studies, large numbers of QTLs are often identified, but only a few of them are significant in across all environments. One of the important reasons for this is the QTL × environment interactions that result in an allele influencing the trait in only certain environments (Gauch et al. 2011). In this study, none of the QTLs was detected in all four environments, although a QTL on chromosome 4AL linked with marker gwm397 was detected in three environments. While some QTLs were significant at the Snyder location (2008 and 2009 environment), others were significant at the McGowan location (2010 and 2011 environment). QTL × environment interactions have been reported for PHS in other studies (Kulwal et al. 2004; Imtiaz et al. 2008; Liu et al. 2011). However, those studies did not offer any solution to deal with inconsistencies of QTL detection across environments. To address this concern and to increase the efficiency of QTL detection under such circumstances, recently Gauch et al. (2011) proposed two new strategies. The additive main effects and multiplicative interaction (AMMI) model proposed by them improved the accuracy of the phenotypic data which increased the likelihood of QTL detection. They demonstrated improved accuracy using the PHS data from the study of Munkvold et al. (2009). The AMMI bi-plot (Fig. 2) of our data clearly separated the Snyder and McGowan locations (these locations are actually in close proximity) in terms of main effects and interaction effects. Therefore, when we analyzed BLUPs of the two locations separately, besides the QTL on 4AL, another QTL linked with marker wPt-3873 (5B/7B) was significant across environments/locations, whereas the QTLs on chromosomes 1BS, 2BS and 2BL were significant at the McGowan location (Table 4).

Functional GWAS

The concept of functional mapping of QTL may provide insight into the genetic control of developmental traits. This has been referred to as time-related mapping (TRM) versus time-fixed mapping (TFM) by Wu et al. (1999). It is logical that during the process of development, different QTLs are expressed at different times in response to the existing environmental conditions and developmental stage (Wu and Lin 2006; He et al. 2010), although they may have a similar effect on the final phenotype. In this study, we used three different periods of after-ripening to perform functional mapping for PHS. Although, the broad sense heritability estimate among the three durations of AR period was highest for the 5-day period (0.70), and can be considered as an optimum time period of AR for the spikes to be tested for PHS, it was not significantly different from that observed for the 3-day period (0.68). Moreover, all the QTLs detected for the 5-day AR period were also detected for the 3-day AR period. Therefore, given similar environmental conditions, the 3-day AR might also be used for PHS evaluation in future studies. As these results were based on the data from one location (2011), additional studies are needed to confirm this.

Comparisons with previous results

All 21 chromosomes of bread wheat have been reported to contain minor or major QTLs for PHS, seed dormancy and other related parameters including grain color, falling number and α-amylase (reviewed by Kulwal et al. 2010). In this study, a total of eight QTLs were significantly associated with PHS. The QTL linked with the markers gwm397 and gwm610 on the long arm of chromosome 4A was highly significant and explained up to 12 % of the variation, and is likely the same QTL on the long arm of chromosome 4A that has been detected in the majority of PHS studies (Flintham et al. 2002). Among these studies, SSR marker gwm397 was linked to a major QTL for PHS

(Ogbonnaya et al. 2008), seed dormancy (Mares et al. 2005; Torada et al. 2005; Tan et al. 2006) and both, PHS and seed dormancy (Chen et al. 2008; Liu et al. 2011; Fig. S2). Besides gwm397, another SSR marker gwm610 was linked with the same QTL in this study. Although, gwm610 was used in many of the earlier studies, it was never reported to be linked with PHS and would be useful for a MAS program aimed at PHS improvement. In our earlier study, a minor QTL in the marker interval gwm397-wmc617 was detected in the Cayuga × Caledonia DH population explaining only 3 % of the variation (Munkvold et al. 2009).

Munkvold et al. (2009) reported a major QTL on the short arm of chromosome 2B in the marker interval barc55-wmc474 that was detected in all 16 environments with a LOD score of up to 27.10 that explained up to 24 % of the variation. Although, this QTL was not consistent in this marker interval (barc55-wmc474) in all environments in this study, based on the earlier results (Munkvold et al. 2009) and our fine mapping studies for this QTL, additional markers were mapped in this region. Based on this study and a related fine mapping study, we were able to resolve this as two different QTLs linked in coupling (unpublished results). Although, neither barc55 nor wmc474 was significant, another nearby SSR marker, barc328, was significant, along with SSR marker stm773 that explained up to 5 % of the variation. Interestingly, another QTL on chromosome arm 2BL linked to DArT marker wPt-2397 was detected in only one of the 16 environments in the earlier study (Munkvold et al. 2009) but was significant in this study. This QTL was linked with the DArT markers wPt-0697 and wPt-666931. This again illustrates the efficiency of AM to detect and validate QTLs in elite breeding germplasm. In both of these studies, this QTL explained up to 4 % of the variation. In the biparental population, out of 15 QTLs detected for PHS, only two of them (one each on 2BS and 6DL) explained more than 10 % of the variation (Munkvold et al. 2009) indicating the complexity of this trait. Similarly, in the present study, out of eight QTLs, only one on 4AL was a major one.

In addition to the important QTLs on chromosome 2BS, 2BL and 4AL, we identified five more QTLs located on chromosome arms 1BS, 2DS, 6DL, 7BS and 7DS. Significant QTLs for PHS and dormancy have been identified on these chromosomes in earlier studies (Kulwal et al. 2004; Munkvold et al. 2009; Knox et al. 2012; Liu et al. 2011). For example, two QTLs for PHS were identified by Munkvold et al. (2009) on chromosome 1B. However, it is difficult to cross reference QTLs in different studies. Comparative analysis of different wheat maps (Roder et al. 1998; Somers et al. 2004; Sourdille et al. 2004; Crossa et al. 2007; data available on GrainGenes) assigned the centromere to different positions leading to differences in chromosome arms assignments, particularly for the markers near the centromeric region. This may be the case for marker wPt-666564 mapped on chromosome 1B, although we putatively assigned it to the short arm of 1B. Similarly, for the marker wPt-8283 mapped on chromosome 7B, the arm location is unclear but based on several published maps, it appears to be on 7BS. Kulwal et al. (2004) identified a QTL for PHS on the short arm of chromosome 2D in the marker interval gwm261-cdo1379 in the ITMI population. Comparison of the maps of Roder et al. (1998), Somers et al. (2004) and that of Munkvold et al. (2009) suggests that the significant QTL associated with marker wPt-6003 identified in the present study on 2DS corresponds to the QTL identified by Kulwal et al. (2004) on 2DS. Similarly, significant marker cfd37 mapped on 6DL identified in this study is the same that was earlier determined to be linked to the PHS QTL by Munkvold et al. (2009). However, in the biparental population this QTL explained 14 % of the variation as compared to 4 % in the association mapping panel used in this study. In a very recent study, Knox et al. (2012) identified two QTLs for germination index in a durum wheat population on chromosome 7B. Although there are other reports of PHS QTLs on chromosome 7B (Kulwal et al. 2004; Mohan et al. 2009; Liu et al. 2011), they have all been identified to be interacting QTLs without any main effect. Therefore, we believe that the main effect QTL identified in this sutdy on 7B is novel in hexaploid wheat. Using a DH population, Munkvold et al. (2009) identified four QTLs on chromosome 7D and one was linked with the marker wPt-0934. According to the unpublished maps from Triticarte Pty Ltd., Australia, the marker, wPt-663918, that was associated with PHS in our study is located ~3 cM from the marker wPt-0934 suggesting that a similar QTL was identified in both of these studies. Although some of the QTLs identified in this study were detected in the same genomic regions identified earlier, it should be noted that the association panel used in this study was unrelated to germplasm in earlier reports except for Anderson et al. (1993) and Munkvold et al. (2009). However, given the statistical issues associated with individual studies and the lack of common markers, direct comparisons between the different studies need to be further verified.

Conclusion

This study has identified a previously reported major QTL for PHS on chromosome 4AL in unrelated germplasm and that was not previously identified in our breeding program. Also, a novel QTL on chromosome 7BS in our elite white winter wheat materials was identified. This highlights the importance of association mapping for identifying QTL on

other chromosomes in germplasm that is more diverse than a biparental population, but still representative of a breeding program. In addition, we have identified another marker (gwm610) linked with this important QTL. This information will be useful for pyramiding important QTLs for PHS resistance in breeding programs along with the QTL on 2BS. This study also illustrates the importance of using elite material in association mapping for adaptive traits of complex inheritance such as PHS for effective use of QTL mapping results in the plant breeding programs.

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