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ORIGINAL PAPER

Identification of quantitative trait loci for productive tiller number and its relationship to agronomic traits in spring wheat

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Abstract Productive tiller number (PTN), defined as the number of tillers that produce spikes and seeds, is a key component of grain yield in wheat. Spring wheat cultivars in the northern Great Plains of North America differ in PTN. The objectives of this study were (1) to determine the relationship of PTN to agronomic traits using recombinant inbred line (RIL) populations derived from crosses Reeder/Conan, McNeal/Thatcher and Reeder/McNeal grown under a range of environments, and (2) to identify and validate quantitative trait loci (QTL) associated with high PTN. Correlation between PTN and plot weight ranged from $r = 0.4$–0.6 among the populations based on combined means over years, and was positive in every environment for all crosses ($P < 0.05$). A genetic map generated for the Reeder/Conan RIL allowed identification of a QTL for PTN consistent over environments, located on chromosome 6B. The QTL on chromosome 6B (*QTn.mst-6B*) explained 9–17% of the variation of PTN and co-segregated with a QTL for yield in the Reeder/Conan RIL. *QTn.mst-6B* was validated by single marker analysis in the McNeal/Thatcher RIL, McNeal/Reeder RIL, and a set of near isogenic line (NIL) developed for *QTn.mst-6B*. The allele for high PTN significantly increased PTN by 8.7, 4, and 13% in the McNeal/Reeder RIL, McNeal/Thatcher RIL and Choteau/Reeder NIL, respectively. The allele for high PTN also had a significant positive effect on plot weight in the McNeal/Reeder RIL. Our results suggest that high PTN, controlled to a significant extent by *QTn.mst-6B*, contributed to increased yield potential over a range of environmental conditions. *QTn.mst-6B* may be useful for improving spring wheat in the northern Great Plains of North America and similar environments.

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Introduction

Climate change affects spring wheat growing environments in the northern Great Plains (Lanning et al. 2010), challenging breeders to identify traits and genes that will allow reliable grain yield under increasing temperatures. Grain yield is a complex trait, controlled by multiple genes and environmental interactions across all plant developmental stages. Grain yield in cereals may be dissected into three primary yield components, including spike number per area, seed number per spike, and seed weight (Ma et al. 2007). Spike number per area is a function of productive tiller number (PTN) per area. PTN, the number of tillers that produce spikes and seeds, is controlled by both tiller initiation and tiller survival to the point of spike production.

Tiller initiation and development has been described as having three stages: (1) initiation of an axillary meristem; (2) development of an axillary bud; and (3) outgrowth of the axillary bud (Schmitz and Theres 2005). In cereal crops, genes controlling axillary meristem development have been identified and characterized. For instance, *teosinte branched* (*tb1*) in maize causes a complete loss of apical dominance, allowing the unrestrained outgrowth of axillary buds (Doebley et al. 1997). *Monoculm1* (*MOC1*) in rice results in a lack of axillary buds (Li et al. 2003). In rye, the monoculm (*mc*) gene on the proximal region of chromosome 6RL controls axillary bud formation (Malyshev

et al. 2001). Several mutations have been reported in barley that affect axillary bud formation and thus tiller production. The recessive mutation *uniculm2* (*cul2*) on chromosome 6HL allows initiation of axillary meristems that fail to develop tillers (Franckowiak 1996; Babb and Muehlbauer 2003). The recessive mutation *absent lower laterals* (*als*) on chromosome 3H results in development of axillary buds for primary tillers, but not for secondary tillers (Dabbert et al. 2009). Spielmeyer and Richards (2004) identified a tiller inhibition gene (*tin*) on the short arm of wheat chromosome 1A, which altered the pattern of axillary bud formation and outgrowth. Kuraparthy et al. (2007) also identified tiller inhibition (*tin3*) on chromosome 3A in wheat, which produces only one main culm compared to the wild-type with many tillers.

Although single genes affecting tiller number have been identified in several cereal crops, tiller number per plant in most segregating populations is inherited as a quantitative trait with low to moderate heritability. Heritability of tiller number per plant was 0.34 and 0.51 in rice (Miyamoto et al. 2004; Rahman et al. 2007), 0.51 in barley (Tapsell and Thomas 1983) and 0.62 in wheat (Li et al. 2002). Quantitative trait loci (QTL) for tiller number and spike number were identified on several wheat chromosomes (Richards 1988; Shah et al. 1999; Kato et al. 2000; Li et al. 2002; Huang et al. 2003; An et al. 2006; Narashimhamoorthy et al. 2006; Kumar et al. 2007; Deng et al. 2011). In two studies, QTL on 4D and 6DL co-segregated with QTL for grain yield (Huang et al. 2003; Kumar et al. 2007).

Not all tillers produce spikes, and many tillers abort before anthesis (Gallagher and Biscoe, 1978). Loss and Siddique (1994) found that many older Mediterranean wheat varieties produce a large number of tillers that are unable to produce spikes, while newer varieties produce fewer total tillers, but more survive to spike production. Pinthus (1966) compared tiller number between vernalized winter wheat and spring wheat and reported that winter wheat had twice as many tillers as spring wheat before heading, but number of tillers at the end of the growing season was similar for winter and spring wheat. Yan et al. (1998) found in rice that QTL affecting tiller number varied depending on the growth stage, with some QTL controlling tiller growth in early stages undetectable at later stages. Therefore, QTL controlling PTN may be more critical for yield improvement than QTL controlling tiller initiation.

Abundant tillering has been predicted to be an undesirable trait for dry-land cereal production due to lack of productive spike formation on later tillers (Donald 1968). Elhani et al. (2007) found that productive tiller number was an important contributor to grain yield under high moisture conditions, but had no detectable effect under rain-fed conditions. However, others have found high PTN is not only a key component for grain yield improvement, but also an important character in relation to phenotypic plasticity in response to drought (Baum et al. 2003; Reynolds et al. 1999). In semiarid region of the northern Great Plains, high-yielding and widely grown spring wheat cultivars differ for tiller production (Hansen et al. 2005). Understanding the genetic basis of PTN and identification of QTL for this trait would be useful for breeders. The objectives of this study were (1) to determine the relationship of PTN to agronomic traits with different recombinant inbred line (RIL) populations under different water regimes and years, and (2) to identify and validate QTL associated with high PTN.

Materials and methods

Plant materials

Recombinant inbred lines (RILs) were developed by single-seed descent starting with the F2 generation from three crosses, including Reeder (PI 613586)/Conan (PI 607549) (WestBred, LLC), McNeal (PI 574642) (Lanning et al. 1994)/Thatcher (CI 10003) and McNeal/Reeder. The parents, McNeal, Reeder and Conan are all widely grown hard red spring wheat cultivars (Montana Agricultural Statistics, 2010), while Thatcher is a historically important cultivar in the Great Plains. A total of 91 Reeder/Conan, 160 McNeal/Thatcher and 50 McNeal/Reeder RIL were derived from individual F6, F5 and F4 plants, respectively. RILs in these three populations were segregating for height because of parental differences at the *Rht* loci. The McNeal/Thatcher population consisted of 80 semi-dwarf and 80 standard height RIL. These RILs were randomized within eight blocks in groups of 20 for either the tall or semi-dwarf genotypes to eliminate the effects of shading. The Reeder/Conan and McNeal/Reeder RIL initially consisted of dwarf, semi-dwarf, and standard height genotypes, but only semi-dwarf and standard height RIL were included in these studies.

Experimental design

The three RIL populations were evaluated with three replications and a randomized complete block design in different irrigation regimes in subsets of years from 2004 to 2009 at the Arthur H. Post Research farm in Bozeman, Montana (latitude 45.41°N, longitude 111.00°W, elevation 1,455 m). The trials were conducted in 2006, 2007 and 2009 for the Reeder/Conan RIL, 2006 and 2007 for McNeal/Thatcher RIL and 2004 and 2005 for the McNeal/Reeder RIL. The total amount of irrigation supplied varied across years, with application of 10.2, 8.9, 12.7, 14.0 and

8.9 cm in 2004, 2005, 2006, 2007 and 2009, respectively, depending on amount of precipitation from April to June in each year. Irrigation was applied prior to heading stage. Each plot consisted of 3.3 m single rows seeded at 2.5 g m^{-1} for rain-fed trials and 3.3 g m^{-1} for irrigated trials. Growing season precipitation and temperature data were collected from the National Oceanic and Atmospheric Administration (NOAA) measurements (National Climatic Data Center 2010).

Phenotypic data collection

Phenotypic data collected in all experiments included PTN, plot weight (kg ha^{-1}), single seed weight (mg) and seed number per spike. For PTN measurement, number of tillers with fertile spikes per 30 cm of each plot was counted once prior to harvesting. Each plot was harvested and each plot weight was adjusted to be 10% moisture such that adjusted plot weight = plot weight × (1 − percent moisture content/100)/0.9.

A subsample of seed was taken from each plot and was analyzed using the Single Kernel Characterization System 4100 (Perten, Huddings, Sweden) to determine single seed weight (mg). For seed number per spike, the number of seed per spike was counted and averaged from ten spikes that were randomly sampled from each plot. This trait was measured only in the Reeder/Conan and McNeal/Thatcher RIL.

Statistical data analysis

Data for each phenotypic trait in each population were analyzed via analysis of variance (ANOVA) using a model for a randomized complete block for each environment (irrigation regime and year). Also, ANOVA was conducted using data combined over environments where the model included environment, replications within environment, entry and environment × entry with PROC GLM (SAS Institute Inc. 2004).

All factors except environment were considered random effects. Narrow-sense heritability for PTN was computed on an entry mean basis as described in Knapp et al. (1985) combined over environments.

Pearson correlations were computed using PROC CORR of SAS (SAS Institute Inc. 2004) between PTN and agronomic traits using entry mean values from each environment and combined over environments for each population.

QTL mapping

Creation of a genetic map for the Reeder/Conan RIL was described previously (Sherman et al. 2010). The map originally contained 232 SSR markers, 190 DArT markers (Akbari et al. 2006), six markers for major genes controlling development (*Ppd-A1*, *Ppd-B1, Ppd D1*, *Rht-B1*, *Rht-D1*, *Vrn-B1*) and three markers for storage proteins. In this study, 18 SSR markers were added to chromosome 6A and 6B and the total size of the map was 2,699.5 cM. Markers were subjected to a Chi-square test for fit to a 1:1 ratio using MapDisto (http://mapdisto.free.fr). Markers with significant distortion were indicated in the genetic map (Sherman et al. 2010). QTL analysis was conducted for PTN using the entry means for each environment by QTL Cartographer (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). QTL were identified with composite interval mapping (CIM) (Zeng 1993, 1994) using the standard CIM model, Forward and Reverse Regression method with a window size of 10.0 cM with probability in and out of 0.1 and a walking speed of 2 cM as described by Sherman et al. (2010). LOD value was set by 1,000 permutations at an experimentwise $P < 0.01$. QTL were established by the map position of the peak LOD score in the interval between the two flanking markers. A one-LOD fall-off (from the QTL peak) method was used to estimate the left-and right-flanking map positions of a confidence interval surrounding the mean QTL map position (Chaky 2003). QTL detected in different environments were considered to be the same if the estimated map position of their peaks was within 20 cM (Maccaferri et al. 2008).

QTL validation

The McNeal/Thatcher RIL, McNeal/Reeder RIL, and a set of near isogenic lines (NILs) were used to verify the effect of the QTL identified from the Reeder/Conan mapping population. For the McNeal/Thatcher RIL and McNeal/Reeder RIL, a single-factor ANOVA was conducted using PROC GLM in SAS (SAS Institute Inc. 2004) where the single factor was the segregating marker locus with two allelic classes. The segregating markers linked to PTN QTL in Reeder/Conan mapping population included PCR markers for *Ppd-B1* (Blake et al. 2009), microsatellite markers wmc453 and barc55 for a QTL on 2B and gwm88 and gwm193 for a QTL on 6B (GrainGenes 2.0, http://wheat.pw.usda.gov/GG2/index.shtml, 2010). Gwm88 and gwm193 were polymorphic between the McNeal/Reeder RIL, while only gwm88 was polymorphic in the McNeal/Thatcher RIL. Entry means of PTN and other traits averaged over environments for each population were used for this analysis. Differences between allele class means were tested with an F ratio for each segregating marker locus for each population.

A set of NIL for a QTL identified on chromosome 6B was generated following the procedure of Blake et al. (2011). An initial cross was made between Reeder (high PTN) and Choteau (PI 633974; lower PTN). An F_4 RIL

population was developed by single seed descent beginning at the F_2 generation. The F_4 RIL population was screened with microsatellite PCR markers for locus gwm88 and gwm193 (GrainGenes 2.0, http://wheat.pw.usda.gov/GG2/indexshtml) to select alleles for the QTL on chromosome 6B previously identified in the Reeder/Conan RIL. Heterozygous F_4 individuals at these loci (referred to as residual heterozygous lines: RHL) were allowed to self-pollinate, and progenies homozygous for the Reeder allele and the Choteau allele were selected. A total of eight F_4 RHL were used to derive NILs. The NIL sets, derived from a single F_4 RHL, are approximately 94% identical, limiting background genetic effects on PTN. NILs derived from same RHL were similar for height, heading date and other morphological traits. Homozygous NILs were evaluated in a randomized block with three replications in 1.7 m single row in the Arthur H. Post Research farm in Bozeman, Montana in 2010. Response variables for the NILs were analyzed using mixed effects ANOVA where the model included block, QTL allele (Reeder vs. Choteau), F_4 family within cross, and entry within QTL allele by F_4 family combination using PROC MIXED in SAS (SAS Institute Inc. 2004). The block, F_4 families, and entry within cross by QTL allele by F_4 family combination were considered random effects while all other effects were fixed. The difference between the Reeder and Choteau QTL allele for each cross was tested using the ESTIMATE statement in SAS.

Results

Environmental conditions

Data were gathered over 5 years for genetic analysis of PTN by evaluating the three RIL populations under rain-fed and irrigated conditions. Table 1 indicates that environmental conditions varied among the years. In 2006 and 2007, above average temperature and below average precipitation, particularly during the grain-fill stage in July and August, resulted in heat and drought stress. In 2007, the conditions were the most severe with 9 days above 35°C maximum temperature and a reduction of precipitation of approximately 36% relative to the 50-year average for this site in July and August. No other year had such an extended period of days with high temperatures and drought conditions. The years 2004, 2005 and 2009 showed similar temperature and precipitation to the 50-year average.

Phenotypic differences

Reeder showed significantly higher PTN than Conan in every environment (Table 2). Both Reeder and Thatcher had higher PTN than McNeal (Table 2). There was significant genetic variation for PTN in Reeder/Conan and McNeal/Thatcher in all environments, with RIL means intermediate to the respective parents (Table 2). Heritability of PTN ranged from 0.65 to 0.75 in the three RIL populations, based on combined means over environments and years (Table 2). As Blake et al. (2009) reported, there was significant genetic variability for grain yield in all populations in all environments (Table 2). Reeder tended to show higher plot weight than Conan and McNeal though there were no significant differences except the 2009 rain-fed and irrigated environments (Table 2). McNeal showed significantly higher plot weight than Thatcher in all environments (Table 2). There was also significant genetic variability for seed weight in the three RIL populations, and for seed number per spike in the Reeder/Conan and McNeal/Thatcher RIL (data not shown).

Correlation analysis

Correlations between PTN and plot weight, seed weight and seed number per spike for the Reeder/Conan, McNeal/Thatcher, and McNeal/Reeder RIL are shown in Table 3. PTN showed a positive correlation with plot weight in all

Table 1 Growing season precipitation (cm) and average monthly temperature (°C) at the Post Research farm in Bozeman, MT

Years	Precipitation (cm)					Average monthly temperature (°C)				≥35°C (day)[a]
	May	June	July	August	Total	May	June	July	August	
2004	6.7	5.7	3.8	4.1	20.2	10.4	14.2	19.8	17.2	1
2005	3.0	7.7	2.7	4.4	17.8	9.4	12.1	19.8	19.1	2
2006	4.4	8.7	1.4	1.8	16.2	11.1	14.8	21.5	18.9	4
2007	12.7	6.7	0.8	1.6	21.7	12.2	16.1	23.4	19.2	9
2009	4.1	6.7	7.1	3.8	21.7	12.1	14.1	18.6	18.4	0
50-year average										
1958–2009	6.7	6.9	3.5	3.2	20.3	10.7	14.8	18.7	18.1	

[a] Number of days recorded greater than or equal to 35°C maximum temperature in July and August

Table 2 Mean of productive tiller number and plot weight for the three different RIL populations and their parents

	Factor	2004		2005		2006		2007		2009		h^{2a}
		RF	IR	RF	IR	RF	IR	RF	IR	RF	IR	
PTN												
Rdr/Con	Rdr[b]	–	–	–	–	83.0**	–	53.8*	–	52.0**	63.0*	0.70
	Con	–	–	–	–	64.2	–	44.3	–	44.6	46.1	
	RIL[c]	–	–	–	–	67.9****	–	50.0**	–	46.4***	54.9***	
McN/That	McN[b]	–	–	–	–	68.3	70.0	46.8*	40.3	–	–	0.75
	That	–	–	–	–	79.8	76.2	55.5	50.9	–	–	
	RIL[c]	–	–	–	–	71.1****	71.3****	52.6**	47.6***	–	–	
McN/Rdr	McN	61.2	89.5	54.0	61.0	–	–	–	–	–	–	0.65
	Rdr	68.3	101	62.7	72.0	–	–	–	–	–	–	
	RIL[c]	68.0	90.5	60.5**	67.3	–	–	–	–	–	–	
Plot weight (kg ha^{-1})												
Rdr/Con	Rdr[b]	–	–	–	–	4,677	–	3,554	–	4,860****	5,229**	
	Con	–	–	–	–	4,079	–	3,184	–	3,816	4,462	
	RIL[c]	–	–	–	–	4,070****	–	3,012****	–	4,069****	4,598****	
McN/That	McN[b]	–	–	–	–	4,656***	5,115****	4,199**	3,109*	–	–	
	That	–	–	–	–	3,602	3,385	3,362	2,670	–	–	
	RIL[c]	–	–	–	–	3,992****	4,110****	3,891****	3,199****	–	–	
McN/Rdr	McN	5,297	7,820	4,705	6,370	–	–	–	–	–	–	
	Rdr	6,005	8,141	4,997	6,423	–	–	–	–	–	–	
	RIL[c]	5,450**	7,412***	4,612**	6,251****	–	–	–	–	–	–	

Significance level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

RF rain-fed, *IR* irrigated, *Rdr* reeder, *Con* Conan, *McN* McNeal, *That* Thatcher

[a] h^2, heritability was calculated based on combined mean over environments

[b] Asterisks in this row indicate parental mean values are significantly different

[c] Asterisks in this row indicate significant difference within RIL population based on analysis of variance

RIL populations across all years and environments. PTN was negatively correlated with seed number per spike in the 2006 Reeder/Conan rain-fed environment and the 2006 McNeal/Thatcher rain-fed and irrigated environments. Seed weight was negatively correlated with PTN in the McNeal/Thatcher RIL in all years and all environments. Negative correlations between seed weight and PTN were also observed in the 2004 and 2005 McNeal/Reeder rain-fed environment and the 2005 McNeal/Reeder irrigated environment. In sum, correlation analysis showed consistent significant positive correlation between PTN and grain yield in all RIL populations across years and environments (Table 3). The correlation between PTN and seed number per spike, and PTN and seed weight, tended to be negative.

QTL analysis

QTL analysis was conducted for each year and environment for the Reeder/Conan RIL population using the genetic map generated by Sherman et al. (2010) with additional markers subsequently added on chromosome 6A and 6B. CIM indicated 11 significant QTL, most occurring in a single environment (Table 4). All QTL from 2007 were observed only in a single environment. QTLs for PTN were observed on each member of the homoeologous group 6 chromosomes. There were two QTL for PTN identified from the 2006 rain-fed and the 2009 irrigated trials on chromosome 6A where the Reeder allele for both QTL was associated with greater PTN, accounting for 12% of variation in both the 2009 irrigated and the 2006 rain-fed environment (Table 4). A single QTL on 6D explained about 12% of the variation. The QTL on 6B were significant in multiple years and environments and were localized within a 21.7 cM region flanked by wPt4716 and wPt3581 (Table 4; Fig. 1). The Reeder allele caused greater PTN and accounted for variation as follows: 15% in 2006 rain-fed, 17% in 2009 irrigated and 9% in 2009 rain-fed environments (Table 4). The 6B QTL co-segregated with plot weight where the Reeder allele was associated with greater yield in the 2009 rain-fed environment (Table 4). Most of the markers within the 6B PTN QTL showed no significant segregation distortion. However, two markers, gwm193

Table 3 Pearson's correlation between productive tiller number and agronomic traits in three RIL populations grown in several environments

Population	Year/environment	Plot weight	Seed weight	Seed number per spike
Rdr/Con	2006/RF	0.39***	−0.19	−0.27**
	2007/RF	0.38***	−0.08	−0.05
	2009/RF	0.55****	−0.14	0.09
	2009/IR	0.32**	−0.13	−0.06
	Combined	0.39***	−0.20	−0.18
McN/That	2006/RF	0.49****	−0.18*	−0.25**
	2006/IR	0.57****	−0.18*	−0.24**
	2007/RF	0.54***	−0.18*	0.11
	2007/IR	0.49***	−0.37***	0.11
	Combined	0.63****	−0.25**	−0.28***
McN/Rdr	2004/RF	0.32*	−0.29*	–
	2004/IR	0.30*	−0.24	–
	2005/RF	0.42**	−0.33*	–
	2005/IR	0.35**	−0.35**	–
	Combined	0.40**	−0.43**	–

Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

RF rain-fed, *IR* irrigated, *Rdr* reeder, *Con* Conan, *McN* McNeal, *That* Thatcher

Table 4 Significant QTL for productive tiller number identified through composite interval mapping of the Reeder/Conan mapping population in environments and years

Chromosome	Flanking markers	Year/environment	Additive effect[a]	R^{2b}	LOD[c]	Coincident QTL
1A	wPt2311-barc269	2006/Rain-fed	−2.78	0.09	2.5	None
2B	PpdB-barc55	2009/Rain-fed	−3.32	0.19	4.0	None
3B	wPt7502-wPt8096	2009/Irrigated	−3.41	0.16	5.0	None
3D	barc316-gwm645	2007/Rain-fed	−3.24	0.19	5.2	None
4B	wPt0391-gwm495.1	2006/Rain-fed	3.06	0.11	3.0	Seed weight
4D	cfd71-wmc457	2007/Rain-fed	−2.40	0.10	3.4	None
5D	Barc345-gwm765	2009/Irrigated	2.17	0.07	2.5	None
6A	gpw7073-gpw3087	2009/Irrigated	2.83	0.12	3.9	None
	gpw4312-gpw4145	2006/Rain-fed	3.20	0.12	4.3	None
6B	wPt4716-wPt3581	2006/Rain-fed	3.70	0.15	5.2	None
		2009/Irrigated	3.43	0.17	5.6	None
		2009/Rain-fed	2.35	0.09	2.9	Plot weight
6D	barc196-cfd88	2006/Rain-fed	3.27	0.12	4.1	None
7B	wmc273-barc303	2007/Rain-fed	2.94	0.15	5.1	None

[a] Additive effect of the Reeder allele

[b] R^2 The phenotypic variation explained by the QTL

[c] LOD logarithm of odds. Only QTL with LOD scores above 2.5 are shown

and barc1008, associated with this QTL were significantly distorted (indicated by asterisk Fig. 1). Although we did not pursue QTL for further analyses that were identified from a single environment or year, several might be of interest. For example, the Reeder allele for the 2B QTL flanked by PpdB and barc55 reduced PTN and accounted for 19% of the variation in the rain-fed environment (Table 4). Reeder carries *Ppd-B1a* that confers photoperiod insensitivity (Blake et al. 2009). Also, a QTL observed on 7B might be of interest as it increased PTN in the extreme 2007 environment.

QTL validation

NIL and RIL populations were used to validate the 6B QTL and confirm consistent effects across backgrounds. Eight pairs of NIL with contrasting alleles at the 6B QTL were developed from heterozygous F_4 plants from a cross between Reeder and Choteau using the method described by Blake et al. (2011). Table 5 presents results of ANOVA for PTN in the Reeder/Choteau NIL. The Reeder allele at the 6B QTL increased PTN by 13% in the Reeder/Choteau NIL ($P < 0.02$). The variation explained by the genotype at

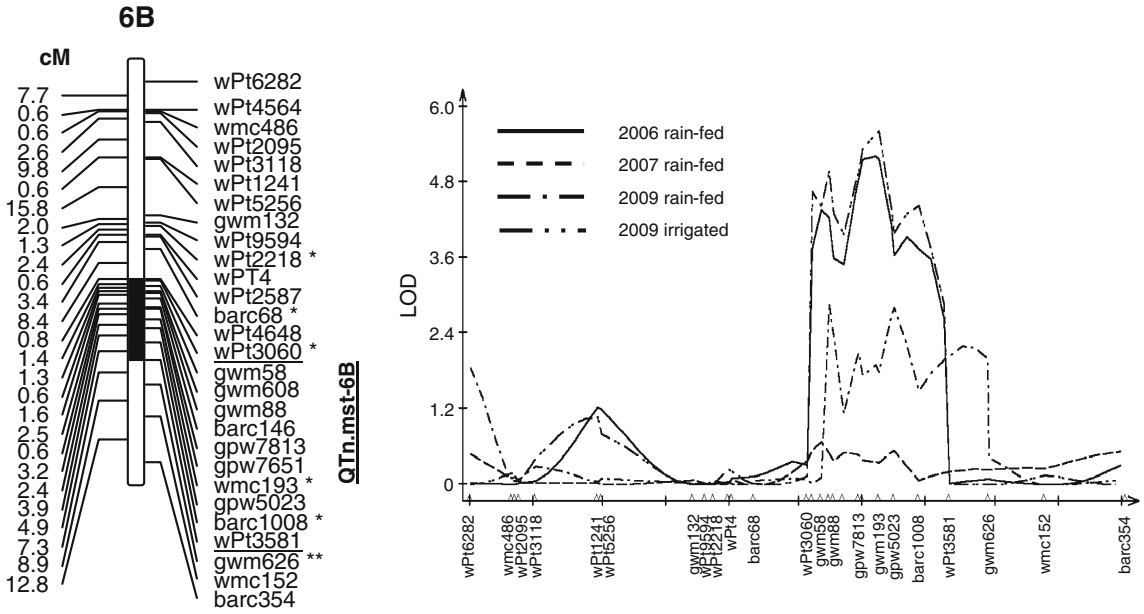

Fig. 1 Linkage map of chromosome 6B from Sherman et al. (2010) with four additional markers (gwm608, gpw7813, gpw7651 and gpw5023) and the removal of co-segregating DArT markers. Chromosome region of *QTn.mst-6B* indicated in black. Flanking markers defining *QTn.mst-6B* are underlined. *Asterisks* at the end of marker name denote significantly distorted loci ($P < 0.05$). Position and LOD score of the QTL for PTN on chromosome 6B in 2006 rain-fed (*normal line*), 2007 rain-fed (*dashed line*) and 2009 rain-fed (*single dotted line*) and irrigated (*double dotted line*) environments are shown on the *right*

Table 5 Analysis of variance to test the effect of alternative alleles at *QTn.mst-6B* based on marker gwm88 and gwm193 using near isogenic lines developed from Reeder/Choteau cross

Analysis of variance				Productive tiller number		
Factor	DF	Mean square	*F* value	Allele	No. NIL[a]	Mean[b]
QTL allele	1	423.2	4.82*	Reeder	36	52.3* (13.0%)
Block	2	80.5	0.74	Choteau	33	46.3
F_4 family	7	209.9	2.47			
Entry (F_4*QTL allele)	14	85.0	0.78			
Residual	37	109.5	–			

Significance levels: * $P < 0.05$

QTL allele refers to the allele state at *QTn.mst-6B*. F_4 family refers to the NIL sets derived from specific heterozygous F_4 plants. Entry (F_4*QTL allele) refers to entry by QTL allele by F_4 family combination

Numbers in brackets show the increasing percentage of Reeder compared to alternate parent

[a] Number of near isogenic lines

[b] Compares the difference between the QTL allele from Reeder and Choteau

marker gwm 88 was significant and greater than any other source of variation. Another marker located within the 6B QTL, gwm193, showed the same result as gwm88. The effect of the 6B QTL Reeder allele was also verified in the McNeal/Reeder RIL through single marker analysis using polymorphic markers gwm88 and gwm193. The effect of the 6B QTL Thatcher allele in the McNeal/Thatcher RIL was also determined in a similar fashion using gwm88 in single marker analysis. Entry means of PTN averaged over environments for each population were used for this analysis. The 6B QTL significantly influenced PTN for the respective RIL populations (Table 6). The Thatcher allele increased PTN by 4% in the McNeal/Thatcher RIL and the Reeder allele increased PTN by 8.7% in the McNeal/Reeder RIL. Due to its consistent effect on PTN and the apparent importance of this QTL in all three RIL populations as well as the NIL, we have designated the 6B QTL *QTn.mst-6B*. We also conducted single marker analysis to determine the impact of *QTn.mst-6B* for agronomic traits in the confirmation RIL populations. Entry means of

Table 6 Single marker analysis for productive tiller number and agronomic traits averaged over environments for each population using gwm88 and gwm193 linked to *QTn.mst-6B*

Population	Allele	No. RIL[a]	PTN[b]	Plot weight (kg ha^{-1})	Seed weight (mg)	Seed number per spike
McN/That	McNeal	89	59.7	3,839	30.0	36.1
RIL	Thatcher	64	62.0 (1.7%)	3,744	29.9	34.6 (−4.2%)
	P value[c]	–	0.04	0.33	0.87	0.01
McN/Rdr	McNeal	26	68.9	5,832	36.1	–
RIL	Reeder	23	74.9 (8.7%)	6,078 (4.2%)	35.4	–
	P value	–	<0.0001	0.04	0.27	–

QTn.mst-6B was identified in Reeder/Conan RIL mapping population. Numbers in brackets show the increasing percentage of Reeder or Thatcher compared to alternate parent

RF rain-fed, *IR* irrigated, *Rdr* reeder, *McN* McNeal, *That* Thatcher

[a] Number of recombinant inbred lines with each allele

[b] Compares the difference between the QTL allele classes based on gwm88 and gwm193 genotype

agronomic traits averaged over environments for each population were also used for this analysis. The Thatcher allele conferring high PTN was also associated with decreased seed number per spike for the McNeal/Thatcher RIL, though there was no association with plot weight (Table 6). The Reeder allele conferring high PTN at *QTn.mst-6B* was associated with increased plot weight in the McNeal/Reeder RIL (Table 6).

Discussion

Measurement of tiller number may be conducted at different stages of development. Assessment of tiller number in vegetative stages provides a measurement of maximum tillering, while assessment at or after heading provides a measurement of productive tiller number. Several genetic studies with mutant populations have focused on pre-heading stages of tiller development (Li et al. 2002; Richards 1988; Spielmeyer and Richards 2004; Kuraparthy et al. 2007; Dabbert et al. 2009), while agronomic studies more often involve measurement of productive tiller number or spike number (Kato et al. 2000; Huang et al. 2003; Quarrie et al. 2006; Kuchel et al. 2007). Moreover, some studies used tiller number per plant, and others used tiller number per area for tillering trait. We report tiller number per area in this paper. Analyses using tiller number per planted seed showed the same results, including a significant positive correlation with yield in every environment for every cross, and an important QTL controlling tiller number on chromosome 6B.

There was a significant positive correlation between PTN and plot weight in each environment for all three RIL populations (Table 3). It is important to note that the experimental environments varied in temperature and precipitation. There was also genetic background variation between populations. Correlations of PTN and plot weight were consistent even with these variations. This result is consistent with the previous studies which reported positive correlations between tiller number and yield in wheat (Sidwell et al. 1976; Kato et al. 2000; Huang et al. 2003; Kumar et al. 2007). Despite the positive correlation with plot weight, there were negative correlations between PTN and seed weight in the McNeal/Thatcher RIL and McNeal/Reeder RIL, and PTN and seed number per spike in the Reeder/Conan RIL and McNeal/Thatcher RIL (Table 3). Other studies have reported similar negative correlations (Kato et al. 2000; Narasimhamoorthy et al. 2006; Deng et al. 2011) and suggested pleiotropic effects among these yield components (Deng et al. 2011). Negative correlations amongst yield traits may make it difficult to improve grain yield by only increasing PTN. Deng et al. (2011) classified wheat cultivars into large-spike versus multi-spike type cultivars, where large-spike cultivars have more seed per spike but fewer spikes than the multi-spike type. In this study, Reeder would be classified as a multi-spike type cultivar compared to McNeal and Conan (Table 1) and showed higher plot weight in every environment (data not shown). However, another multi-spike type cultivar, Thatcher, did not show a yield advantage compared to McNeal. Blake et al. (2009) reported that Reeder showed longer green leaf duration after heading (GLDAH) than McNeal, and McNeal showed longer GLDAH than Thatcher. In their study, longer GLDAH was associated with higher grain yield and kernel weight in the Reeder/McNeal RIL and the McNeal/Thatcher RIL. It is possible that multi-spike type cultivars need to possess longer GLDAH to support grain filling of many spikes in order to show a yield advantage.

We identified significant QTL on all group 6 chromosomes, whereby the Reeder allele showed a significant positive effect on PTN (Table 4). QTL for PTN observed across environments were identified on 6B in the Reeder/Conan mapping population (Table 4). Other studies have reported QTL for tiller number on chromosome 6A and 6D

(Li et al. 2002; Huang et al. 2003; An et al. 2006; Kumar et al. 2007). An et al. (2006) identified a QTL for tiller number on chromosome 6A flanked by wmc179 and wmc256. The marker wmc256 is linked to wmc753 which is located within the 6A QTL flanked by gpw4312 and gpw4145 reported here (Table 4; GrainGenes 2.0, http://wheat.pw.usda.gov/GG2/index.shtml). The marker wmc256 is also located on the long arm of chromosome 6A based on Chinese Spring deletion map (GrainGenes 2.0, http://wheat.pw.usda.gov/GG2/indexshtml). Therefore, one of the 6A QTLs we identified in this study is probably a verification of the QTL as previously reported by An et al. (2006). Molecular markers within *QTn.mst-6B* (gwm88, gwm193 and gwm608) and a flanking marker of the 6D QTL (barc196) are located on long arm of each chromosome based on Chinese Spring deletion map (GrainGenes 2.0, http://wheat.pw.usda.gov/GG2/indexshtml). Therefore, these QTL may represent homoeologs of a gene controlling PTN. In barley, Babb and Muehlbauer (2003) positioned *cul2* on chromosome 6HL. This gene co-segregates with molecular marker cdo524 that is closely linked to gwm88. gwm88 is linked to *QTn.mst-6B* reported here (GrainGenes 2.0,http://wheat.pw.usda.gov/GG2/index.shtml). Moreover, Malyshev et al. (2001) identified monoculm growth habit (*mc)* on chromosome 6RL in rye (*Secale cereale L.)* that also appears to occupy a similar chromosomal position. These studies support the possibility that the chromosome 6 QTLs for PTN identified in the present study and the barley and rye genes controlling tiller number are homoeologous.

QTL identified in multiple environments and genetic backgrounds may be most useful for plant breeding. The only significant QTL for PTN observed in three environments was *QTn.mst-6B*. It was also validated in the McNeal/Reeder RIL, and the Reeder/Choteau NIL, indicating effectiveness in multiple genetic backgrounds. Effectiveness of QTL in eight different NIL pairs from Reeder/Choteau confirms the primary cause of increased PTN in this cross is *QTn.mst-6B*. The observation of an allele at the same locus from a different background (Thatcher) with similar effect supports the importance of *QTn.mst-6B*. (Table 6). The results from the McNeal/Thatcher RIL population provide further confirmation for a QTL at this genomic location, though any relationship between the Thatcher and Reeder alleles for high PTN cannot be inferred. The Thatcher allele at *QTn.mst-6B* had about half the effect as the Reeder counterpart, which might indicate the Thatcher allele is different. However, due to the possibility of epistatic effects from Thatcher, the nature of the relationship between the Reeder allele and the Thatcher allele is unknown at this time. Nevertheless, *QTn.mst-6B* has a stable effect on PTN between genetic backgrounds and environments. More QTL for PTN were identified in 2009, which were cooler and wetter during the grain-fill stage than 2006 and 2007. Cool temperatures along with adequate moisture may have allowed higher tiller survival and thus higher numbers of productive tillers. QTL identified in 2009 alone, located on chromosomes 2B, 3B and 5B might have a primary effect on tiller initiation. The only QTL identified in 2007 were unique to that year. The severe high temperature and drought conditions during the grain-fill stage in 2007 caused abrupt senescence and negatively impacted tiller number. The QTL on 7B, that increased PTN under the stress conditions, might be of particular interest. By combining with the more environmentally stable QTL on 6B, PTN might be improved in a broader range of environments.

Two of the RIL populations and the NIL population in this study had Reeder as a parent, and the Reeder allele at *QTn.mst-6B* conferred increased PTN and yield potential in RIL populations. Although no PTN QTL on 6B has been previously reported, several QTL for yield on chromosome 6B have been identified (Marza et al. 2006). Our results suggest that selection for the positive allele at *QTn.mst-6B* in crosses involving Reeder is likely to increase PTN and plot weight. Conversely, there was no significant effect on plot weight for alternative alleles at this QTL for the McNeal/Thatcher RIL. It is likely that the negative effect of the Thatcher allele for high PTN on seed number per spike at *QTn.mst-6B* (Table 6) offset the positive effect on increased PTN and resulted in no effect on yield in the McNeal/Thatcher RIL.

Crop modeling efforts have suggested that low tiller number may be beneficial for wheat productivity in water-stressed environments (Donald 1968). However, others have suggested that high tiller survival may be related to increased tolerance to drought stress (Reynolds et al. 1999). An et al. (2006) showed significant correlation between root dry weight and tiller number in a wheat population derived from parents which differed in drought tolerance. As the wheat root system develops in a coordinated pattern along with tillers (Weaver, 1926), plants with more tillers may develop more roots, therefore high tiller number may contribute tolerance to drought stress through increased root development. Deng et al. (2011) also discussed that compared to large-spike type cultivars, multi-spike type cultivars usually have a more stable and higher grain yield especially under stress environments such as drought and high salt. Baum et al. (2003) reported that under stress conditions, the number of productive tillers contributes largely to grain yield in barley.

In conclusion, our results showed a consistent positive correlation between PTN and plot weight under drought and heat stress conditions as well as well-watered conditions for three spring wheat populations. *QTn.mst-6B,* for high productive tiller number on chromosome 6B from Reeder, was consistent across environments and

populations, and increased plot weight in RIL involving Reeder. *QTn.mst-6B* may be useful for improving spring wheat in the northern Great Plains of North America and similar environments.

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