Impact of a major gene for stem solidness on agronomic performance of spring wheat near-isogenic lines

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List of Abbreviations: PCR, polymerase chain reaction; NIL, near-isogenic line; WSS, wheat stem sawfly; QTL, quantitative trait locus

Abstract

The primary trait in wheat to prevent damage caused by the wheat stem sawfly (*Cephus cinctus* Norton) is antibiosis facilitated by solid stems. The pith of solid stems impedes development of larvae, often resulting in their death inside the stem. A key question regarding solid stems is ~~its~~ the possible impact on grain yield potential due to photosynthate partitioning to stem pith ~~rather than to~~instead of to grain. Molecular markers for the major gene controlling stem solidness, *Qss.msub-3BL*, were used to develop near-isogenic lines (NIL) for alleles at *Qss.msub-3BL* in six genetic backgrounds. The NIL were grown in replicated trials in twelve locations that varied for yield potential in Montana, Washington and Idaho. There was no significant impact of the solid stem allele on grain yield based on mean performance over recurrent parents and locations. Individually, ~~S~~solid-stemmed NIL were significantly lower yielding in one of six crosses. Based on means over all crosses, the solid-stemmed NIL had lower yield in only one high-yielding environment. These results suggest that in general the allele for solid stems at *Qss.msub-3BL* does not result in yield reduction. Development of solid-stemmed cultivars with yield potential similar to the best hollow-stemmed cultivars is a reasonable goal for wheat breeding programs in areas impacted by the wheat stem sawfly.

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Wheat (*Triticum aestivum* L.) genotypes vary for the amount of pith found inside stems. Solid-stemmed genotypes have stems completely filled with pith, while hollow-stemmed genotypes have no pith inside the stem. Intermediate types are referred to as having semi-solid stems. The solid-stemmed characteristic is the primary control measure for an important insect pest of wheat, the wheat stem sawfly (WSS) (*Cephus cinctus* Nort.) (Weiss and Morrill, 1992; Beres et al., 2011). The WSS females lay eggs inside of young wheat stems early in the growing season. The feeding larvae migrate to the base of the plant at the end of the season, and cut a notch inside the stem leading to stem lodging. The combination of resource depletion by the feeding insect and lodging results in severe losses of yield and seed quality. Solid stems impede the progress of the WSS larvae, leading to high mortality rates, reduced feeding damage, and lower stem cutting.

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Identification of solid stems as a control mechanism for the WSS (Platt and Farstad, 1946; Platt et al., 1948) prompted considerable research regarding the genetic basis of the trait. Early genetic studies using crosses and aneuploid stocks pointed to a limited number of genes with major effects, also influenced by genes of lesser effect (reviewed in Wallace and McNeal, 1966). More recent molecular mapping allowed identification of a major quantitative trait locus on chromosome 3B, *Qss.msub-3BL* (Cook et al., 2004). This locus controlled 76% of the variation in a population of recombinant inbred lines (RIL) derived from a solid- by hollow-stemmed winter wheat cross. A major effect QTL at the same genomic location has been identified in durum wheat (*Triticum turgidum* L.) (Houshman et al., 2007). Kalous et al. (2011) showed that *Qss.msub-3BL* was the major contributor to variation in stem solidness for an association mapping panel of spring wheat lines. Lanning et al. (2006) identified a second QTL with lesser effect on solid stems on chromosome 3D. Traits such as plant height also ~~have an~~ impact ~~on~~ the degree of stem solidness in lines possessing *Qss.msub-3BL* (Lanning et al., 2012). The level of stem solidness is also affected by the environment, with lower levels of pith expression in cool wet conditions (Hayat et al., 1995).

The impact of solid stems on other agronomic traits has been investigated in several studies, with conflicting results. Allocation of photosynthate to pith in stems rather than to grain has been hypothesized to lower harvest index and yield potential (Weiss and Morrill, 1992). Solid-stemmed varieties have historically yielded less than hollow-stemmed ones in the absence of WSS (Weiss and Morrill, 1992). McNeal et al. (1965) found a negative genetic correlation between stem solidness and grain yield in a cross between the original solid-stemmed cultivar Rescue and hollow-stemmed lines. Later work involving crosses with improved solid stemmed lines showed that there was no negative genetic correlation between stem solidness and grain yield (McNeal and Berg, 1979). In fact, there was a positive correlation between stem solidness and grain yield when moisture availability was not yield-limiting. Similarly, Hayat et al. (1995) showed that there was no genetic correlation between stem solidness and yield in six populations. They suggested that the low yield of solid-stemmed cultivars was not related to the genes for solid stems but rather to unlinked deleterious genes from the original solid-stemmed accession. Cook et al. (2004) showed no association between the major solid stem locus on 3BL *Qss.msub-3BL* and grain yield based on a winter wheat RIL population, while Lanning et al. (2006) showed no impact on yield for the secondary gene on chromosome 3D.

At least one study has suggested that solid stems may have a positive impact on grain yield in stress environments. Saint Pierre et al. (2010) compared a set of 36 spring wheat lines from the CIMMYT breeding program with different levels of stem solidness under a range of conditions. Solid-stemmed lines produced higher yields under stress conditions than hollow-stemmed lines.

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The amount of pith in stems was positively correlated with amount of water soluble carbohydrates in both well-watered and drought conditions. There was a positive association between amount of pith and grain yield under drought stress. Results of Damania et al. (2007) ~~were opposite to~~conflicted with those of Saint Pierre (2010), ~~in~~ as ~~that~~ solid-stemmed landraces of wheat from Turkey were less prevalent in the driest provinces. Damania et al. (1997) suggested that WSS distribution patterns may have had a larger effect than abiotic conditions on the occurrence of solid stems in landraces.

The question of genetic association between solid stems and grain yield is an important one. Yield deficiencies associated with solid stems lessens grower adaptation of solid-stemmed cultivars. Many growers will accept a degree of WSS damage in order to grow the highest-yielding cultivar. This is problematic, as larvae that complete their life cycle to the point of stem cutting will also overwinter, leading to abundant WSS populations in the next year. A key question for breeders is whether it is feasible to expect that solid-stemmed cultivars can be developed with no yield penalty relative to the best hollow-stemmed lines. For this study, sets of near-isogenic lines differing for the major solid stem QTL *Qss.msub-3BL* were developed in six spring wheat backgrounds. The lines were tested in 12 environments in the intermountain west of the United States that varied in level of stress to determine the impact of stem solidness on ~~other~~ agronomic traits.

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Materials and Methods

~~The donor parent for ~~T~~t~~he solid stem allele at *Qss.msub-3BL* ~~was ~~from‘Choteau’ (PI 633974) (Lanning et al., 2004) was backcrossed into six hollow-stemmed wheat lines.. Recurrent hollow-stemmed parents included two hard red spring wheat cultivars, ‘Hank’ (developed by Westbred, a unit of Monsanto) and Norpro (developed by Agripro, Syngenta). Five backcrosses were made for the Hank lines, and three backcrosses for the ‘Norpro’ lines. Four soft white spring wheat lines were used as recurrent parents, including ‘Cataldo’ (PI 642361) (Chen et al., 2009), IDO671, IDO644, and WA8008. WA8008 has been released as ‘Whit’ (PI 653841) (Kidwell et al., 2009). Five backcrosses were made for all of the soft wheat parents.

A marker-assisted backcrossing program was used to introgress the Choteau allele for stem solidness at *Qss.msub-3BL* into the six recurrent parents. DNA was isolated from backcross progeny starting at the BC_1 generation using the procedure of Riede et al. (1996). DNA was amplified via the polymerase chain reaction (PCR) using microsatellite primer set gwm340, which is closely linked to *Qss.msub-3BL* (Cook et al., 2004). Phenotypic distributions for the degree of stem solidness conferred by alternative alleles at -*Qss.msub-3BL* overlap, and thus exact map distances cannot be computed. However, Cook et al. (2004) observed a single clear recombinant based on a population of 96 doubled-haploid lines. PCR products were visualized on ethidium bromide-stained polyacrylamide gels or with the Li-Cor DNA Analysis System (Sherman et al., 2010). Homozygotes for the Choteau allele at *Qss.msub-3BL* were selected using PCR in F_2 progeny from the last backcross generation~~.~~, which was BC_5 for Hank, Cataldo, IDO671, IDO644, and ~~WA 8808~~Whit, and BC_3 for Norpro. Progeny from homozygous F_2 plants were increased for two to three generations to provide seed for testing.

Randomized complete block experiments with three replications were planted in twelve ~~locations~~ environments that did not have WSS pressure (Table 1). Seeding rate was 60 seed m^{-1} for rainfed nurseries and 90 seed m^{-1} for irrigated nurseries. Entries included two backcrossed-derived lines with the Choteau allele at *Qss.msub*-3BL for each of the six recurrent parents, as well the recurrent parents and the donor parent Choteau. The Bozeman 2011 location contained single row plots while the remaining locations consisted of 3 to 4 row plots approximately 3 m in length. All rows within a plot were harvested to obtain grain yield. Weather conditions and traits evaluated at each environment are shown in Table 1. Heading date was recorded as the day after January 1 when 50% of the heads were emerged from the flag leaf sheath. Date of flag leaf senescence was recorded as the day after Jan 1 when 50% of the flag leaves were completely senesced. Green leaf duration after heading was calculated as date of flag leaf senescence minus heading date. Plant height was recorded from the soil surface to the top of the spike of three main tillers, excluding awns. Stem solidness was obtained using five stems per plot, pulled randomly near crop maturity. A cross section was cut through the center of each internode. Five internode measurements were obtained per stem using a 1 to 5 scale, where 1 designates hollow and 5 designates completely solid stem. Internode scores were summed for each stem to give a number between 5 (hollow) and 25 (completely solid). Single stem scores were averaged for one final reading ~~per~~ for each plot. Number of productive tillers (tillers with seed-bearing spikes) was obtained near maturity for a 30 cm section of each plot. Biomass was measured by cutting a 1 m section of each plot at ground level, weighing the bundle prior to threshing. A 2.4 m section was assayed in Bozeman in 2011. The weight of threshed grain was obtained. Harvest index was calculated as bundle grain weight/biomass. Test weight was measured using a Fairbanks grain weighing scale (Fairbanks Scales, Kansas City, MO). Grain protein percentage

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was obtained using a Foss Infratech 1241 Grain Analyzer (Foss Analytical AB, Hoganis, Sweden). The Single Kernel Characterization System (SKCS 4100, Perten Instruments, Springfield, IL.) was used to characterize kernel weight~~. –~~ using ~~F~~fifty seeds from each field replicate~~ were tested~~.

Grain yield, test weight, grain protein, and stem solidness~~ heading date and plant height~~ was ~~obtained~~ measured at ~~all 12~~11 environments and heading date and plant height~~stem solidness~~ at all 12~~1~~ environments. Other traits were measured at a subset of the environments, including productive tiller number (~~seven~~ six environments), number of seeds per spike (nine environments), harvest index (~~six~~ five environments), kernel weight (~~seven~~ six environments), date of flag leaf senescence (five environments), and green leaf duration after heading (five environments).

Data for the two near isogenic lines within each replication with the solid allele at *Qss.msub-3BL* from Choteau were averaged prior to analysis. Data for all response variables were analyzed via analysis of variance where the model was that for a randomized block design combined over environments using PROC MIXED in SAS (SAS Inst. Inc, 2010). The entries source of variation was partitioned into sources due to recurrent parent, allele class, their interaction, and interactions with environments. Data was also analyzed for a randomized block design where entries was included as a non-~~partinioned~~partitioned source of variation to obtain an estimate for the ~~cooeficient~~coefficient of variation (CV) using Proc GLM.

The near-isogenic lines were also planted as three-replication randomized complete block experiments at three environments with significant WSS infestation. Environments were Loma MT in 2012 and 2013 and Amsterdam MT in 2012. Experiments were planted as hill plots with

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ten seeds representing a plot as described by Sherman et al. (2010). Percent cutting was analyzed using PROC MIXED in SAS (SAS Inst. Inc. 2010).

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Results

Replicated trials containing NILs for alleles at *Qss.msub-3BL* were grown in 12 diverse environments where a range of temperature and water conditions were encountered (Table 1). The dryland nursery at Huntley in 2012, and dryland nurseries at Lind in 2012 and 2013 received less than 25 cm of water during the year. Several trials, including irrigated nurseries in Bozeman (2012 and 2013), and dryland nurseries in Bozeman (2012) and Pullman (2012) received over 40 cm of annual water. Average daily temperatures also varied during the growing season, from 11.6 C in Bozeman in 2012 to 16.1 C in Lind and Aberdeen.

The Choteau allele conferring solid stems was successfully transferred into six spring wheat backgrounds using marker-assisted selection. Based on a scale of 5-25, where 5 is hollow and 25 is completely solid, solid stem scores for NIL with the Choteau allele at *Qss.msub-3BL* ranged from 15.0 to 19.1 over the six recurrent parents and eleven locations (Table 2). Near-isogenic lines with the Choteau allele were more solid than their respective recurrent parent for all six parents ($P < 0.001$). The mean solid stem score for Choteau in these trials was 23.0 (data not shown). Grain yield was not significantly different among the NIL for five of the six crosses and when averaged over all parents. Exceptionally, grain yield was significantly higher for hollow-stemmed Hank relative to the solid-stemmed NIL ($P < 0.001$). Six locations were used to obtain harvest index (Table 1). No significant differences between allele classes were observed for harvest index based on the mean over the six crosses. However, solid-stemmed NIL in the Norpro background had a lower harvest index than Norpro itself ($P < 0.05$). The difference in harvest index for the hollow- and solid-stemmed NIL approached significance for the Hank NIL also ($P = 0.08$).

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Irrigation was applied by overhead ~~sprinlers~~sprinklers to experiments in Bozeman in 2012 and 2013 and Aberdeen in 2013, while the remaining experiments were ~~dryland~~rainfed. Variation in precipitation at the ~~dryland~~rainfed environments led to greater than three-fold differences in grain yield among the environments. Allele by ~~enivironment~~environment interaction was significant for stem solidness score, ~~and grain yield~~seeds per spike and grain protein. Data from each environment for stem solidness, yield, and harvest index is reported in Table 3. ~~Variation in precipitation at the dryland environments led to greater than three-fold differences in grain yield among the environments (Table 3).~~ Differences in stem solidness caused by alleles at *Qss.msub-3BL* were apparent in all environments ($P < 0.001$). Grain yield did not vary significantly between allele classes based on means over all six crosses and ~~the twelve~~eleven environments (Table 2). However, grain yield was significantly higher for the hollow-stemmed versus solid-stemmed NIL at the irrigated environment in Bozeman in 2012 ($P <0.05$). Harvest index ~~was~~ did not vary for the stem types in any of the ~~six~~five environments for which this data was obtained based on means over the six recurrent parents.

Mean performance of the NIL for additional agronomic traits based on means over the crosses and environments is shown in Table 4. The yield components seed weight, seeds per spike, and number of productive tillers did not vary between the hollow-stemmed and solid-stemmed NIL ($P < 0.05$) (Table 4). Solid-stemmed NILs were shorter by 0.9 cm and later heading by 0.6 d than the hollow-stemmed lines ($P < 0.001$). The later heading date led to shorter green leaf duration after heading for the solid-stemmed lines. No differences between stem types were seen for test weight or grain protein content ($P > 0.05$).

None of the twelve environments used for collection of agronomic data were infested by the wheat stem sawfly. However, the NIL were planted as hill plots in three WSS-infested locations.

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The solid-stemmed NIL showed less stem cutting than the susceptible recurrent parents due to the sawfly in all three environments and based on mean over environments (Table 5). Percent cutting for the cultivar ~~Choteaa~~Choteau at ~~xx~~6.0% was significantly less than that of the solid-stemmed NILs.

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Discussion

A negative relationship between traits is a historical challenge for plant breeders. Gain due to selection for one trait may be accompanied by losses for a negatively correlated trait. For example, the negative relationship between grain yield and grain protein has been a long-standing challenge for wheat breeders. Single QTL often have opposite effects on yield and protein (Tsilo et al., 2011; Li et al., 2009; Sherman et al., 2014). In some cases, genes for disease resistance are associated with decreased yield potential (Brown, 2002), including the wheat leaf rust resistance gene *Lr34* (Singh and Huerta-Espina, 1997). A cost of genes for resistance to insects due to allocation of carbon resources has also been reported (Baldwin et al. 1990; Han and Lincoln, 1994). These negative relationships impact the ability of breeding programs to make simultaneous gains in multiple traits.

Performance of cultivars with contrasting phenotypes is often used to make inferences regarding the relationship between traits in the absence of genetic studies. One such example is with the solid stem phenotype used as a form of resistance to the wheat stem sawfly. Genes conferring stem solidness were introduced into hard red spring and winter wheat in the 1940's from a landrace originally collected in Turkey (Platt and Farstad, 1946). The first solid-stemmed cultivar, Rescue, was lower yielding than its hollow-stemmed counterparts in the absence of WSS. There has been a steady improvement in yield potential for both solid and hollow-stemmed lines since that time, though current solid-stemmed cultivars continue to be lower yielding than contemporary hollow-stemmed lines. One possible explanation ~~for this observation~~ is that the genes that confer~~s~~ solid stems also cause lower yield, perhaps due to diversion of photosynthate from developing grain to pith formation in the stem. Alternatively, the generally lower yield of solid-stemmed cultivars may be due to the fact that the first solid-

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stemmed cultivars were genetically inferior ~~to~~ than the best available hollow-stemmed cultivars, and at equal rates of improvement, the current hollow-stemmed lines remain superior to their solid-stemmed counterparts. This effect may be enhanced by the fact that there is much more effort concentrated in breeding for increased yield in hollow-stemmed cultivars relative to solid-stemmed types on a regional and national scale.

Hayat et al. (1995) developed RIL populations from crosses between solid-and hollow-stemmed cultivars where the solid-stemmed cultivars included Rescue (Platt et al., 1948), Fortuna (Lebsock et al., 1967) and Lew (McNeal and Berg, 1977). Though the solid-stemmed parents tended to be lower yielding than the hollow-stemmed parents, there was no genetic correlation between stem solidness and grain yield in any of the crosses. Similar studies conducted by other authors have provided mixed results, with some authors observing a negative correlation between stem solidness and yield (McNeal et al., 1965), and others observing no correlation (Lebsock and Koch, 1968; McNeal and Berg, 1979; Cook et al., 2004). Saint Pierre et al. (2010) showed that a set of solid-stemmed cultivars tended to be superior in yield potential to hollow-stemmed cultivars in water stressed conditions based on two years of data from Ciudad Obregon, north-west Mexico.

A single QTL, referred to as *Qss.msub-3BL*, was identified by Cook et al. (2004) as controlling about 75% of the variation for stem solidness in a winter wheat RIL population. The QTL also controls most of the variation for stem solidness in spring wheat (Kalous et al., 2010), and a QTL that maps to the same location has been shown to control most of the variation for stem solidness in durum wheat (Houshmand et al., 2007). The availability of a tightly linked molecular marker provided an opportunity to develop near-isogenic lines that varied for alleles at *Qss.msub-3BL*, and to conduct replicated trials at several locations in Montana, Washington and

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Idaho that varied in yield potential. Table 2 shows that the backcrossing program using MAS for transferring the Choteau allele at *Qss.msub-3BL* resulted in a similar increase in stem solidness in all six backgrounds. The effect of the allele for solid stems at *Qss.msub-3BL* did not have a significant impact on yield based on means over all recurrent parents, but did have a negative impact on yield for solid-stemmed NIL derived from Hank. No measured physiological or phenological trait shown in Table ~~5~~ 4 (data not shown) was uniquely impacted in Hank relative to the other recurrent parents. The effect of the allele for solid stems on harvest index for ~~Hank~~ Norpro approached significance (P =.0~~8~~9), with solid stemmed NIL showing a lower value, ~~. A lower harvest index was also observed for the solid-stemmed NIL in the Norpro background,~~ suggesting greater carbon allocation to stems in these lines. However, no effect of the solid stem allele was observed on grain yield for Norpro. One of the solid-stemmed NIL in the Norpro background is currently being marketed as 'SY Tyra' for WSS control in Montana and North Dakota.

A key focus of this research was to determine the impact of the major gene for solid stems on grain yield under diverse climatic conditions. There was no significant relationship between the solid stem allele and grain yield based on the mean over crosses and locations. Regression of the difference between yield of hollow-stemmed minus solid-stemmed NIL on mean nursery yield showed a positive but non-significant slope (P = 0.09) (data not shown). However, at three of four of the trials in lowest yielding, most water-stressed environments at Lind WA and Huntley MT, the solid-stemmed NIL had numerically higher grain yield. The only environment that showed a yield disadvantage to the solid-stemmed allele at *Qss.msub-3BL* was the high yielding irrigated trial in Bozeman in 2012. The solid-stem NIL had significantly fewer seeds per spike (P= 0.007) than hollow lines at this location but the yield components productive tiller number

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and kernel weight showed no difference between solid NIL and hollow lines. The allele for stem solidness was correlated with a few physiological traits, including plant height and green leaf duration after heading (Table 4). Physiological and phenological differences are likely to be environment specific in terms of their impact on grain yield. Our results suggest that differences at *Qss.msub-3BL* were typically not sufficient to alter grain yield potential.

The allele for stem solidness had the expected result of decreasing damage caused by the WSS (Table 5). This level of resistance may be referred to as partial, and would likely be sufficient in moderately or minimally infested locations. The most widely grown solid-stemmed cultivar is Choteau, the donor of the allele for solid stems at *Qss.msub-3BL*. The stem solidness score for Choteau of 23.0 is considerably higher than that seen for the NIL in this study, and Choteau typically shows greater resistance to WSS. Percent cutting for Choteau in the three WSS environments for this study was 6.0%, versus an average of 21.8% for the NIL containing the allele for solid stems at *Qss.msub-3BL* (Table 5). Thus, other alleles for stem solidness in addition to the major gene at *Qss.msub-3BL* are also important. Lanning et al. (2006) found a modifying gene on chromosome 3D that increased stem solidness did not negatively affect grain yield in a spring wheat recombinant inbred line population. The impact of other genes that modify the effects of *Qss.msub-3BL* on grain yield potential was not addressed by this study, thus introgression of additional alleles for solidness may impact grain yield.~~.~~ However, this study supports the idea that introduction of the major gene for stem solidness at *Qss.msub-3BL* will not impact grain yield in most backgrounds and most environments. Based on current information, a solid-stemmed cultivar with similar yield potential to the best hollow-stemmed cultivars is a reasonable goal for breeders and a reasonable expectation for wheat growers.

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Table 1. Temperature and total water applied for 12 environments used to compare near-isogenic lines varying for alleles at *Qss.msub-3BL.*

		Water applied (cm)		Temperature(C)	Measured Traits†	
Environment	year	annual (Sept-Aug)	Apr-Jul	Apr-Jul	phenologic/morphologic	grain
Bozeman-~~dryland~~rainfed	2011	42.7	24.7	11.7	HD, PH, ~~PTN, HI,~~ SS,FLS, GLDAH,	~~GY, KW, TW, GP~~
Bozeman-rainfed	2012	22.9	14.1	14.1	HD, PH, PTN, HI, SS,FLS, GLDAH, SPS	GY, KW, TW, GP
Bozeman-irrigated	2012	40.7	31.9	14.1	HD, PH, PTN, HI, SS,FLS, GLDAH	GY, KW, TW, GP
Bozeman-rainfed	2013	27.8	17.9	12.6	HD, PH, PTN, HI, SS,FLS, GLDAH, SPS,	GY, KW, TW, GP
Bozeman-irrigated	2013	58.3	48.4	12.6	HD, PH, PTN, HI, SS,FLS, GLDAH, SPS,	GY, KW, TW, GP
Huntley	2012	21.3	8.8	16.6	HD, PH, PTN, SS, SPS	GY, KW, TW, GP
Huntley	2013	31.1	21.8	14.3	HD, PH, PTN, HI, SS, SPS	GY, KW, TW, GP
Lind	2012	23.0	11.9	15.4	HD, PH, SS, SPS	GY,TW, GP
Lind	2013	22.2	5.2	16.1	HD, PH, SS, SPS	GY,TW, GP
Pullman	2012	45.1	11.2	13.3	HD, PH, SS, SPS	GY,TW, GP
Pullman	2013	37.4	12.5	13.5	HD, PH, SS, SPS	GY,TW, GP
Aberdeen	2013	49.5	41.3	16.1	HD, PH	GY, TW, GP

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† HD = Heading date (days from Jan 1), PH = plant height, PTN = Productive tiller number, GY = Grain yield, HI = Harvest index, SS = Stem solidness, KW = 1000 kernel weight, GP= Grain protein, SPS = Seed per spike, FLS = Flag leaf senescence, GLDAH = FLS-HD

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Table 2. Comparison of near-isogenic lines varying for alleles at *Qss.msub-3BL* for six recurrent parents. Stem solidness data was from eleven environments, grain yield was from ~~twelve~~ eleven environments, and harvest index was obtained from ~~six~~ five environments.

	Stem Solidness (5-25)			Yield (kg ha^{-1})			Harvest Index (%)		
Parental Background	**Hollow**	**Solid**	**P value**	**Hollow**	**Solid**	**P value**	**Hollow**	**Solid**	**P value**
Hank	10.2	19.1	***<0.001***	3668.1 ~~3699.7~~	3469.7 ~~3404.5~~	***0.03***~~***0.002***~~	46.0~~47.1~~	45.2~~45.5~~	*0.40*~~*0.08*~~
Norpro	8.5	18.2	***<0.001***	3929.9 ~~3917.3~~	3995.0 ~~3998.5~~	*0.48*~~*0.38*~~	47.1~~47.6~~	45.4~~45.8~~	*0.09*~~*0.03*~~
Cataldo	9.4	15.9	***<0.001***	4022.6 ~~3969.4~~	3897.8 ~~3900.2~~	*0.17*~~*0.45*~~	48.7~~49.1~~	49.3~~50.1~~	*0.55*~~*0.24*~~
IDO644	9.3	15.9	***<0.001***	4574.6 ~~4621.5~~	4663.5 ~~4649.4~~	*0.33*~~*0.76*~~	49.9~~51.2~~	48.8~~50.1~~	*0.28*~~*0.21*~~
IDO671	8.9	15.0	***<0.001***	4626.4 ~~4656.6~~	4505.1 ~~4562.8~~	*0.18*~~*0.31*~~	46.9~~48.1~~	47.7~~48.8~~	*0.42*~~*0.45*~~
Whit	9.5	17.4	***<0.001***	4011.4 ~~3965.6~~	4051.1 ~~4020.9~~	*0.66*~~*0.55*~~	45.1~~45.9~~	45.3~~45.7~~	*0.84*~~*0.87*~~
Mean	9.3	16.9	***<0.001***	4138.8 ~~4138.2~~	4097.0 ~~4091.0~~	*0.26*~~*0.19*~~	47.3~~48.2~~	47.0~~47.7~~	*0.41*~~*0.15*~~
~~Coeff. Var.~~ CV[†]	11.5			11.0			7.3		

[†] CV=Coefficient of Variation

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Table 3. Comparison of near-isogenic lines varying for alleles at *Qss.msub-3BL* for twelve environments based on means over six recurrent parents.

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	Stem Solidness (5-25)			**Yield (kg ha^{-1})**			**Harvest Index (%)**		
Environment	**Hollow**	**Solid**	**P value**	**Hollow**	**Solid**	**P value**	**Hollow**	**Solid**	**P value**
2011									
Bozeman rainfed	7.7	14.6	***<0.001***	nd~~4132.3~~	nd~~4005.1~~	~~*0.36*~~	nd~~52.6~~	nd~~51.2~~	~~*0.15*~~
2012									
Bozeman rainfed	7.6	16.1	***<0.001***	4240.7	4177.5	*0.61*	51.4	49.9	*0.10*
Bozeman irrigated	5.9	10.8	***<0.001***	6348.9	6098.8	***0.05***	44.6	43.3	*0.13*
Huntley	7	17.1	***<0.001***	2192.3	2253.4	*0.62*	nd	nd	
Lind	13.4	22.4	***<0.001***	1677.5	1702.9	*0.84*	nd	nd	
Pullman	12.4	18.2	***<0.001***	4810.3	4651.5	*0.21*	nd	nd	
2013									
Bozeman rainfed	8.5	15.6	***<0.001***	3936.5	3977.4	*0.75*	51	51.9	*0.25*
Bozeman irrigated	7	13	***<0.001***	6299.7	6327.5	*0.83*	46.9	48	*0.17*
Huntley	7.1	13.8	***<0.001***	3106.8	3231.5	*0.32*	42.6	41.6	*0.21*
Lind	15.3	24.2	***<0.001***	1503.6	1439.4	*0.61*	nd	nd	
Pullman	10.2	20.5	***<0.001***	4619.7	4532.3	*0.49*	nd	nd	
Aberdeen	nd	nd		6791.2	6675.0	*0.36*	nd	nd	
Mean	9.3	16.9	***<0.001***	4138.8~~4138.2~~	4097.0~~4091.0~~	*0.26~~0.19~~*	47.3~~48.2~~	47.0~~47.7~~	*0.41~~0.15~~*
~~Coeff. Var~~CV[†]~~.~~	11.5			11.0			7.3		

[†] CV=Coefficient of Variation

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Table 4. Comparison of near-isogenic lines varying for alleles at *Qss.msub-3BL* for six recurrent parents. The allele for solid stems was contributed by Choteau.

Traits	**PTN (no. m^{-2})**	**PH (cm)**	**TW (kg m^{-3})**	**HD (days)**	**FLS (days)**	**GLDAH (days)**	**GP (g kg^{-1})**	**KW (mg)**	**SPS (no.)**
No. Environments	~~7~~6	12	~~12~~11	12	5	5	~~12~~11	~~7~~6	9
Hollow	555.7~~540.3~~	69.5	772.8~~772.5~~	173.3	220.6	35.2	~~122~~121	35.2~~35~~	40.1
Solid	557.6~~542.2~~	68.6	770.8~~771.2~~	173.9	220.6	34.5	122	35.0~~34.9~~	39.5
P value	*0.87~~0.92~~*	***0.003***	*0.06~~0.10~~*	***<0.001***	*0.92*	***0.001***	*0.~~27~~30*	*0.42~~0.45~~*	*0.08*
~~Coeff. Varr.~~CV[††]	16.6	4.1	1.6	0.4	0.6	4.7	3.1	4.8	9.2

[†] HD = Heading date (days from Jan 1), PH = plant height, PTN = Productive tiller number, GY = Grain yield, HI = Harvest index, SS = Stem solidness, KW = 1000 kernel weight, GP= Grain protein, SPS = Seed per spike, FLS = Flag leaf senescence, GLDAH = FLS-HD

[††] CV=Coefficient of Variation

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Table 5. Comparison of near-isogenic lines varying for alleles at *Qss.msub-3BL* for percent stem cutting by the wheat stem sawfly in three environments based on means over six recurrent parents.

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	Near-Isogenic Lines			Choteau
Environment	**Hollow**	**Solid**	***P value***	
2012 Amsterdam	28.9	19.0	***0.02***	6.3
2012 Loma	37.1	24.8	***0.002***	2.6
2013 Loma	30.1	21.5	***0.03***	9.2
Mean	32.0	21.8	***<0.001***	6.0
~~Coeff. Var.~~CV[†]	4.8			

[†] CV=Coefficient of Variation

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