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Genetic analysis of heading date in winter and spring wheat

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Abstract Climate change will have severe effects on wheat production, but crop phenology can be an important component of wheat adaptation. In this study, elite soft winter wheat and hard spring wheat (HSW) populations were phenotyped for heading date (HD) in North America and Tanzania (HSW only). All lines were genotyped with common single nucleotide polymorphism markers to compare the genetics and prediction accuracy of genomic selection (GS) for HD in winter and spring wheat. Lines were tested under diverse environments and the HSW germplasm was assessed for their early maturity performance in Africa. Two clusters of environments were formed for each population. One cluster consisted of southern environments and the other consisted of northern environments. The latter produced a more narrow range of HD than the southern cluster. Thirteen highly significant ($p < 0.0005$) quantitative trait loci (QTLs) for HD were detected in two populations. Within each population, the QTL effects were consistent between clusters of environments. Within each population, GS model developed using data from one cluster of environments could predict HD in the other cluster. The prediction accuracy of GS between two populations was minimal. Similarly, only a few minor effects QTL were in common between the two populations. Additionally, we identified 15 spring wheat genotypes with HD earlier than commercial Tanzanian wheat varieties. These genotypes could be used as a resource for creating early HD wheat varieties for Tanzania.

Keywords Clustering of environments · Genomic selection · Heading date · Kompetitive allelic specific

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Introduction

Wheat (*Triticum aestivum* L.) is a global source of staple grain, and has contributed immensely to the development of human civilization. Presently, global wheat demand is increasing, especially in sub-Saharan Africa, where the consumption rate for wheat is increasing more rapidly than for any other cereal grain (http://www.world-grain.com), resulting in a growing gap between domestic wheat production and consumption in Africa over the last several decades.

Tanzania and the rest of east Africa are among the most fragile regions in the world and are facing the impacts of climate variation induced by climate change (Challinor et al. 2007), which has increased the frequency, duration and timing of drought and heat stress in Africa (http://www.unep.org). Drought can reduce wheat yield through ovule abortion, pollen sterility, shriveled seeds and kernel abortion (Rosenzweig et al. 2001). Foulkes et al. (2007) estimated average yield of wheat under normal water supply conditions to be 8 metric tons/hectare, but severe yield reductions can be caused by drought, especially when it occurs after anthesis. In order to develop new cultivars adaptive to environmental changes, breeders should take into account the potential effects of drought and heat stress (Ceccarelli et al. 2010).

The wide adaptability of wheat is partly due to genetic diversity in flowering time and maturity (Law and Worland 1997; Lewis et al. 2008). Wheat phenology is controlled by the vernalization and photoperiod sensitive loci, as well as earliness per se gene*s* (Zikhali and Griffiths 2015). Combinations of these genes contribute to differences in flowering and maturity time in wheat, taking into account environmental conditions (Gomez et al. 2014; Guedira et al. 2014; Kamran et al. 2013; Sukumaran et al. 2016; van Beem et al. 2005).

In order to flower, photoperiod sensitive wheat cultivars require long day-length and the response to photoperiod is important for their adaptation to different environments (Snape et al. 2001). Three major loci for photoperiod sensitivity in wheat are located on chromosomes 2A (*Ppd-A1*), 2B (*Ppd-B1*) and 2D (*Ppd-D1)* (Beales et al. 2007; Díaz et al. 2012; Iqbal et al. 2007; Kamran et al. 2014; Khlestkina et al. 2009; Wilhelm et al. 2009). Recessive alleles at these loci enhance photoperiod sensitivity, whereas the dominant alleles reduce or eliminate photoperiod response (Whitechurch and Slafer 2001). The *Ppd-D1* locus has the largest effect on photoperiod sensitivity (Beales et al. 2007; Kamran et al. 2013, 2014). A fourth locus (*Ppd-B2*) was mapped on chromosome 7B (Khlestkina et al. 2009). Low photoperiod sensitivity is important for plants grown in latitudes below 45° where the day-length can be short (Dyck et al. 2004). Photoperiod insensitive wheat is also important in short day-length areas, where spring wheat is mainly produced, to allow multiple crop harvests per year (Mohler et al. 2004).

Vernalization response in wheat is controlled by multiple gene series designated *Vrn-1*, *Vrn-2, Vrn-3 and Vrn-4*. There are three *Vrn-1* loci located on chromosome 5A (*Vrn-A1*), 5B (*Vrn-B1*) and 5D (*Vrn-D1*) (Law et al. 1976; Sourdille et al. 2000; Barrett et al. 2002; Yan et al. 2003). Three *Vrn-3* loci are located on chromosomes 7A, 7B, and 7D (Yan et al. 2006; Bonnin et al. 2008; Wang et al. 2009) whereas one *Vrn-4* locus is located on chromosome 5D (Kato et al. 2003). The *Vrn-2* gene series are dominant for winter growth habit wheat, whereas *Vrn-1, Vrn-3,* and *Vrn-4* are dominant for spring growth habit (Yan et al. 2004).

Earliness per se genes can influence wheat flowering time and maturity, independent of photoperiod and vernalization genes (Lewis et al. 2008; Gomez et al. 2014). These genes are important for the wide adaptation of wheat to various environments (Snape et al. 2001; Gomez et al. 2014). Worland (1996) summarized the regions of the wheat genome that have earliness per se genes, reporting such genes on chromosomes 3A, 2B, 2D, 4D, 6B, 6D and 7B. A QTL study by Sourdille et al. (2000) found important chromosome regions associated with flowering time in wheat on chromosomes 2B, 5A and 7B. Additionally, a study conducted by Hoogendoorn (1985) found chromosome regions controlling flowering in wheat, and identified earliness per se genes on chromosomes 3A, 4A, 4D, 6B and 7B. Hanocq et al. (2004) identified flowering or heading time QTLs on chromosomes 2B, 2D, 5B and 7A. Wheat chromosome groups 2, 5 and to a lesser extent, group 7, are generally known to have major effect on wheat development (Law and Worland 1997). Earliness per

se genes affect flowering after the vernalization and photoperiod requirements are satisfied (Flood and Halloran 1984).

Crop variability in flowering time can be exploited to select cultivars adaptive to different climates and be used to maximize yield potential under various environmental conditions (Lewis et al. 2008). Wheat cultivars with a short growing season may have the ability to escape the effect of drought, which usually occurs toward the end of the growing season in Africa (Araus et al. 2008). Breeding lines with better adaptation to different environments could be enhanced via marker assisted selection (MAS). Conventional MAS utilizes molecular markers that are in linkage disequilibrium (LD) with genes or QTLs controlling a trait, and hence can be used to select individuals with desirable traits. Conventional MAS is most useful for chromosome regions with large trait effect and can be repeatable over environments and genetic backgrounds. Genomic selection (GS) utilizes all markers distributed throughout the genome to predict the breeding value of an individual and hence can be useful for traits controlled by many small effect genes (Meuwissen et al. 2001). It is suggested that GS can be a useful tool to reduce the duration of a breeding cycle (Heffner et al. 2009; Jannink et al. 2010). Using GS to predict HD values from different environments and populations could assist breeders in selecting cultivars adaptive to specific environments.

Indirect selection for maturity can be used if data from one environment can predict maturity in another. In addition, genetic information from one population and environment could be useful to understand that trait in a different environment and population. The objectives of this study were to: (1) assess the commonality of the genetics for HD trait in winter and spring wheat grown in different environments, (2) evaluate prediction accuracy of GS for HD between different environments and market classes, and (3) identify wheat germplasm with early maturity in Tanzania.

Materials and methods

Plant materials

Two populations, a soft winter wheat population (SWW) and a hard spring wheat population (HSW) were used in the study. The SWW population contained 273 elite winter wheat genotypes generated by the Triticeae Coordinated Agricultural Project (TCAP) described by Huang et al. (2016). Briefly, the SWW lines and check variety "Branson" were planted in 16 environments in the USA between 2012 and 2013 (Table 1). An augmented design was used for each replication at each location. Only one replication was used at each site except at Wooster Ohio and Warsaw Virginia, where three replications were included. The check variety "Branson" was repeated eight times per block. At the Wooster and Warsaw locations all treatments received 28 kg of N per hectare at fall planting time, and two replications received low nitrogen treatment (45 kg of N per hectare) in spring, while the third replication received full nitrogen treatment (101 kg N per hectare). All other locations received full nitrogen treatment in the spring. Heading date for SWW was the day when a line attained Feekes growth stage 10.5 (e.g., when 50% of the spikes had emerged from the boot). This date was expressed in Julian days.

The HSW population contained 249 elite spring wheat line generated by the TCAP (Supplemental Table 1). The lines were planted in twelve different environments in the USA in 2012 and 2013 and also in Arusha Tanzania in 2013 and 2014 (Table 2). The study was conducted using an augmented design with one replication at each location. Five check varieties were repeated within each block. Heading date was recorded as the date when a line attained at Feekes stage 10.5 and was expressed as days from planting.

Genotyping

Both populations were genotyped the with 90,000 single nucleotide polymorphism (SNP) marker panel through Infinium iSELECT (Wang et al. 2014). For both the SWW and HSW wheat, markers were filtered with missing value larger than 5%, and minor allele frequency less than 10%. A total of 13,198 SNPs for SWW and 17,303 SNPs for HSW population were retained for use in association analysis. We also performed SNP tagging for both populations to obtain a subset of independent markers. Details of the SWW were described by Huang et al. (2016). The subset of markers being used was 3919 SNPs in SWW (Huang et al. 2016), and was 5508 SNPs in HSW. These subsets of independent markers were used to compute

Table 1 Summary for Soft Winter Wheat (SWW) and Hard Spring Wheat (HSW) growing environments, their main effects on heading date (Julian days in SWW and days from planting in HSW), and environmental cluster assignment within each population

Pop[a]	Year	Town, state, province, or country	Latitude, longitude	Code	Main effects	Cluster	Environmental conditions
SWW	2012	Wooster, Ohio	Lat: 40.875, Long: − 81.888	12OWL[b]	3.1	North	Low N
	2102	Wooster, Ohio	–	12OWM	3.4	North	High N
	2013	Wooster, Ohio	–	13OWL	14.6	North	Low N
	2013	Wooster, Ohio	–	13OWM	14.8	North	High N
	2013	Custar, Ohio	Lat: 41.284 Long: − 83.844	13ONM	16.3	North	High N
	2013	Fremont, Ohio	Lat: 41.364 Long: − 83.155	13OVM	15.6	North	High N
	2012	Columbia, Missouri	Lat: 38.951 Long: − 92.334	12MOM	− 10.3	South	High N
	2013	Columbia, Missouri	–	13MOM	16.5	North	High N
	2012	Warsaw, Virginia	Lat: 37.960 Long: − 76.761	12VAL	− 21.78	South	Low N
	2012	Warsaw, Virginia	–	12VAM	− 21.4	South	High N
	2013	Warsaw, Virginia	–	13VAL	− 5.1	South	Low N
	2013	Warsaw, Virginia	–	13VAM	− 5.1	South	High N
	2012	Lexington, Kentucky	Lat: 38.040 Long: − 84.503	12KYM	− 11.1	South	High N
	2013	Lexington, Kentucky	–	13KYM	5.2	South	High N
	2012	Queenstown, Maryland	Lat: 38.991 Long: − 76.158	12MDM	− 14.4	South	High N
	2013	Queenstown, Maryland	–	13MDM	− 0.3	South	High N
HSW	2012	Davis, California	Lat: 38.526 Long: − 121.773	2012 CADAVIRR	53	South	Irrigated
	2012	Davis, California	–	2012 CADAVNIR	53	South	Non-irrigated
	2013	Arusha, Tanzania	Lat: 3.367 Long: 36.683	2013 TAARU	− 51	South	Irrigated
	2014	Arusha, Tanzania	–	2014 TAARU	− 47	South	Non-irrigated
	2012	Bozeman, Montana	Lat: 45.676 Long: − 111.157	2012 MTBOZ	− 51	North	Non-irrigated
	2013	Bozeman, Montana	–	2013 MTBOZ	− 53	North	Non-irrigated
	2012	Saskatoon, Canada	Lat: 52.117 Long: − 106.65	2012 SASAS	− 55	North	Non-irrigated
	2012	Huntley, Montana	Lat: 45.928 Long: − 108.246	2012 MTHUN	− 65	North	Non-irrigated
	2013	Huntley, Montana	–	2013 MTHUN	− 17	North	Non-irrigated
	2013	El Centro, California	Lat: 32.808 Long: 115.446	2013CA IMPIRR	− 13	North	Irrigated
	2013	El Centro, California	–	2013CA IMPNIRR	− 13	North	Non-irrigated
	2013	Wooster, Ohio	Lat: 40.875, Long: − 81.888	2013 OHWOO	− 59	North	Non-irrigated

[a]Pop: *SWW* soft winter wheat, *HSW* hard spring wheat

[b]*M* medium Nitrogen treatment, *L* low Nitrogen treatment in the SWW population

Table 2 Mean squares (MS), F-tests, variance components, and broad-sense heritability (H) of heading date for soft winter wheat (SWW) and hard spring wheat (HSW) populations over all environments, over the environments in the South cluster of environments, and over the North cluster of environments

Pop.	Source	All environments			South cluster			North cluster		
		df	MS	F[b]	*df*	MS	F	*df*	MS	F
SWW	Gen[a]	287	94	26	8	99	28	287	15	14
	Env	15	51,418	14,300	287	23,678	6756	6	10,460	1000
	Error	4286	4		2586	2586	3	2013	1	
	σ^2_{Gen}		6			11			2	
	σ^2_{Env}		179			82			36	
	σ^2_{Error}		3.6			4			1	
	# Env		16			9			7	
	H		0.96			0.96			0.93	
HSW	Gen	248	184	7	248	398	24	248	163	58
	Env	11	415,079	15,783	3	852,139	51,826	7	91,083	32,099
	Error	2521	26		743	16		1530	3	
	σ^2_{Gen}		14.3			94.2			3.5	
	σ^2_{Env}		1686			3416			485.2	
	σ^2_{Error}		14.3			16.7			2.8	
	# Env		12			4			8	
	H		0.92			0.96			0.91	

[a] σ^2_{Gen}: genotypic variance; σ^2_{Env}: environmental variance; σ^2_{Error}: error variance; # Env: number of environments within the cluster

[b] All F tests were significant at $p < 0.001$

kinship matrix and population structure matrix. Both the SWW and HSW populations were further genotyped with Kompetitive allele specific polymerase chain reaction (KASP) markers for the *Vrn-A1*, *Vrn-B1*, *Vrn-D3, Ppd-A1*, *Ppd-B1*, and *Ppd-D1* loci. An additional *Vrn-D1* locus conferring spring growth habit was also genotyped in the HSW population (Grogan et al. 2016).

Data analysis

In both populations, phenotypes were adjusted for block effects. A two-step approach was used to obtain the best linear unbiased prediction (BLUPs) of each line, as described for the SWW population by Huang et al. (2016). The block effects were first adjusted within each replication in each environment and mean of each genotype was obtained within each environment, then a mixed model with genotype, environment, and error term was included to fit the model. We estimated the broad sense heritability (H) for HD using the formula:

$$H = \frac{\sigma_g^2}{\sigma_g^2 + \sigma^2_{error}/e}$$

where σ_g^2 is variation due to genotype, σ^2_{error} is error variance due to both genotype × environment interaction (GEI) and error variance, and e is the number of environments.

The matrix for GEI effects from the SWW and HSW data sets was each generated, and Wards minimum variance was used to cluster the environments within each population using PROC CLUSTER in SAS (SAS Institute Inc. 2008). Two clusters of environments were identified for each population: a "South" cluster consisting of the southern environments and a "North" cluster consisting of the most northern environments. Association analysis was performed using mixed linear model in R (R Development Core Team 2008) with package Genomic Association and Prediction Integrated Tools (GAPIT Lipka et al. 2012). Principal component analysis (PCA) was conducted in R using prcomp function. The PC scores of each lines were used to correct for

population structure with the number of PCs selected by GAPIT, and a kinship matrix (K) was used to account for relatedness between individuals. The principal component scores were generated in R and the K matrix was generated using R package rrBLUP (Endelman 2011). The association analysis model was:

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Q}\mathbf{w} + \mathbf{S}\alpha + \mathbf{Z}\mathbf{v} + \mathbf{e}$$

where **y** is a vector of observed phenotypes, **Xβ** is the non-genetic fixed effect (mean); **Qw** is a fixed effect with q principal component scores and with **w** being the vector of principal component effects, **Sα** is a fixed effect with **S** being the matrix of marker scores and **α** being a vector of marker effects; **Zv** is the random polygene effect with **Z** being the matrix relating observations to their polygene effect, and **v** being the vector of polygene effects (Yu et al. 2006). Based on population structure and scree plot, two principal component scores were used for both populations. The total number of markers being used for association analysis was 13,198 for the SWW and 17,030 for the HSW populations. For both populations, we analyzed HD (1) over all environments, (2) North environments only, and (3) South environments only.

We conducted LD analysis between significant ($p < 0.0005$) SNPs using the R packages genetics (Warnes and Leisch 2005) and LDheatmap (Shin et al. 2006).

The alleles at the *Ppd-A1*, *Ppd-B1*, *Ppd-D1*, *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-D3* loci were determined using KASP markers in both populations with standard protocols of the USDA Small Grains Genotyping Lab (Rasheed et al. 2016). These markers were filtered and only those with minor allele frequency greater than 0.10 were analyzed using single marker analysis. Thus markers for *Ppd-A1*, *Ppd-B1*, *Ppd-D1* and *Vrn-D3* loci were retained and analyzed in the SWW population, and markers for *Ppd-B1*, *Ppd-D1*, *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Vrn-D3* loci were retained in the HSW analyses. Analysis for LD, as described above, was conducted between KASP markers and the SNP markers that were significant from the association analysis on the same chromosome.

Genomic selection (GS) analysis was performed in R using rrBLUP package (Endelman 2011). A common set of 8754 markers scored in both SWW and in HSW populations were used for GS and each individual's genomic estimated breeding value (GEBVs) was calculated. Prior to GS, missing values for all markers were imputed using the *A.mat*() function (Endelman 2011). We used ten-fold cross validation to estimate GS accuracy, which was estimated as the correlation between the GEBVs and the observed phenotypic values. The GS accuracy was also estimated for between population predictions and for between environmental cluster predictions within each population.

Results

SWW phenotypes and GEI

We analyzed HD expressed in Julian days in the SWW population. Winter wheat in southern environments break dormancy earlier than when they are grown in northern environments, and thus have an earlier HD when expressed as Julian days. This does not mean that the time period between breaking dormancy and heading is shorter in the southern environments than in the North. Heading date expressed in Julian days does not estimate total vegetative time period, which in winter wheat involves an extended period of dormancy. The use of Julian days in the SWW population will have a large impact on estimating environmental main effect, but no impact on the relative value of the main effects of genotypes, QTL or GEI effects.

In the analysis of variance in the SWW population, we found both genotypic and environmental effects were highly significant ($p < 0.0005$) (Table 2). As data were averaged over replications prior to the analysis of variance (ANOVA), the error variance was due to both GEI and error effects: genetic variance was 1.58 times greater than the error variance (Table 2).

As was reported in Huang et al. (2016), two clusters of environments were identified for HD in SWW. One cluster contained the six Ohio environments plus Missouri 2013 and was termed the North cluster (Fig. 1a). The other cluster had all the environments from southern US and was termed the South cluster. Replications from the same environment that differed only by nitrogen treatment always clustered together. All of the environments in the South cluster had negative main effects except 13KYM, whereas all environments in the North cluster had positive main effects (Table 1). Using ANOVA, the GEI variance

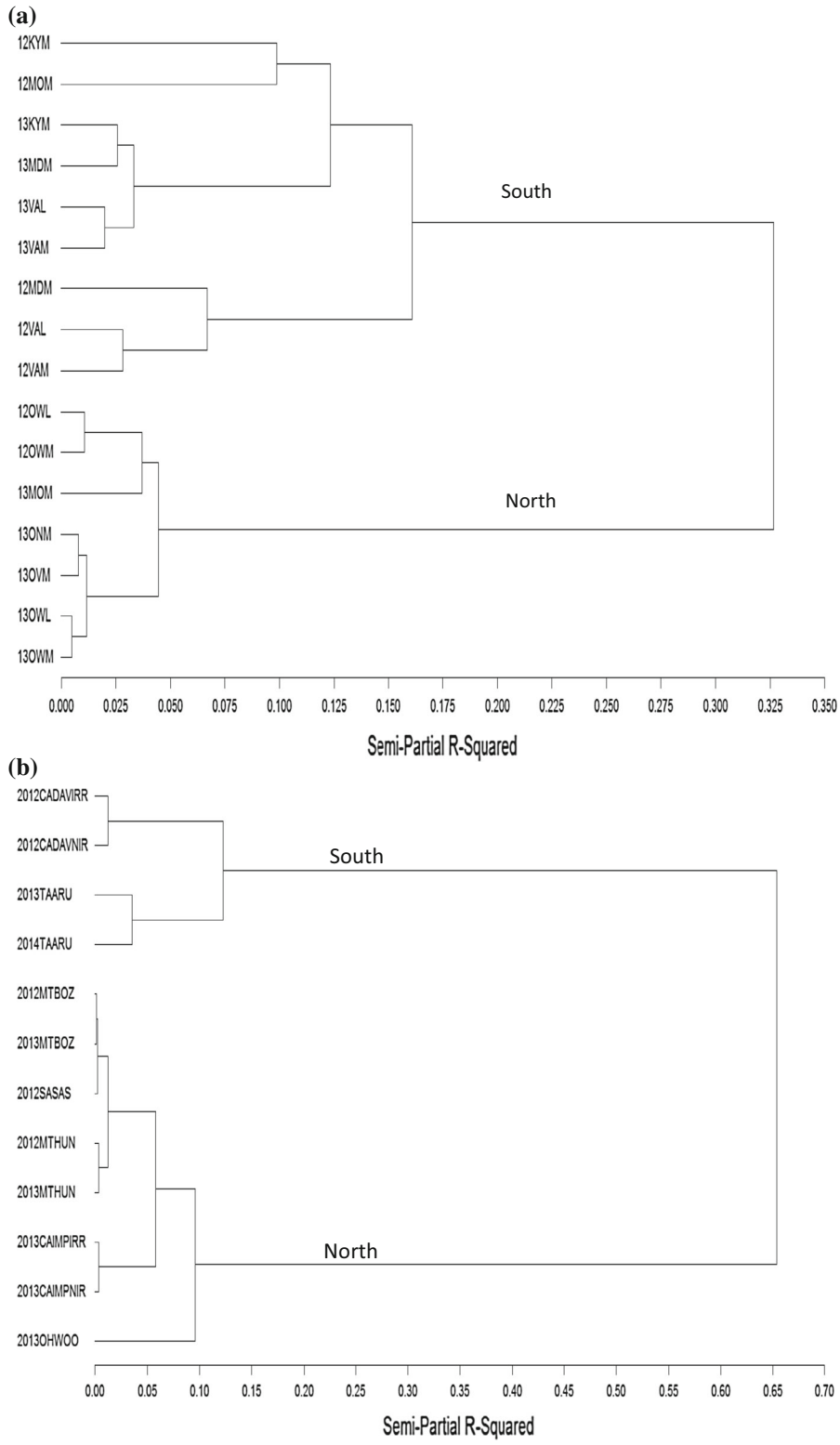

Fig. 1 Clustering of the environments for the Soft Winter Wheat (SWW) population (**a**) and for the Hard Spring Wheat (HSW) population (**b**) using the matrix of genotype × environment interaction values with Ward's minimum variance criteria

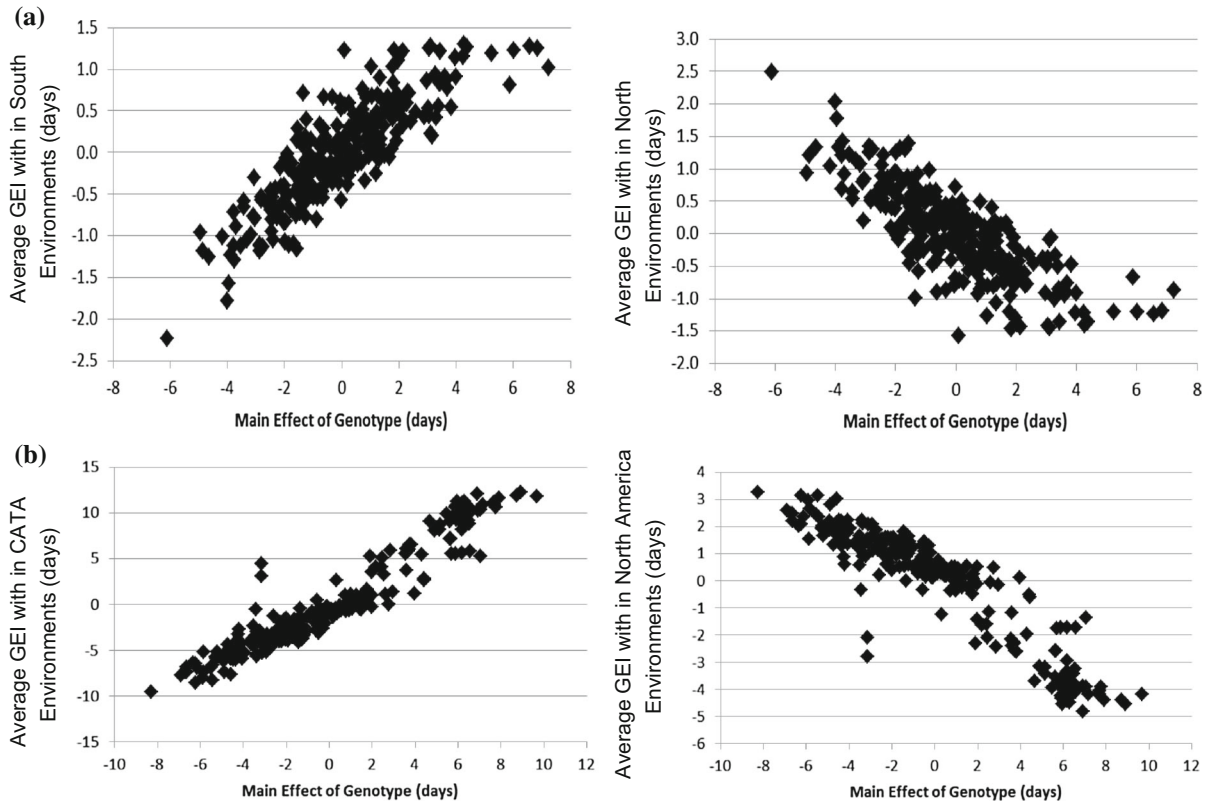

Fig. 2 Plot of main effects of Soft Winter Wheat genotypes **a** over environments versus their average genotype by environment interaction with South or North environments; and **b** main effects of Hard Spring Wheat genotypes versus their average genotype by environment interaction with South or North environments

was partitioned and 58% of the total GEI variance was due to genotype × cluster effects and 42% of the GEI variance was due to genotype × environment effects within clusters (data not shown).

In SWW, the estimated heritability for HD was greater than 0.92 for overall environments and within each cluster (Table 2). Genetic variance for HD in the South was greater than in the North (Table 2). There was a significant positive correlation ($r = 0.83$) between the genotype main effects over the North and over the South environments. The correlation of the main effects of genotypes with their GEI effects was 0.85 within South environments and was − 0.80 within North environments (Fig. 2a): the late genotypes had positive interactions with the south environments, but had negative interactions with the North environments; early genotypes had negative interactions with south environments and positive interactions with the North environments (Fig. 2a). These results indicate that early heading genotypes are later than expected, based on main effects, when planted in the North, and would head out earlier than expected when planted in the South. HD for late genotypes are earlier than expected in the North and are later than expected when planted in the South.

HSW phenotypes and GEI

Heading date for the HSW population was recorded as days from planting to Feekes 10.5 growth stage. This system has a different interpretation than HD recorded as Julian days in the SWW population. For HSW, HD is an estimate of the duration of the vegetative period of a line.

The analysis of variance in HSW over all environments revealed that genotype and environment effects were highly significant ($p < 0.0005$, Table 2). The 12 HSW environments were placed into two distinct clusters (Fig. 1b, Table 1). One cluster contained eight northern HSW environments and the two El

Centro, California environments: this cluster was named the North cluster. The second cluster contained two environments from Davis, California and two environments from Tanzania and was named the South cluster (Fig. 1b). The El Centro and Davis California sites had very different main effects on HD, though the clustering was based solely on GEI values. The four environments in the South cluster had very different main effects, with the Tanzania sites having large negative main effects and the California sites having large positive effects (Table 1). The GEI variance was partitioned and 86% of the total GEI variance was due to genotype × cluster effects and 14% of them was due to genotype × environment effects within clusters (Results not shown). Genetic variance for HD in HSW was much greater in the South environments than in the North cluster (Table 2). Similar to the trend we observed in SWW, the later heading HSW genotypes tended to have a positive interaction with the South environments (Fig. 2b) and a negative interaction with the North environments (Fig. 2b), whereas earlier genotypes tended to have the opposite GEI pattern (Fig. 2b).

The estimated heritability for HD in HSW was 0.88 over all environments, 0.96 in the South cluster, and 0.91 in the North cluster (Table 2). This corresponded to what we observed in the SWW population, where greater genetic variance existed in the South environments that in the North environments (Table 2).

Association analyses

For the SWW and HSW populations, we analyzed 13,198 and 17,030 markers, respectively (Tables 3

Table 3 Total number of markers and number of significant ($p < 0.05$) markers for heading date for each chromosome in the soft winter wheat (SWW) and hard spring wheat (HSW) populations

Chromosome	Number of markers		Number of significant markers					
	SWW	HSW	SWW			HSW		
			All	North cluster	South cluster	All	North cluster	South cluster
1A	700	1129	35	24	32	94	13	40
1B	1337	1341	37	37	37	58	36	62
1D	358	412	19	2	26	6	43	6
2A	662	956	27	21	25	38	32	28
2B	1365	1673	31	41	27	82	57	149
2D	484	476	72	47	53	1	11	8
3A	676	839	6	14	6	22	25	23
3B	998	1124	68	86	62	31	25	32
3D	132	183	14	35	13	38	14	44
4A	486	851	23	27	24	18	52	17
4B	515	568	23	34	18	42	60	39
4D	40	67	3	4	3	3	6	NA
5A	735	956	33	40	35	49	64	42
5B	1064	1699	72	66	73	177	165	137
5D	133	172	NA	1	NA	3	12	2
6A	808	990	59	75	68	43	103	17
6B	991	1229	34	38	24	46	12	44
6D	111	149	21	16	20	3	5	3
7A	772	1044	19	41	13	60	62	55
7B	729	997	62	71	55	26	60	25
7D	102	175	10	9	10	23	4	23
Total	13,198	17,030	668	729	624	863	861	796

Significance in each population is summarized by analysis over all environments and then within each cluster of environments (North and South)

Table 4 Summary of marker information for the soft winter wheat (SWW) and hard spring wheat (HSW) populations (1) overall environments, (2) over environments in the South cluster, and (3) over environments in the North cluster. Significance is defined as $p < 0.05$

	SWW	HSW
Number of markers used in association analysis	13,198	17,030
Number of markers significant for one or more traits	976	1588
Number of markers significant for all three traits	386	174
Number of markers significant overall environments	668	863
Range of r^2 values overall environments	0.21–0.27	0.22–0.30
Range of |a| overall environments	0.29–0.94	0.52–1.57
Number of markers significant in North cluster	729	796
Range of r^2 values in North cluster	0.16–0.19	0.23–0.30
Range of |a| for North cluster	0.17–0.46	1.25–3.66
Number of markers significant in South cluster	624	861
Range of r^2 values in South cluster	0.23–0.29	0.14–0.19
Range of |a| for South cluster	0.39–1.33	0.18–0.52

and 4). In SWW, approximately 36.7, 53.0, and 10.3% of markers were from the A, B, and D genomes, respectively. In HSW, approximately 39.7, 50.7, and 9.6% of the markers were from the A, B, and D genomes, respectively (Table 3). The correlation of the number of markers per chromosome between the two populations was 0.96, indicating similar genome coverage in both populations (Table 3).

We considered both the false discovery rate (FDR) probability values and the unadjusted probability value from GAPIT association analysis when assessing QTL. Over different environments (either all environments, North only, or South environments only), the FDR is very conservative and only 14 and 5 markers had FDR probabilities less than 0.05 in the SWW and in the HSW, respectively. The heritability for all traits was high (> 0.87) in both populations, so it is almost certain that many markers with FDR values greater than 0.05 are associated with real QTL, whose effects are simply not large enough to pass the very stringent FDR criteria. One of our objectives was to compare genetic architecture of HD over North and South environments and between the SWW and HSW populations; the FDR seemed poorly suited for that objective due to a seemingly high type II error rate. Thus, we used unadjusted probability values to define "significant" ($p < 0.05$), "very significant" ($p < 0.005$) and "highly significant" ($p < 0.0005$) SNP-QTL association (Table 4).

We analyzed HD over all environments, over North environments only, and South environments only. In the SWW population, we found a total of 976 markers (7.40%) were significant ($p < 0.05$) in at least one of the three HD analyses and 386 were significant in all three (Table 4). The correlation of allele effects in the North or South cluster of environments was 0.96 for the 967 markers that were significant ($p < 0.05$) in either cluster. The regression of the allele effects of these markers in the South cluster of environments onto the allele effects in the North cluster was significant ($p < 0.05$, $y = 0004 + 2.13x$) suggesting allele effects in the South were 2.13-fold greater than those in the North. In the HSW population, a total of 1588 markers (9.32%) were significant ($p < 0.05$) in at least one of the three analyses though only 174 were significant in all three (Table 4).

The correlation of allele effects in the North and South cluster of environments was 0.73 for the 1483 markers that were significant ($p < 0.05$) in either cluster. The regression of the allele effects of markers from the South environments onto those of the North environments was significant ($p < 0.05$, $y = -0.006 + 1.11x$) although the slope was close to unity, suggesting that allele effects in the South cluster were similar to those in the North cluster.

In both populations, the distribution of significant ($p < 0.05$) markers for HD was very similar to the distribution of all markers over chromosomes: 28–41% from the A genome, 47–61% from the B genome, and 9–21% from the D genome (Table 3). In total, 29 markers were highly significant ($p < 0.0005$) in at least one analysis in the SWW and eight markers were highly significant in the HSW. In either population, the identification of independent QTLs within a chromosome was clear, as the LD r^2 values between

highly significant markers from the same chromosome were either greater than 0.4 or close to zero.

The LD analysis coupled with the marker position from consensus map suggested there were eight and five highly significant ($p \leq 0.0005$) and independent QTL for HD in the SWW and the HSW population, respectively (Table 5). In SWW, these eight QTL were significant ($p < 0.05$) in all three analyses. Single-marker analysis revealed that the *Ppd-A1*, *Ppd-B1*, *Ppd-D1* and *Vrn-D3* markers were significant ($p < 0.05$) in at least one HD analysis within the SWW population (Table 6). *Vrn-D3* was significant in the North environments but not the South environments. The LD analysis indicated that the *Vrn-D3* locus were marginally associated with QTL8 (Table 6). All three *Ppd* loci were significant ($p < 0.05$) in SWW in the North and South environments as well as overall environments (Table 6). The LD analysis indicated that *Ppd-A1* was strongly associated with markers for QTL3 (Table 6).

In HSW, just one marker (QTL12) was highly significant ($p < 0.0005$) in all three analyses whereas the other four (QTL9, 10, 11, and 13) were significant ($p < 0.05$) only for overall HD environments and in the South environments (Table 5). The *Ppd-B1* and *Ppd-D1* loci were highly significant ($p < 0.0005$) for HD in the HSW South environments (Table 6) and overall environments. The LD analysis indicated that *Ppd-B1* and *Ppd-D1* were slightly associated with markers for QTL11 and QTL12, respectively (Table 6). The *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* loci were significant ($p < 0.05$) for HD in the North environments (Table 6) whereas the *Vrn-D3* locus was significant in the South environments.

The eight highly significant ($p < 0.0005$) QTL in the SWW population and the five QTL from the HSW population all had R^2 values less than 0.1 and the absolute value for allele effects did not exceed 3.8 days (Table 5). In both populations, the R^2 values and allele effects were higher in analyses of the South clusters of environments, which produced greater range of HD and greater genetic variance than in the North environments.

Of 8754 markers scored in common between the two populations, 657 and 808 markers were significant ($p < 0.05$) in at least one of the three analyses in the SWW population and in the HSW population, respectively. Between the sets of 657 significant markers in SWW and 808 significant markers in HSW, 98 markers were common. The correlation of absolute values of allele effects of these 98 markers in the HSW and SWW population, estimated from the three different analyses, ranged from 0.02 to 0.36. Within SWW, there were 29 highly significant markers ($p < 0.0005$) in at least one of the three analyses: 18 of these were scored in the HSW population, and all had p values greater than 0.05. In the HSW, there were eight highly significant markers ($p < 0.0005$) that were also scored on the SWW where they were not significant in any analysis.

In the SWW population, 29 markers were found to be highly significant ($p < 0.0005$) in at least one analysis and were further selected for LD analysis to assess their independence. In the HSW population, eight markers were identified as highly significant ($p < 0.0005$) in at least one analysis and were used in LD analysis.

Genomic selection

The GS accuracy of HD for the SWW or the HSW populations, estimated in overall environments, North environments only, or South environments only, ranged from 0.41 to 0.51 (Table 7). In the SWW, GS accuracy was greater in the South environments than in the North cluster, whereas the opposite trend was observed in HSW (Table 7). Using one-time predictions, we found that, within each wheat market class, data from one cluster of environments could predict the phenotype in the other cluster of environments (Table 7). Data from one wheat market class could not predict the phenotypes of lines in the other class (Table 7).

Line selection in Africa

The HD in Tanzania ranged from 45.2 to 89.3 days and the average was 63.5 days. We noted 63 lines in the HSW had significantly ($p < 0.05$) earlier HD values than the mean HD, based on a least significant difference analysis. We identified 15 lines from the HSW population that had an earlier HD in northern Tanzania than the existing commercial cultivars (50 days). The 15 earliest HSW came from breeding programs based in California, Idaho, South Dakota, Montana, and Canada, representing the entire north–south range sampled in the HSW populations.

Table 5 Summary of highly significant markers ($p \leq 0.0005$) for heading date in the soft winter wheat (SWW) and hard spring wheat (HSW) populations, their chromosome positions (Pos, in cM), R^2, and effects (days) overall, in the North cluster and in the South cluster (SWW)

Pop	QTL	Best marker	Chr	Pos (cM)	All environments			North			South		
					Prob.	R^2	a	Prob.	R^2	a	Prob.	R^2	a
SWW	1	Excalibur_c95327_126	1B	142	0.0002	0.041	0.7	0.0025	0.030	0.4	0.0002	0.042	1.0
	2	RAC875_c56811_258	1D	35	0.0003	0.039	0.6	0.0017	0.032	0.3	0.0003	0.038	0.8
	3	RAC875_c14944_555	2A	250	0.0004	0.039	0.6	0.0004	0.041	0.3	0.0006	0.035	0.7
	4	Excalibur_rep_c103747_193	5A	448	< 0.0001	0.059	0.9	0.0009	0.035	0.4	< 0.0001	0.065	1.2
	5	BS00020982_51	5B	290	0.0001	0.050	− 0.7	0.0007	0.038	− 0.3	< 0.0001	0.050	− 0.9
	6	BS00063415_51	6B	375	0.0017	0.030	0.5	0.0002	0.045	0.4	0.0042	0.024	0.7
	7	Ex_c12057_797	7B	246	0.0005	0.037	0.6	0.0003	0.043	0.4	0.0008	0.033	0.8
	8	Excalibur_c22419_460	7D	206	0.0003	0.039	− 0.7	0.0010	0.035	− 0.4	0.0003	0.038	− 0.9
HSW	9	CAP12_c1979_117	1A	887	0.0008	0.037	1.0	0.1409	0.008	0.2	0.0005	0.040	2.6
	10	CAP7_c2791_231	2A	1063	0.0008	0.037	1.4	0.1796	0.006	0.2	0.0004	0.042	3.5
	11	BobWhite_c41535_52	2B	748	0.0001	0.054	1.2	0.6906	0.001	0.0	< 0.0001	0.068	3.2
	12	IWA989	2D	190	< 0.0001	0.091	1.6	0.0001	0.056	0.4	< 0.0001	0.083	3.7
	13	Kukri_rep_c101179_404	7D	259	0.0016	0.032	0.8	0.4333	0.002	0.1	0.0005	0.040	2.3

Table 6 Summary of single point analyses of markers for photoperiod and vernalization genes in the soft winter wheat (SWW) and the hard spring wheat (HSW) panels, their chromosome locations, and linkage disequilibrium (LD) values with the markers on the same chromosome that were associated with the heading date QTL shown in Table 5

Pop[a]	Locus	Chr	Possible QTL association	LD (r^2)	*p* value		
					All environments	North	South
SWW	*Ppd-A1*	2A	QTL3[b]	0.56	0.0011	0.0000	0.0326
	Ppd-B1	2B	NA	NA	0.0093	0.0269	0.0094
	Ppd-D1	2D	NA	NA	< 0.0001	0.0133	< 0.0001
	Vrn-D3	7D	QTL8	0.15	0.0682	0.0001	0.4217
HSW	*Ppd-B1*	2B	QTL11	0.10	0.0000	0.0047	0.0000
	Ppd-D1	2D	QTL12	0.13	0.0000	0.0910	0.0000
	Vrn-A1	5A	NA	NA	0.0749	0.0000	0.3821
	Vrn-B1	5B	NA	NA	0.0089	0.0146	0.0150
	Vrn-D1	5D	NA	NA	0.1595	0.0001	0.4328
	Vrn-D3	7D	QTL13	0.01	0.0051	0.4779	0.0003

[a]Pop: Population for Soft Winter Wheat (SWW) or Hard Spring Wheat (HSW)

[b]QTL numbers from Table 5

Discussion

Wheat flowering process is controlled by vernalization, photoperiod response and earliness per se genes. In this study, vernalization requirement was confounded between the winter (SWW) and spring (HSW) wheat populations. Within each population, we identified two distinct clusters of environments corresponding to northern and southern test sites. In each population, the South cluster of environments produced a larger range of HD than the North environments. However, in both populations, the phenotypic values were highly, positively correlated between the North and South environments. Thus, the

Table 7 Accuracy of genomic selection within and between soft winter wheat (SWW) and hard spring wheat (HSW) populations and cluster of environments

Training population (TP)		Prediction population		*r*
SWW	All environments	Same as TP, used tenfold CV		0.48
	North environments	Same as TP, used tenfold CV		0.43
	South environments	Same as TP, used tenfold CV		0.49
HSW	All environments	Same as TP, used tenfold CV		0.51
	North environments	Same as TP, used tenfold CV		0.51
	South environments	Same as TP, used tenfold CV		0.41
SWW	All environments	SWW	North environments	0.81
	All environments		South environments	0.92
	North environments		South environments	0.75
HSW	All environments	HSW	North environments	0.61
	All environments		South environments	0.89
	North environments		South environments	0.53
SWW	All environments	HSW	All environments	− 0.20
	North environments		North environments	− 0.10
	South environments		South environments	− 0.21
HSW	All environments	SWW	All environments	− 0.09
	North environments		North environments	− 0.02
	South environments		South environments	− 0.12

A tenfold cross-validation (CV) was used when the training population and prediction population were the same

separation of environments into clusters based on GEI appeared to be due to differences in genotype, rather than lack of correlation of phenotypic values between clusters. This was supported by the estimated genetic variance within each cluster of environments in each population (Table 2).

The effect of photoperiod genes varies with the latitude of the growing environment (Kamran et al. 2014). In SWW, the differences in variation in HD between the South and North clusters of environments is possibly due to the difference in magnitude of effects for photoperiod and earliness per se between the two clusters. This was observed as the *PpD-D1* and *Ppd-B1* loci had greater effects on HD in the South environments than the North environments, though the opposite was observed for *Ppd-A1* (Table 6). Kamran et al. (2014) reported that photoperiod genes influence heading more in lower latitudes than in northern latitudes, due to shorter day-length in the South. In the North environments, the SWW lines break dormancy later in the calendar year when day-length is longer than when they are grown in the South. Thus the day-length requirement for the photoperiod genes is met sooner in the North than in the South and the photoperiod genes may cause less variation for HD in the North than in the South. Earliness per se genes contribute to regional adaptation (Griffiths et al. 2009). In this study, the earliness per se genes would likely have similar effects in the North and South, as indicated by the correlation of the marker effects in the North and South environments.

Similarly, the differences in variation for HD shown by the two clusters of environments for the HSW population could also be due to differences in the effects of *Ppd* genes and the effects of growing temperature between the two clusters. The environments in the North cluster of HSW environments all experience long days immediately after crop emergence in early May, such that the day-length requirement for flowering would be met quickly and thus the *Ppd* genes would likely have a small effect on heading. Wheat lines in the HSW South cluster of environments were planted when day-length was short, therefore the photoperiod-sensitive wheat lines would not be flowering until the day-length exceeds a threshold. This likely explains why the *Ppd-B1* and *Ppd-D1* loci were only highly significant ($p < 0.0005$) for HD in the South environments of the HSW.

The assayed *Vrn* loci all had significant ($p < 0.05$) effects on HD in the HSW and SWW populations, with some being important in the North environments and some in the South environments. It is clear why the *Vrn-D3* locus would be significant in the SWW population in the North environments. It is difficult to explain why the *Vrn* loci have a significant effect on HD in the HSW environments. Perhaps there are some effects of the *Vrn* loci on HD in HSW due to copy number variants (Díaz et al. 2012).

The results in this study are supported by previous findings. Worland and Snape (2001) suggested that adaptation of spring wheat to different agro-climate conditions is highly influenced by *Ppd* genes. It has also been reported that lack of fulfillment of photoperiod requirement delays flowering in photoperiod-sensitive spring wheat (Kamran et al. 2014) and this delay would have occurred in the South environments in our study.

We used unadjusted probability values for classifying QTL instead of FDR probability values, since the FDR was too stringent and it likely produced a high type II error rate in this study. The use of unadjusted *p-values* increases the potential rate of type I error, which is a concern for identifying real QTL. Yet, reducing type II error is beneficial for analysis of genetic architecture for polygenic traits. Bernardo and Yu (2007) suggested that the effectiveness of marker-assisted selection was improved when more markers were added to a model with relaxed probability values to reduce type II error, indicating that many markers with p values greater than 0.05 can be marking real QTL. In each population, a similar proportion of markers was significant ($p < 0.05$) and had a similar distribution across chromosomes. The genetic variance explained (R^2) and allele effects were generally low in both populations even for highly significant ($p < 0.0005$) markers.

In both populations, significant ($p < 0.05$) QTL effects estimated in the South and North clusters of environments were highly correlated ($r = 0.97$ in the SWW population; $r = 0.73$ in the HSW population). Thus, the results from one set of environments validated the other, suggesting that repeatable marker effects were identified even when using a probability of 0.05. The QTL effects in both populations were two- to five-folds larger in the South cluster of environments, which produced the greatest range of phenotypes and tended to have short day-length, than

in the North cluster of environments. Heading date appeared to be controlled by the same genes in both clusters of environments, suggesting that the earliness per se genes have similar effects within a class of wheat, regardless of different environments though the magnitude of effects for these genes varied. In contrast, there was weak evidence for common HD QTL between the SWW and HSW populations, as the correlation of SWW and HSW allele effects was close to zero, and the few QTL that appeared in common had only a minor effect on HD in either population. Two QTL on chromosomes 4A and 5A did affect HD with probability values of less than 0.005 in both populations. The other four possible coincident QTL had probability values greater than 0.05 in at least one population.

Some QTLs (QTL3 and QTL8 in SWW; QTL12 in HSW were located near *Ppd* or *Vrn* genes (Table 6) and thus may be coincident with those loci. Other *Ppd* and *Vrn* loci were located on chromosomes with QTL but did not appear to be associated with the markers for those QTL. In HSW, the QTL located on chromosomes 2B (QTL11) and 2D (QTL12) had an allele effect of greater than 3.15 days in the short day-length South environments. These were associated with the *Ppd* genes (Table 6).

The HSW QTL12 located on chromosome 2DS is in disequilibrium with *Ppd-D1*. This QTL had an effect on HD of 0.40 in overall environments but 3.7 days in the South environments, where photoperiod genes should have a large effect on HD. *Ppd-D1* is reported to have a great effect on photoperiod sensitivity (Beales et al. 2007; Kamran et al. 2014).

The markers for the highly significant ($p < 0.0005$) QTLs (QTL8 and 13) identified in this study on chromosome 7D (Table 5) were slightly associated with vernalization gene *Vrn-D3*, (LD $r^2 = 0.1$, Table 6). *Vrn-D3* together with *Vrn-A1* and *Vrn-B1* were reported to have effects on heading date in previous studies (Law et al. 1976; Sourdille et al. 2000; Barrett et al. 2002; Yan et al. 2003; Kiss et al. 2014). The QTLs on chromosomes 6B (QTL6 in SWW) and 2B (in QTL11 in HSW) are in regions, where earliness per se genes were reported in previous studies (Scarth and Law 1983; Worland 1996; Snap et al. 2001).

There is often a complex interaction among *Ppd*, *Vrn*, and earliness per se genes as well as interactions of these genes with the environments (Gomez et al. 2014; Gororo et al. 2001; Kamran et al. 2013; Sukumaran et al. 2016; van Beem et al. 2005). As the SWW and HSW were grown in very different environments this may be the reason for lack of common QTLs between the two populations. Moreover, the SWW and HSW are genetically very different from one another based on the first two principal component scores (Fig. 3). The presence or absence of particular *Vrn* alleles may affect the expression and value of *Ppd* and earliness per se genes (Iqbal et al. 2007; Sukumaran et al. 2016) and may explain why marker effects in the HSW and SWW showed little commonality.

Using genomic selection to predict HD among different environments and populations could assist breeders to select cultivars with adaptation to specific environments. Our results suggest that GS models can accurately predict HD between different environments within HSW or within SWW, but predictions between SWW and HSW would not be useful. The HSW results could be useful for wheat breeding in Africa. Much African wheat germplasm originates from CIMMYT and other non-local programs. Results in this study reveal that GS models built using data from other trials in other countries would appear to be able to predict HD and maturity data in Africa. Thus African breeders can collaborate with HSW breeders worldwide to find early maturing lines with confidence that HD data

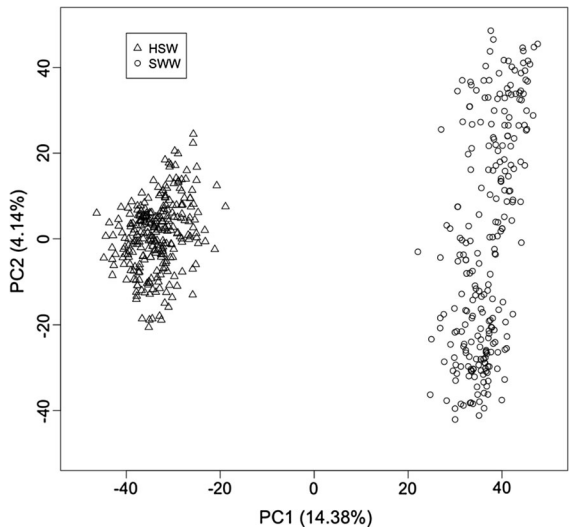

Fig. 3 Graph of first two principal components from the analysis of the Soft Winter Wheat (SWW) and Hard Spring Wheat (HSW) populations using a common set of 8754 markers

from those programs can predict HD in Africa. Tanzania has very short growing season and early maturing wheat cultivars are desirable to avoid season-ending drought. We identified 15 genotypes from the HSW population that have HD at least 4.8 days earlier than that of standard checks for northern Tanzania. These 15 genotypes were selected based on BLUPs from phenotypic analysis of Tanzania environment only, the presence of highly significant markers for early HD, and also the GEBVs developed from the South set of HSW environments. There remained genetic variation among these 15 lines at two of the HSW QTLs, one of which had a large effect on HD in South environments. Thus crossing amongst these 15 lines should produce transgressive segregants that would have even earlier HD than that found in the 15 lines.

The selected lines can be used in the Tanzanian wheat-breeding program to develop early heading populations adapted to wheat growing environments in Tanzania. Heading date is a highly heritable trait even when based on individual plant, and phenotypic selection could be used to breed for early wheat lines for Tanzania. But association analysis and genomic selection techniques could still be helpful in identifying genotypes with novel alleles for early HD, especially if the individual plants were being genotyped for selecting more complex traits such as yield. The selections made as a consequence of this work will be used to create early flowering wheat cultivars adapted to wheat growing environments in northern Tanzania.

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Compliance with ethical standards

Conflicts of interest The authors of this study declare that there is no conflict of interest for this study.

Ethical standard This research complies with the current laws of the United States of America.

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