Identificaton of microsatellite markers associated to a solid stem QTL in wheat
by Jason Patrick Cook

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Sciences
Montana State University

Abstract:
The research that was conducted involved phenotyping and genotyping a doubled haploid (DH) winter wheat population derived from a ‘Rampart’ (solid stems) X ‘Jerry’ (hollow stem) cross to identify molecular markers linked to solid stem genes, which provide wheat stem sawfly resistance. Additionally, the DH population was used to determine if a relationship exists between stem solidness and other important traits, such as yield. The DH population was genotyped using GWM and BARC microsatellite primers that spanned the whole-wheat genome. To efficiently genotype the population, bulked segregent analysis was used to identify polymorphism between groups of solid stem and hollow stem individuals. Four microsatellite markers (GWM247, GWM340, GWM547, and BARC77) were found linked to a single solid stem QTL (designate Qss.msub-3BL) on chromosome 3BL. Linear regression analysis showed Qss.msub-3BL contributes at least 76% of the total variation for stem solidness. GWM247, GWM340, GWM547 are more closely linked to Qss.msub-3BL then BARC77. Additionally, linear regression analysis showed no relationship between Qss.msub-3BL and other traits. Also, trait to trait correlation analysis revealed no correlation to stem solidness and other traits except for plant height. It is hoped that GWM247, GWM340, and GWM547 will be useful for selecting solid stem varieties without deleterious affects on yield.

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APPROVAL

of a thesis submitted by

Jason Patrick Cook

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Dr. Luther E. Talbert _____ (Signature) 4/21/03 Date

Approved for the Department of Plant Sciences and Plant Pathology

Dr. Norman F. Weeden _____ (Signature) 4/21/03 Date

Approved for the College of Graduate Studies

Dr. Bruce R. McLeod _____ (Signature) 4-21-03 Date

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ABSTRACT

The research that was conducted involved phenotyping and genotyping a doubled haploid (DH) winter wheat population derived from a 'Rampart' (solid stems) X 'Jerry' (hollow stem) cross to identify molecular markers linked to solid stem genes, which provide wheat stem sawfly resistance. Additionally, the DH population was used to determine if a relationship exists between stem solidness and other important traits, such as yield. The DH population was genotyped using GWM and BARC microsatellite primers that spanned the whole-wheat genome. To efficiently genotype the population, bulked segregent analysis was used to identify polymorphism between groups of solid stem and hollow stem individuals. Four microsatellite markers (GWM247, GWM340, GWM547, and BARC77) were found linked to a single solid stem QTL (designate *Qss.msub-3BL*) on chromosome 3BL. Linear regression analysis showed *Qss.msub-3BL* contributes at least 76% of the total variation for stem solidness. GWM247, GWM340, GWM547 are more closely linked to *Qss.msub-3BL* then BARC77. Additionally, linear regression analysis showed no relationship between *Qss.msub-3BL* and other traits. Also, trait to trait correlation analysis revealed no correlation to stem solidness and other traits except for plant height. It is hoped that GWM247, GWM340, and GWM547 will be useful for selecting solid stem varieties without deleterious affects on yield.

CHAPTER 1

INTRODUCTION

Wheat stem sawfly (WSS), *Cephus cinctus* Norton, is a pest that inflicts severe economic damage to the winter and spring wheat, *Triticum aestivum* L., production areas of the Northern Great Plains of North America. The pest is a native species of North America, originally preferring wild grasses, primarily *Agropyron* spp (Criddle 1923). In the early 1900's, wheat stem sawfly switched to wheat as its primary host (Wallace and McNeal 1966). Damage caused by WSS is two-fold and only inflicted by the larva. Larva will first tunnel inside the stem, feeding on vascular tissue and parenchyma cells (Holmes 1954). The larval tunneling and feeding disrupts water and nutrient translocation to the developing kernels, causing up to 22% decrease in test weight and more then 1% loss in protein content (Holmes 1977). Secondly, when the larva is mature it migrates towards the base of the stem and cuts a ring or girdle around the stem wall. The girdling weakens the stem, substantially increasing lodging with consequent yield loss (Morrill et al.1992).

Despite considerable effort to control WSS proliferation and migration with cultural, chemical, and biological methods, only plant host resistance has proven to be effective. Plant host resistance is found in wheat accessions that have stems filled with pith, referred to as solid stems (Kemp 1934). The pith impedes larval growth and migration, greatly reducing stem cutting and population abundance (Wallace and McNeal 1966). The first publicly released WSS resistant cultivar was 'Rescue' (Stoa 1947).

Cytogenetic and inheritance analyses have determined that several genes may control solid stem expression. Larson and MacDonald (1959) identified the presence of potential genes for stem solidness on chromosomes 3B, 3D, 5A, 5B, and 5D. Inheritance studies have shown that 3 or 4 genes cause stems to be solid, but one gene in particular appears to account for more then twice the genetic variation compared to the other two or three genes (McNeal 1956, McKenzie 1965).

Acceptance of WSS resistant varieties has been minimal in areas where WSS population levels are low or non-existent. Solid stem varieties yield significantly less then hollow stem varieties in areas where little WSS pressure is present (Weiss and Morrill 1992). Early research showed a significant negative correlation between stem solidness and yield (McNeal et al.1965). However, more recent studies have indicated that the negative correlation between stem solidness and yield was not significant (Hayat et al. 1995). Hayat's data attributes low yield in solid stem varieties to the poor genetic background of the solid stem source rather then pleiotropy or deleterious linkage.

Breeding high yielding WSS resistant cultivars is problematic because of the subjectivity of solid stem scoring and variation of expression due to environmental effects. Marker Assisted Selection (MAS) could enhance the accurate identification of breeding lines with solid stem genes. By using molecular markers to ensure the presence of solid stem genes, backcrossing would become a viable option for developing WSS resistant wheat varieties in high yielding genetic backgrounds.

Microsatellite markers have become a popular DNA marker system in wheat (Plashke et al. 1995, Roder et al. 1995, Bryan et al. 1997). A microsatellite map

developed by Roder et al. (1998) demonstrates that microsatellite loci are evenly distributed across the wheat genome providing excellent coverage for marker analysis. Recently, several microsatellites have been identified linked to both pest and disease resistance in wheat (Chantret et al. 2000, Huang et al. 2000, Liu et al. 2001, Ghislain et al. 2001, Liu et al. 2002). This report details the identification of microsatellite markers closely linked to a stem solidness gene in wheat. The markers may be suitable for MAS of WWS resistant wheat varieties.

CHAPTER 2

LITERATURE REVIEW

History of Wheat Stem Sawfly

Wheat stem sawfly (WSS), *Cephus cinctus* Norton, is a native insect of North America, preferentially living in areas where annual precipitation ranges from 250-500 mm (Weiss and Morrill 1992). Originally, the insect inhabited large-stemmed native grasses (Ainsle 1920, Criddle 1923). In 1895, sawfly larva was observed in several native grass species in the Northwest Territories of Canada (Ainslie 1920). Observations in 1905 and 1906 indicated sawfly preferentially inhabited *Agropyron* spp grasses in Wyoming and the Dakotas (Ainslie 1920). By 1908, sawfly was found to inhabit native grasses as far west as Oregon and as far south as California and Nevada (Ainslie 1929).

In the late 1800's, farmers began to cultivate the native grasslands of the Northern Plains for wheat production. As the abundance of native grasses dwindled and the abundance of wheat increased the insect was forced to adapt to spring wheat as its primary host (Criddle 1922, Ainslie 1929). The first report of sawfly damage in spring wheat occurred in 1895 at Moose Jaw, Saskatchewan (Ainslie 1920). Subsequent reports in 1900, from Bozeman, Montana; in 1907, from Minot, North Dakota; and in 1908, from Manitoba and Saskatchewan, indicated wheat stem sawfly was becoming a potential pest of spring wheat production (Ainslie 1920). By 1908 and 1910, severe economic damage was reported in Minot, ND and Bainville, MT respectively (Ainslie 1929, Montana Agricultural Experiment Station and Montana Extension Service 1946). Severe losses

were also reported in the Southern Prairie Provinces of Canada in 1926 and 1931 (Atkinson 1931, King 1929). From 1943 –1955, the economic impact of sawfly increased as its area of infestation expanded in Montana, North Dakota, and Canada (Mills 1945, Montana Agricultural Experiment Station and Montana Extension Service, 1946, Bird 1955). By 1954, annual losses to spring wheat production had reached $17 million in Montana and North Dakota (Davis 1955).

Initially, winter wheat escaped sawfly damage due to early maturation (Wallace and McNeal 1966). Unfortunately, sawfly adapted to the growth pattern of winter wheat between 1970 and 1985 (Morrill and Kushnak 1996). By 1985, consistent reports of sawfly infestation in winter wheat were documented (Morrill and Kushnak 1996). Presently, wheat stem sawfly is the primary economic pest for winter and spring wheat production in Montana, North Dakota, and the Southern Prairie Provinces of Canada.

Insect Morphology

Adult

Adult sawflies are slender and approximately 1cm long (Morrill 1995). The insect has a black body with yellow markings on the abdomen (Wallace and McNeal 1966). Sawfly have two pairs of clear wings that appear golden in the sunlight (Morrill 1995).

In late May to early June, adult sawfly begin to emerge from wheat stubble. The male sawfly generally emerges before the female sawfly (Holmes 1982). The duration of

emergence can last 3 to 4 weeks (Morrill et al. 1992). Environment dictates the timing of emergence with ideal conditions combining a warm moist May, a hot June, adequate moisture for vigorous plant growth, and sporadic dry periods to allow sawfly to emerge (Seamans 1945). Emergence also coincides with the host plant growth stage suitable for ovipositing. Once the sawfly emerges, it typically lives 5 to 8 days (Wallace and McNeal 1966).

After emergence, the female sawfly will seek suitable stems, one that is young, succulent, elongating, and has a diameter between 2.8 and 3.4 mm, for depositing its eggs (Holmes and Peterson 1960). Using Zadoks et al. (1974) growth stage code, Morrill and Kushnak (1996) indicated that the plant growth stages susceptible to ovipositing started at growth stage 31 (first detectable internode) and ended at stage 40 (boot). Sawfly are relatively weak flyers capable of traveling no further then 2 km (Morrill 1995). The female will typically oviposit its eggs into stems that are in close proximity to the site of emergence (Criddle 1911, Ainslie 1920, Holmes 1975). Adults are most active during the day when the temperature ranges from 17°-32° C and wind speed is minimal (Seamans 1945). Once the female finds a suitable stem, it will insert its saw-like ovipositor through the stem tissue and oviposit the egg (Wallace and McNeal 1966). A female sawfly will deposit one egg per stem and is capable of laying eggs in approximately 30 stems, depending on lifespan and vigor of the female sawfly (Ainslie 1920). Although a female sawfly will deposit only one egg per stem, subsequent female sawfly may also deposit eggs into the stem (Wallace and McNeal 1966). The developing

larvae will compete with one another until only one remains (Holmes 1954, Weiss and Morrill 1992).

Wheat stem sawfly is haplodiploid; the genome of the female has 18 chromosomes and the male has nine chromosomes (Mackay 1956). Sex of the sawfly is determined by selective egg fertilization at the time of oviposition (Flanders 1946). Typically, equal numbers of male and female sawfly persist in the environment (McGinnis 1950). However, male dominated populations can occur if late emerging female sawflies are not able to find mates (Jacobson and Farstad 1952).

The Egg and Larva

Wheat stem sawfly eggs are crescent-shaped, glossy, and milky-white in color (Ainslie 1920). The size of the egg depends on the size of the female sawfly. Eggs are typically 1.00 – 1.25mm long and 0.33-0.42mm wide (Ainslie 1920). The egg will incubate inside the stem for approximately seven days before the larva hatches (Ainslie 1920).

Newly emerged larvae are colorless and transparent until they begin feeding on plant tissue giving them a yellow green coloration (Wallace and McNeal 1966). The larva head is easily identified by its pale brown coloration, eyespots, and dark brown four-pointed mandibles (Wallace and McNeal 1966). Average length of the larva is 2.24 mm and an average width is 0.28 mm (Wallace and McNeal 1966). As the larva develops, it progresses through four to five instars (Ainslie 1920, Farstad 1940).

Sawfly larvae obtain nutrition by migrating up and down the stem feeding on plant tissue. Holmes (1954) found that parenchyma tissue makes up the majority of the ingested plant material, however as the larva matures, it might ingest vascular tissue as well. Sawfly larvae are cannibalistic when they encounter either sawfly eggs or another feeding larva. The larva that is the lowest in the stem usually destroys all other larvae and eggs above it. Because eggs are usually laid first in the lower portion of the stem, the first larva to develop is most likely to survive (Wallace and McNeal 1966). Only one larva will survive within a stem.

Completion of larval development usually coincides with plant senescence. As the plant begins to senesce, visible and infrared light transmitted though the stem wall changes, triggering the larva to migrate towards the stem base (Holmes 1975). Once the larva reaches the stem base, it will cut or girdle a V-shaped notch near the soil surface. After girdling, a frass plug approximately 4 mm in length is compactly inserted directly below the V-shaped notch (Wallace and McNeal 1966). The plug adds rigidity to the stem, forcing the stem to break cleanly where the V-shaped notch is cut, creating a stub (Wallace and McNeal 1966). If the stem collapsed upon itself, adult sawfly would not be able to emerge the following spring (Ainslie 1920). The remaining stub and frass plug provides an overwintering site for the sawfly larva, protecting it from extreme environmental conditions (Salt 1946a, Holmes and Farstad 1956). Inside the stub, the larva will form a transparent cocoon and enter obligatory diapause (Wall 1952, Villacorta et al. 1971). In the spring, larvae will pupate after spending a minimum of 90 days at 10°

C in diapause (Salt 1947). Pupation lasts 7 to 14 days and then the adult will emerge (Criddle 1922, Holmes 1954).

Plant Hosts

Wheat stem sawfly larvae have been found in several cultivated and native plant species. The preferred cultivated host is *Triticum aestivum* (common wheat), however sawfly will also infest other *Triticum* spp. such as *T. compactum, T. spelta, T. sphaerococcum, T. carthlicum, T. dicoccum, T. durum,* and *T. monococcum* but with limited success primarily due to narrow stem diameters (Wallace and McNeal 1966). Sawfly will also infest *Hordeum vulgare* L. (barley), *Secale cereale* L. (rye), *Avena sativa* (oats), and *Linum usitatissimum* L., Linaceae (flax), but larva mortality is usually high and in the case of oats mortality is nearly 100% (Farstad 1944, Farstad and Platt 1946, Wallace and McNeal 1966).

Along with cultivated plant species, sawfly will also infest many native plant species as well. It is well documented that *Elymus* spp. are preferred by sawfly (Criddle 1923). *Agropyron* species that have been infested include *E. caninum, E. cristatum, E. dasystachyum, E. elongatum, E. intermedium, E. repens,* and *E. smithii* (Wallace and McNeal 1966). Other native species such as *Beckmannia syzigachne, Bromus inermis* and *Bromus secalinus,* to name a few, have had larvae detected in their stems (Wallace and McNeal 1966). Female sawfly will typically shun grasses with narrow stems (Wallace and McNeal 1966). Variation of grass phenology at the time of sawfly

emergence will dictate which grass species will be most likely infested with sawfly larvae (Wallace and McNeal 1966).

Crop Damage

Damage inflicted by wheat stem sawfly is two-fold and only caused by the larva inside the stem. First, larval feeding will damage vascular tissue disrupting carbohydrate and water translocation to the developing kernels (Holmes 1954). Evidence of carbohydrate translocation disruption can be observed by the presence of darkened spots, caused by the accumulation of carbohydrates, on the sub-nodal regions of the stem (Morrill et al. 1992). Reduction of carbohydrate and water translocation reduces kernel weight and numbers. Kernel weight reduction ranges from 2.8 – 10%, depending on the wheat variety (Morrill et al. 1992). Other studies have shown kernel weight reductions to be 10.8 – 22.3% (Holmes 1977), 5 – 20% (McNeal et al. 1955), and 3% (Munro et al. 1947). Holmes (1977) also observed a reduction in grain protein content that ranged between 0.6 – 1.2%.

The sawfly larva causes additional damage when it reaches maturity and ceases to feed. At the end of the growing season the larva will migrate to the base of the stem and cut a V-shaped notch or girdle nearly completely through the stem wall (Holmes 1975). Wind will induce the cut stem to break away causing extensive lodging (Weiss and Morrill 1992). Lodging increases the difficulty of harvesting the grain and also reduces grain quality (Holmes 1977).

Methods of Control

Since the inception of sawfly as a pest in wheat, substantial effort has been put forth to control the pest. Cultural, chemical, and biological strategies have been studied for their effectiveness. The single most effective means of control is solid stem resistant wheat cultivars. Cultural, chemical, and biological strategies alone have not been found to be economically effective because of the biology of sawfly. Emergence of adult sawfly is sporadic over a 3 to 4 week period, making it very difficult to eradicate all of the adults at one time. Also, the stem and soil protects the larva from desiccation while it feeds during the growing season and freezing during its winter dormancy. If sawfly infestation is not reduced below 7 to 9%, an infestation of 70 to 80% will likely occur the following year (Holmes 1982).

Cultural Control

Initial efforts for managing sawfly were focused on the use of cultural methods of control. Norman Criddle, a farmer hired by the Manitoba provincial government, initiated the first studies for controlling sawfly. From extensive research, Criddle (1911, 1913, 1915, 1922) proposed several strategies including: tillage, early mowing of rye grasses, refraining from disturbing grasses that are hosts to wheat stem sawfly parasites, planting trap crops in which larvae will not survive, planting non-host crops, early harvesting, and swathing. Since Criddle's research, further studies have produced mixed results for the effectiveness of cultural management techniques in controlling sawfly.

Shallow tillage, alternative seeding dates, swathing, and crop rotations have constituted the majority of the strategies chosen for cultural control research.

Shallow tillage, at depths less then 0.3 meters, is a common technique for weed control, but also has been extensively studied for sawfly management (Callenbach and Hansmeir 1944, Mills 1945, Holmes and Farstad 1956, Morrill et al. 1993). The purpose of shallow tillage is to disturb the soil surrounding stems cut by sawfly larva, exposing the overwintering larvae to the harsh environment (Holmes and Farstad 1956). Salt (1946, 1961a, 1961b) found that freezing and desiccation of larvae in exposed wheat stems significantly increased mortality. Both fall and spring tillage were studied for their effectiveness, however spring tillage appeared to be less effective because larva would sometimes re-enter diapause and emerge the following year (Church 1955, Holmes and Farstad 1956). Morrill et al. (1993) conducted a study, using shallow tillage in the fall, which showed larval survival rate in exposed stems to be 7.3% and 8.0% in 1990-1991 and 1991-1992, respectively. The drawback of using shallow tillage is the difficulty in freeing an adequate number of stems from the soil to sufficiently reduce sawfly populations below an economic threshold (Morrill et al. 1993). Large-scale tillage can also be disadvantageous because it reduces the amount of snow captured to increase soil moisture, and soil erosion may occur (Morrill et al. 1993).

Altering seeding dates has been shown to reduce sawfly infestation. The objective of altered seeding dates is to de-synchronize wheat development and sawfly emergence (Weiss et al. 1987), and is accomplished by seeding winter wheat early or delaying the seeding of spring wheat. By planting winter wheat early, the plants will be to

advanced (boot stage), and by delaying spring wheat seeding, the plants should be too immature (prior to stem elongation), at the time of adult emergence making it difficult for the female sawfly to find a suitable host. Callenbach and Hansmeier (1944) recommended seeding spring wheat after May 20 in highly infested sawfly areas. There are, unfortunately, risks associated with altering seeding dates. Late planting subjects spring wheat to higher possibility of moisture stress. Low levels of moisture will result in significant crop losses due to low germination. Losses may also occur if the plants are actively growing during July, which is one of the hottest and driest months of the year.

The use of swathing has long been considered a potential method for reducing sawfly-inflicted damage (Criddle 1922, Callenbach and Hansmieier 1944, Mills 1945). The primary purpose of swathing is to cut and windrow grain before lodging occurs to increase yields. Swathing was also studied for its potential in reducing sawfly population levels. Holmes and Peterson (1965) found no significant reduction of sawfly populations after swathing at the recommended grain moisture level of 35%. The larva had successfully migrated to the base of the stem before the grain was swathed. They determined that swathing would have to occur when the grain moisture levels were between 55 to 61% to adequately reduce sawfly numbers.

Dodds (1957) and Molberg (1963) observed that swathing grain before moisture levels dropped below 35% and 38% respectively, would reduce yield and test weight. Molberg (1963) reported losses as high as 14 bushels per acre from grain that was swathed at 55% moisture. Dodd (1957), however, found no significant yield differences in grain that was swathed at moisture levels ranging from 35.4% and 40.9%. The

potential risk for yield and test weight loss when grain is swathed at high moisture levels has prevented farmers from this method for reducing sawfly populations. However, swathing at the recommended grain moisture level of 35% is widely used in areas that are highly infested with sawfly to help reduce losses associated with lodging.

Crop rotations are a proven method for reducing sawfly populations and the damage that they inflict on wheat (Munro 1944, Callenbach and Hansmeier 1945, Butcher 1946). Using crop rotations with non-susceptible hosts limits the opportunity for female sawfly to oviposit and produce progeny, thereby reducing sawfly populations. There are several hosts, including flax, oats, and mustards that are not susceptible to sawfly infestation (Platt and Farstad 1946). Additionally, hosts, such as fall rye that are minimally affected by sawfly infestation, can also be used (Wallace and McNeal 1966). Unfortunately, economics associated with continuous planting of wheat makes it undesirable for producers to rotate a large amount of acreage into a non-host crop (Weiss and Morrill 1992).

Chemical Control

Insecticides have been thoroughly investigated for controlling wheat stem sawfly, including both foliar and systemic seed treatments. Foliar treatments are applied by spraying the insecticide onto the foliage of a growing crop. Systemic seed treatments are applied to seeds prior to planting and are translocated through the plant as it develops. Neither foliar nor systemic insecticides have provided acceptable control of wheat stem sawfly (Holmes and Hurtig 1952, Skoog and Wallace 1964, Wallace and McNeal 1966).

Wallace (1962) evaluated the systemic insecticide heptachlor. He reported sawfly larval mortalities ranging from 61.2% to 96.3% in 'Thatcher' spring wheat with most mortality occurring in the early instar larvae. Holmes and Peterson (1963a) also evaluated heptachlor on 'Thatcher' and reported inconsistent larval control. They concluded that heptachlor was only effective in the lower two internodes on early instar larvae. Mature larvae in higher internodes could tolerate heptachlor and successfully lodge the host plant.

In a more recent study, three foliar insecticides were evaluated for sawfly control. Blodgett et al. (1996) evaluated Lorsban 4E-SG (chlorpyrifos), Furadan 4F (carbofuran), and Warrior 1E (lambdacyhalothrin) in winter wheat at various rates. The insecticides were sprayed directly on 2 to 3 node winter wheat during peak sawfly emergence. Fifty stems were randomly chosen from each plot to determine the level of plot infestation. No significant differences were recorded in larvae per stem between control and treated plots.

Adult and larval biology of wheat stem sawfly makes control with conventional insecticides difficult and uneconomical. Sawfly larvae are protected from insecticides inside the stem, which make foliar insecticides impractical for larval control. It is also difficult to control adult sawflies with foliar insecticides because they emerge sporadically over a 3 to 4 week period, so a single insecticide application has little effect on reducing ovipositing females. While possible to kill sawfly adults with foliar insecticides, targeting the adults would require applications at three to five-day intervals

over the entire adult emergence cycle. This is prohibitively expensive in a wheat production system.

Biological Control

Use of biological controls, primarily parasitic insects, has been unsuccessful. In native grasses, wheat stem sawfly is attacked by nine species of hymenopterous parasites (Holmes et al. 1963). Two species, *Bracon cephi* (Gahan) and *Bracon lissogaster* (Muesebeck), have been found to parasitize sawfly in wheat (Somsen and Luginbill 1956, Holmes et al. 1963). The female parasite will seek sawfly larva by tapping on the stem with its antennae to determine the location of the larva (Somsen and Luginbill 1956). Once detected, the parasitoid will insert its ovipositor through the stem to paralyze the larva and place an egg on top of the larva. The egg will hatch, producing a larval parasite that feeds on the sawfly larva (Nelson and Farstad 1953). *Bracon cephi* and *Bracon lissogaster* have two generations per year in native grasses, but in wheat, the second generation is often not completed, possibly due to grain harvesting (Criddle 1923, Somson and Luginbill 1956, Holmes et al. 1963). Loss of the second generation limits the population size of the sawfly parasites, which therefore decreases the ability of the parasite to control sawfly.

Attempts with biological control agents from abroad have also occurred. In 1930, approximately 6,000 adult *Collyria calcitrator* (Gravenhorst), an egg parasite from Europe, was released in Saskatchewan (Smith 1931). Unfortunately, the released parasites never became established. Further releases of *Collyria calcitrator* over a nine-

year period were also unsuccessful (Weiss and Morrill 1992). *Bracon terebella* (Wesnsen), a European hymenopterous larval parasite, was released in the 1950's, and it also failed to be established (Davis et al. 1955). The reasons for the establishment failures have never been fully understood. The most likely explanation may be European parasitoids are not adapted to the North American climate. Overall, biological agents may hold promise for controlling wheat stem sawfly, however, current parasitoid population levels are insufficient to effectively reduce sawfly numbers.

Host Plant Resistance

Host plant resistance is the single most effective strategy for controlling sawfly in wheat (Roberts 1954, Holmes and Peterson 1962, Weiss and Morrill 1992). Resistance enables the plant to repel or tolerate pest infestation without causing a significant negative impact on productivity. Sawfly resistance in wheat was identified when a positive correlation between stem solidness and reduced sawfly damage was observed (Shchegolev 1926, Kemp 1934, Farstad 1940, Eckroth and McNeal 1953, Holmes and Peterson 1962). The first observation of sawfly resistance in solid stem wheat was reported in the 1920's. Shchegolev (1926) tested rye, barley, wheat, and oats and found solid stem wheat to be resistant to sawfly. A further investigation by Kemp (1934) concluded solid stem wheat could reduce sawfly damage to inconsequential levels. The potential for developing wheat stem sawfly resistant wheat compelled the Canadian government to collect solid stem accessions for the development of an agronomically suitable sawfly resistant cultivar for the Northern Plains. A solid stem spring wheat

cultivar from Portugal, S-615, was crossed with a hollow stem spring wheat cultivar 'Apex' to generate a solid stem cultivar, 'Rescue' (Stoa 1947). 'Rescue' was initially released in Canada in 1946 and then in the United States in 1947 (Wallace and McNeal 1966). It was reported that the first year 'Rescue' was used in a highly infested sawfly area, damage was reduced to 5% while hollow stem varieties sustained nearly 95% losses (Platt et al. 1948). The success of 'Rescue' has prompted further development of solid stem cultivars, including winter wheat, with 'Rescue' being the solid stem source.

Even though 'Rescue' was successful in reducing sawfly damage, it possessed poor agronomic characteristics. Yields were generally 8 to 15% less then hollow stem varieties in areas with low sawfly infestation, and it lacked good milling and baking qualities (Stoa 1947). The low yield potential has caused reduced grower acceptance of solid stem cultivars. However, when sawfly infestations are high, solid stem varieties will yield equal to or greater than their hollow stem counterparts (Weiss and Morill 1992). By developing higher yielding solid stem cultivars, grower acceptance would likely increase.

Solid Stem Wheat

Stem solidness in wheat is caused by the development of pith inside the stem. The solid regions of the stem resist sawfly infestation and cause high rates of larval mortality (Holmes and Peterson 1962). How wheat with solid stems resist infestation or cause sawfly mortality is not clearly known, however several studies have been conducted to determine the cause of resistance and mortality. One study analyzed

whether female sawfly had a reduced preference for laying eggs in solid stems. Farstad (1951) observed fewer eggs were laid in solid stem versus hollow stem wheat, however if the only available host was solid stem wheat, the sawfly would deposit eggs into it as well. Other studies focused on how the egg and larva inside the host might be affected by solid stems. A study by McGinnis and Kasting (1961) analyzed whether pith was deficient in essential nutrients causing the larvae to die from malnutrition. The study found no significant differences in dry matter or nitrogen content between pith in solid stem varieties and the tissue found in walls of hollow stem wheat. They believed solid stem wheat kills larvae by desiccation. Holmes and Peterson (1960, 1961) studied the susceptibility of eggs to destruction in solid stems, and they also reported that eggs and larvae appeared to be vulnerable to desiccation. Holmes and Peterson (1962) also suggested that the pith might impede larvae movement, causing starvation due to lack of cells to ingest. The highest sawfly mortality rates in solid stem wheat have been shown to occur after the larva has fully matured (Wallace and McNeal 1966). This could be due to the impediment of larvae movement by the pith, frass, and nodal plates (Farstad 1940, Holmes and Peterson 1962, Morrill et al. 1994). The restricted movement prevents the larvae from reaching the base of the stem, which exposes them to freezing temperatures during the winter, resulting in nearly 100% mortality (Morrill et al. 1994).

Environmental Effects

Environmental factors can affect the degree of stem solidness, which potentially reduces sawfly resistance. Platt (1941) and Platt et al. (1948) reported stem solidness was affected by changes in light, temperature, moisture, and plant spacing. Holmes et al.

(1960) found that shading from the two-leaf to boot stage reduced the solidness of the bottom internode. Other research on 'Rescue' showed that in the greenhouse, 4,000 foot-candles of supplemental light maintained stem solidness, but 1,500 foot-candles of supplemental light did not (Roberts and Tyrell 1961). Further studies conducted by Luginbill and McNeal (1954) reported the effect of fertilizers on 'Rescue'. Phosphorous applied alone caused increased sawfly stem cuttings, whereas potassium applied with both phosphorous and nitrogen reduced sawfly cutting. Nitrogen applied alone had no significant effect on sawfly cutting.

Stem Solidness Inheritance

Stem solidness is considered to be a highly heritable trait. A study conducted by Lebsock and Koch (1968) reported stem solidness heritability estimates in wheat ranging from 60% to 95%. Another study by McNeal and Berg (1979) reported 73% heritability for stem solidness. The number of genes that control the development of solid stems and whether the genes are recessive or dominant is uncertain. Engledow and Hutchinson (1925) conducted a stem solidness inheritance study, which concluded the solid stem trait was dominant and controlled by one gene. Another study by Platt et al. (1941) reported, however, that three recessive genes were the controlling factors for stem solidness. Putnam (1942) studied the inheritance of stem solidness in tetraploid wheat. He indicated that stem solidness was controlled by one partially dominant gene. A recent study in durum that was conducted by Clarke et al. (2002) reported a single dominant gene controls stem solidness.

McNeal (1956) studied inheritance of stem solidness by crossing 'Rescue' (solid stem) with 'Thatcher' (hollow stem). He found that 'Thatcher' and 'Rescue' were different by one major gene and several modifying genes, which affected stem solidness. The major gene was found to have an effect equal to two and one-half times that of all minor modifying genes. A study by McKenzie (1965) agreed with the study by McNeal (1956) concerning the presence of a single major gene and several minor genes. McKenzie (1965) studied inheritance of stem solidness by crossing two hollow stemmed ('Red Bobs' and 'Redman') and two solid stemmed ('C.T.715' and 'S-615') spring wheat cultivars. He reported one major gene and three minor genes were influencing stem solidness.

Further research, conducted by McNeal et al. (1957), examined F_2 progeny from crosses made between 'Rescue' and four solid stem wheat accessions from Portugal. They reported that each Portuguese wheat accession contained the same major gene for solid stem expression that was found in 'Rescue'. However, three of the Portuguese accessions varied slightly for the level of stem solidness of 'Rescue'. McNeal attributed the variation to the addition or loss of minor genes that affect stem solidness. Wallace et al. (1969) reinforced McNeal's hypothesis when he studied a group of solid stem Portuguese spring wheat accessions and reported that the accessions may possess different or additional genes from those found is 'S-615', the source of Rescue's stem solidness.

Cytogenetic analysis has further indicated that there are several genes controlling stem solidness. Larson (1952, 1959a) compared monosomic F_2 lines derived from

crossing 'Chinese Spring' (hollow stem) X 'S-615' (solid stem) with normal F_2 lines for solid stems. She found in 'Chinese Spring' that chromosomes 2A, 2D, 6D, and 7D carry genes for hollow stem and chromosome 4B has a gene for stem solidness. No genes for stem solidness were detected in S-615, leading Larson to postulate solid stem genes were probably recessive. Further analysis by Larson and MacDonald in 1959b, using monosomic lines of 'S-615', showed that chromosomes 3B, 3D, 5A, 5B, and 5D carried genes for solid stem expression, and chromosomes 2D, 6D, and 7D have genes for hollow stem. Lines monosomic for 3B and 3D were less solid in the top internode, and lines monosomic for 5A, 5B, and 5D were less solid in the bottom four internodes.

In 1962, Larson and MacDonald reported the development of monosomic lines of 'Rescue'. They found 'Rescue' has fewer chromosomes affecting solid stem development than 'S-615'. Chromosomes 3D, 5B, and 5D did not make the stem more solid and chromosomes 2D and 7D did not make the stem more hollow as in 'S-615'. It was revealed, however, that chromosome 3B has a very important gene for stem solidness (cited by Wallace and McNeal 1966). The presence of a major gene on chromosome 3B was reinforced by Larson and MacDonald (1963). They reported results from an analysis of F8 lines that were selected from F5 hexaploid plants of a 'Rescue' (*T. aestivum*) X 'Golden Ball' (*T. durum*) cross. Their work also suggested a major gene for stem solidness on chromosome 3B.

Pleiotrophic Effects of Solid Stems

Even though solid stem wheat is the best form of wheat stem sawfly control, producers are reluctant to grow the resistant varieties because of yield loss compared to

their hollow stem counterparts (Weiss and Morrill 1992). There was concern that solid stems were related to low yields (Wallace and McNeal 1966). McNeal et al. (1965) reported solid stems and yield were negatively correlated (-0.846 and –0.825) in two tests of backcross lines derived from a 'Thatcher' X 'Rescue' cross. Other studies however, have indicated there is no relationship between yield and stem solidness (Lebsock and Koch 1968, McNeal and Berg 1979, Hayat et al. 1995). Hayat et al. (1995) attributed the low yield in solid stem wheat to the poor genetic background contributed by the solid stem source, rather than pleiotropy or deleterious linkage.

Microsatellites

Microsatellites, or simple sequence repeats (SSR's), are found interspersed in the genomes of all eukaryotes and have emerged as an important source of co-dominant genetic markers (Wang et al. 1994). They are a class of sequences consisting of tandem repeats, such as $(GT)_n$ or $(CT)_n$, with a basic motif of less then six base pair (Litt and Luty 1989). It was observed that microsatellites show a high frequency of variation, or polymorphism in the number of repeats in different individuals, probably due to slippage during DNA replication (Tautz et al. 1986). Polymorphism can be observed at a specific locus using polymerase chain reaction (PCR), where primers are developed that flank the tandem repeat sequence allowing the amplification of a specific microsatellite locus. Microsatellites have been shown to be highly informative and locus specific (Condit and Hubbell 1991, Wu and Tanksley 1993, Smulders et al. 1997).

Wheat has a very limited intraspecific level of polymorphism compared to other plant species (Chao et al. 1989, Kam-Morgan et al. 1989, Liu et al. 1990, Cadalen et al. 1997). Microsatellites, however, have a higher level of polymorphism and informativeness in wheat then any other marker system (Plaschke et al. 1995, Roder et al. 1995, Bryan et al. 1997, Roder et al. 1998). Several microsatellite maps have been constructed, revealing an even distribution of microsatellite loci along all chromosome arms, thus providing excellent coverage of the wheat genome (Korzun et al. 1997, Peil et al. 1998, Roder et al. 1998).

Use of Microsatellites in Identifying Genes

The development of microsatellite markers and maps has provided a useful tool for identifying genetic markers associated with agronomic and grain quality genes and quantitative trait loci (QTL) in wheat. A study, reported by Korzun et al. (1998), identified a microsatellite locus, *wms261*, that is 0.6 cM distal to the *Rht8* dwarfing gene on chromosome 2DS. Another study conducted by Prasad et al. (1999) analyzed 100 recombinant inbred lines and screened them with 232 microsatellite primer pairs. They detected a significant association between a microsatellite locus, *wmc41*, and a QTL for protein content, which accounted for 18.73% of the variation. Varshney et al. (2000) looked for associations between grain weight and microsatellite markers. From their analysis, microsatellite *Xwmc333* was found to be associated with a grain weight QTL on chromosome 1AS, which accounted for 15.09% of the variation for grain weight.

Microsatellites have also been used to identify genetic markers linked to disease and insect host plant resistance genes in wheat. Huang et al. (2000) identified a microsatellite marker associated with the powdery mildew resistance gene *Pm24*. The microsatellite locus, *Xgwm337*, located on chromosome 1D, was found to be 2.4 cM from *Pm24*. *Xgwm337* was shown to be diagnostic and therefore potentially useful for pyramiding two or more genes for powdery mildew resistance in a single genotype. Liu et al. (2001) used microsatellites to identify markers linked to Russian wheat aphid resistance genes. Microsatellite *Xgwm111*, located on wheat chromosome 7DS, was reported to be tightly linked to the Russian wheat aphid resistance genes *Dn1, Dn2, Dn5*, and *Dnx*. Another microsatellite marker, *Xgwm635*, located on the long arm of chromosome 7D marked the location of the Russian wheat aphid resistant gene *Dn8*. Lastly, a microsatellite locus *Xgwm642* marked and identified a Russian wheat aphid resistant gene *Dn9* on chromosome 1DL .

Marker Assisted Selection (MAS)

Once markers identifying genes of interest have been found, molecular genetics can be integrated with traditional methods of artificial selection of phenotypes by applying marker-assisted selection (Lande and Thompson 1990). Cultivar improvement predominantly has resulted from phenotypic selection wherein superior genotypes have been identified only through replicated testing in diverse environments. Plant breeders have been restricted to the use of phenotypic selection, because little is known of the genetic identity and chromosome location of most genes controlling most important

agronomic traits. Molecular marker technology offers the tools needed to identify, select, and combine favorable alleles via genotypic selection. Marker assisted selection could aid in the development of resistant cultivars by producing genotypes with more stable and durable resistance.

CHAPTER 3

MATERIALS AND METHODS

Plant Materials

A doubled haploid (DH) mapping population for stem solidness was derived from a cross of two hard red winter wheats, PI 593889 ('Rampart'), a solid stem genotype and PI 632433 ('Jerry'), a hollow stem genotype (Knox et al. 2000). The DH mapping population contained 96 lines generated from the F_1 generation.

In 2001, the DH population was planted at Bozeman, MT. The elevation at the experimental site is 1,439 m and the soil is an Amsterdam silt loam. The population was planted in single row non-replicated plots for seed increase. The plots were 1.5 m long with row spacing of 60 cm. The seeding rate varied among the lines. Planting occurred on 10 October 2000 and harvest occurred on 06 August 2001. Precipitation received from 01 October 2000 to 02 July 2001 was 311 mm. Preliminary stem solidness data were obtained.

In 2002, the 96 DH winter wheat lines and four check varieties ('Rampart', 'Jerry', 'Judith', and 'Norstar') were planted in a 10 X 10 lattice design with three replications at two Montana locations: Bozeman and Moccasin. At Bozeman, the experimental site was the same as 2001. The plots had four rows and were 3.3 m long with row spacing of 30 cm. The seeding rate was 67.2 kg ha^{-1}. Planting occurred on 30 September 2001 and harvest was on 16 August 2002. Precipitation received from 01 October 2000 to 02 July 2001 was 316 mm. Traits evaluated at Bozeman included stem

solidness, yield, test weight, protein content, emergence, winter survival, heading date, height, and lodging.

Moccasin has an elevation of 1,307 m and the soil is a Judith clay loam. The plots had five rows and were 2.4 m long with row spacing of 30 cm. The seeding rate was 67.2 kg ha^{-1}. Planting occurred on 24 September 2001 and harvest occurred on 09 August 2002. Precipitation received from 01 October 2000 to 02 July 2001 was 235 mm. Measured traits included stem solidness, yield, test weight, protein content, emergence, winter survival, heading date, and height.

In 2002, the 96 DH lines were also planted for observation in a randomized complete block single row design at Williston, ND. The Williston site has an elevation of 640 m and the soil is a Max loam. The plots were 2 m long with row spacing of 30 cm. Planting occurred on 11 September 2001 but they were not harvested. Precipitation received from 01 October 2000 to 02 July 2001 was 208 mm. Measured traits were stem solidness and winter survival.

To evaluate for stem solidness, ten stems were randomly selected from each plot. The stems were cross sectionally cut in the center of five internodes. The level of pith at each internode was rated on a previously established scale ranging from one to five; one was considered hollow and five was solid (Fig. 1) (O'Keefe et al. 1960, Wallace et al. 1973). Ratings for each of the five internodes were summed providing a total stem solidness score ranging from 5 – 25, where five indicated hollow and 25 was solid.

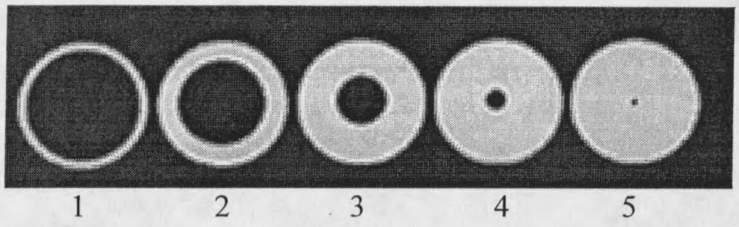

Fig. 1 Diagram of stem solidness rating. 1 = hollow, 5 = Solid (McNeal, 1956).

Yield was obtained by harvesting with a plot combine. Test weight was measured on a Seedburo (Chicago, IL) test weight scale. Grain protein content was obtained on whole grain samples using an Infratec (Tecator, Höganäs, Sweden) whole kernel analyzer. Heading date was the number of days from 1 January to when 50% of the heads in a plot were completely emerged from the flag leaf sheath. Emergence, winter survival, and lodging were measured as a percent of the total plot.

Microsatellite Evaluation

Potential microsatellite markers associated with stem solidness genes were identified by screening the DH population using bulk segregant analysis (BSA) as described by Michelmore et al. (1991). A total of six DNA bulks were assembled, three contained DNA from lines rated as hollow (<10) and three contained DNA from lines rated as solid (>20). Each bulk contained equal concentrations of DNA from six individual DH lines. The DNA was extracted from young leaf tissue by method of Riede and Anderson (1996). Markers identifying polymorphisms between the hollow and solid parents and bulks were used to screen the entire DH population to determine linkage between the marker and a solid stem gene.

Primers designed from microsatellite markers from two sources were utilized to screen the DH winter wheat population. The primers screened included a set of 230 GWM microsatellite primers developed by Roder et al. (1998), and 168 BARC microsatellite primers, provided by the USDA – ARS and U.S. Wheat and Barley Scab Initiative (http://www.scabusa.org/research_bio.html). The PCR amplification protocol consisted of a 25µl reaction volume subjected to thermocycler program of 94°C - 4 min; 30 cycles of: 94°C – 1 min, 50°C, 55°C, or 60°C – 1 min (annealing temperature appropriate for each primer set), 72°C - 1:20 min; 7 min at 72°C – 7 min.

Physical Mapping

Nulli-tetrasomic lines of 'Chinese Spring' (Sears 1954) were used to verify the location of microsatellites used for screening the DH population. Additionally, two chromosome 3BL deletion lines of 'Chinese Spring', 3BL-7 and 3BL-11, were used to physically map the position of *Xgwm247, Xgwm340, Xgwm547* and *Xbarc77* on chromosome 3BL. The development and nomenclature of the deletion stocks are described by Endo and Gill (1996). The deletion lines break point is indicated by their fraction length (FL), which was calculated by dividing the length of the deletion segment with the total arm length. All deletions are distal from the break points.

Statistical Analysis

Data were analyzed by mixed effects analysis of variance first performing a separate analysis for each environment and then combining the analysis over

environments using PROC MIXED in SAS (SAS Institute Inc. 1988). Locations were considered fixed and all other factors and their interactions in the model were considered random effects. Least squares entry means were obtained by fitting the same model in PROC GLM in SAS (SAS Institute Inc. 1988) for each location and combined over locations. The proportion of variation among the entry means accounted for by the microsatellite marker was obtained as the ration of sum of squares for marker class divided by sum of squares for entries using the least squares entry means. Correlations among traits were computed using least squares entry means for each location and combined over locations using PROC CORR in SAS (SAS Institute Inc. 1988).

CHAPTER 4

RESULTS AND DISCUSSION

Ninety-six DH lines were developed from a 'Rampart' X 'Jerry' cross. The DH lines were raised at Bozeman and Moccasin, MT and Williston, ND in 2002. Agronomic data was acquired from all three locations, however the primary emphasis was on stem solidness. Combined means across locations for stem solidness showed the stem solidness of 'Rampart' (mean = 20.3) was significantly different ($P<0.01$) from the stem solidness score for 'Jerry' (mean = 6.3) (Fig. 2). The combined means of the solid stem scores from the DH lines ranged from 5.7 to 20.2.

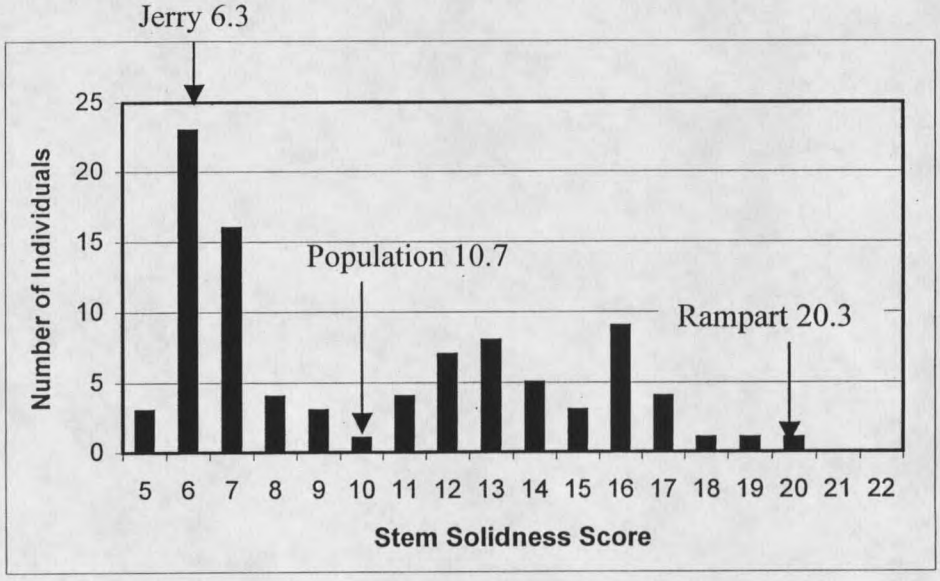

Fig. 2 Histogram of 2002 combined stem solidness score means from all locations for DH population developed from a Rampart X Jerry cross.

Correlations among Traits

Stem solidness has been found to be associated with several agronomic traits (Stoa 1947, McNeal et al. 1965, Wallace and McNeal 1966, Weiss and Morrill 1992). The association between stem solidness and yield is of great concern. Some studies have shown a negative correlation between stem solidness and yield (McNeal et al. 1965, Wallace and McNeal 1966, Weiss and Morrill 1992). Other studies however have shown no correlation between stem solidness and yield (Lebsock and Koch 1968, McNeal and Berg 1979, Hayat et al. 1995). Correlations between various traits measured in the DH winter wheat population were calculated (Tables 1, 2, 3).

Table 1. Correlations (r) between agronomic traits of DH progeny derived from Rampart X Jerry cross. Correlations conducted on 2002 combined means across all locations.

	Yield	Stem Solidness Score	Winter Survival	Plant Height	Test Weight	Lodging	Heading Date	Protein Content
Stem Solidness Score	0.006	-	-	-	-	-	-	-
Winter Survival	-0.027	-0.12	-	-	-	-	-	-
Plant Height	-0.291**	-0.21*	0.07	-	-	-	-	-
Test Wt.	0.137	-0.01	0.21*	0.41**	-	-	-	-
Lodging	-0.35**	-0.08	-0.12	0.68**	0.08	-	-	-
Heading Date	-0.317**	0.10	0.10	0.03	-0.06	-0.18	-	-
Protein Content	-0.691**	0.01	0.07	0.27**	0.03	0.30**	-0.03	-
Emergence	0.174	-0.03	-0.04	0.09	0.10	0.09	-0.14	-0.00

*,** Significant at the 0.05 and 0.01 levels, respectively

Table 2. Correlations (r) between agronomic traits of DH progeny derived from Rampart X Jerry cross. Correlations conducted on 2002 means across Bozeman.

	Yield	Stem Solidness Score	Winter Survival	Plant Height	Test Weight	Lodging	Heading Date	Protein Content
Stem Solidness Score	0.02	-	-	-	-	-	-	-
Winter Survival	-	-	-	-	-	-	-	-
Plant Height	-0.29**	-0.28**	-	-	-	-	-	-
Test Wt.	0.21*	-0.04	-	0.47**	-	-	-	-
Lodging	-0.24*	-0.14	-	0.71**	0.17	-	-	-
Heading Date	-0.40**	0.09	-	0.08	-0.03	-0.21**	-	-
Protein Content	-0.62**	0.04	-	0.19	-0.08	0.18	0.16	-
Emergence	0.20	-0.11	-	-0.27*	-0.19	-0.16	-0.14	-0.10

*,** Significant at the 0.05 and 0.01 levels, respectively

Table 3. Correlations (r) between agronomic traits of DH progeny derived from Rampart X Jerry cross. Correlations conducted on 2002 means across Moccasin.

	Yield	Stem Solidness Score	Winter Survival	Plant Height	Test Weight	Heading Date	Protein Content
Stem Solidness Score	-0.03	-	-	-	-	-	-
Winter Survival	0.06	-0.13	-	-	-	-	-
Plant Height	-0.08	-0.17	-0.01	-	-	-	-
Test Wt.	0.06	0.04	0.03	0.34**	-	-	-
Heading Date	-0.15	0.11	-0.37**	-0.08	-0.15	-	-
Protein Content	-0.75**	0.02	-0.04	0.27**	0.06	-0.16	-
Emergence	-0.03	0.03	-0.17	0.23*	0.12	-0.06	0.11

*,** Significant at the 0.05 and 0.01 levels, respectively

Results from the trait correlation analysis across all locations revealed there was no significant correlation between stem solidness and yield ($r < 0.01$) (Table 1). This lack of correlation agrees with Hayat et al. (1995) who also reported no significant correlation between stem solidness and yield. Correlation analysis did reveal, however, that plant height was negatively correlated to stem solidness ($r = -0.21$). Plant height was the only measured trait that was significantly correlated to stem solidness. Significant negative correlations between stem solidness and plant height were also reported by McKenzie (1965) and Lebsock and Koch (1968).

Several other significant correlations between traits were detected using the combined means across all locations (Table 1). Highly significant negative correlations were observed between yield and plant height ($r = -0.29$), lodging ($r = -0.35$), heading date ($r = -0.32$), and protein content ($r = -0.69$). Winter survival was significantly correlated to test weight ($r = 0.21$), and plant height had highly significant correlations with test weight ($r = 0.41$), lodging ($r = 0.68$) and protein content ($r = 0.27$). Lodging was found to have a highly significant correlation to protein content ($r = 0.30$).

Significant correlations between trait means at the three individual experimental locations showed some variability compared to correlations derived from the combined means across locations. However, results from all individual locations failed to show any additional significant correlations between stem solidness and measured traits. The only trait that had a significant correlation with stem solidness at an individual location, Bozeman, was plant height ($r = -0.28$).

The correlation between stem solidness and winter hardiness ($r = -0.11$) at Williston, ND was nonsignificant (data not shown). Although the data is limited, this provides evidence that winter-hardy solid stem cultivars could be developed.

Marker Identification

From a total of 398 microsatellite primer pairs evaluated for polymorphism between the two parental genotypes 'Rampart' and 'Jerry', 312 provided scorable amplification products. Of these primers, 87 detected polymorphism between the parental genotypes (Table 4). Using the 87 polymorphic primers, we conducted bulk segregant analysis (Michelmore et al. 1991) on six pooled-DNA samples, each consisting of six DH lines representing the two tails of the solid and hollow stem distribution derived from preliminary data obtained in 2001 (Fig. 3). Of the 87 polymorphic microsatellite primers, GWM247, GWM340, GWM547, and BARC77 exhibited amplification profiles characteristic of the solid and hollow stem parents in the corresponding bulks. This suggested an association between stem solidness and these markers.

Table 4. Polymorphism observed in Rampart X Jerry DH winter wheat population among two microsatellite primer sets.

Microsatellite Library	Number of Primers	Scorable Amplification Product	Polymorphic (Rampart vs. Jerry)
GWM	230	207	59
BARC	168	105	28
Totals:	398	312	87

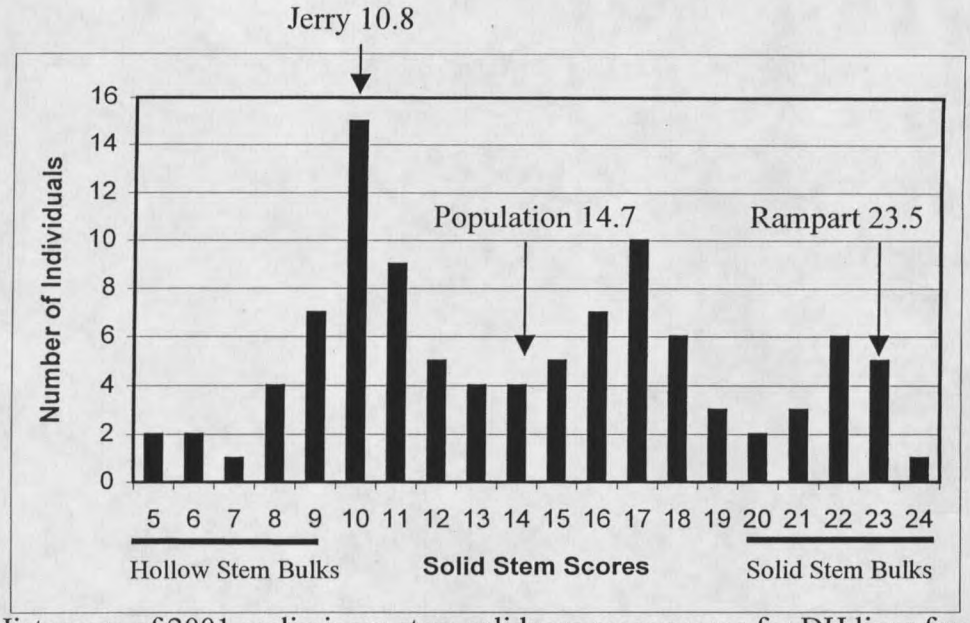

Fig. 3 Histogram of 2001 preliminary stem solidness score means for DH lines from the Rampart X Jerry cross. Stem solidness means were used to select individual DH lines for either hollow or solid stem bulks.

To further confirm this association, we conducted selective genotyping (Lander and Botstein 1989) of individual DH lines comprising the six bulks. Results from analyzing GWM247, GWM340, and GWM547 revealed that 17 of the 18 DH lines within the three solid stem bulks had a profile identical to the solid stem parent, whereas all 18 DH lines comprising the three hollow stem bulks had a profile identical to the hollow stem parent. The lone solid stem DH line, which did not have the solid stem parental profile, was considered a putative recombinant. Results from analyzing BARC77 revealed that 12 of the 18 DH lines within the three solid stem bulks showed a profile identical to the solid stem parent, whereas 14 of the 18 DH lines comprising the three hollow stem bulks matched the hollow stem parental profile. This confirmed an association between the markers and stem solidness, however a stronger relationship between stem solidness and GWM247, GWM340, and GWM547 was apparent than with

BARC77. Subsequently, the 96 DH lines were genotyped using these four microsatellite primers. Results from genotyping revealed three of the DH lines were heterozygous at the *Xgwm247* and *Xgwm340* loci. These three lines were removed from the QTL analysis and the remaining segregation data was used for QTL analysis.

Observations of the amplified PCR products of GWM247 and GWM340 suggest the two primer pairs may be amplifying the same locus. The banding pattern derived from the two primer pairs is very similar except that amplified fragments from GWM340 are smaller then those derived from GWM247 (Table 5). Additionally, the forward primer sequence and microsatellite motif is the same for both markers, though the reverse primer sequences are different (Table 5). Based on the fragment size difference between GWM247 and GWM340 it seems likely that the reverse primer of GWM247 is located upstream from the GWM340 reverse primer. To verify that GWM247 and GWM340 amplify the same locus, the amplified products from the two primer pairs should be sequenced and compared to determine their homology.

QTL Analysis

Loci *Xgwm247*, *Xgwm340*, *Xgwm547*, and *Xbarc77* were analyzed using a single-marker linear regression approach. The regression of stem solidness was highly significant for all markers, indicating a linkage between the microsatellite markers and a QTL for stem solidness (designated *Qss.msub-3BL*). Marker loci *Xgwm247, Xgwm340,* and *Xgwm547* all had a R^2 value of 0.76 suggesting the markers are cosegregating with each other and are linked to a QTL that contributes at least 76% of the total variation for

Table 5. Description of microsatellites associated with stem solidness in a 'Rampart' X 'Jerry' DH population.

Markers	Primer sequences (Forward and Reverse)	Motif	T_m (°C)	Chromosome	Fragment Size[a] (bp)
GWM247	GCAATCTTTTTTCTGACCACG ATGTGCATGTCGGACGC	$(GA)_{24}$	55	3B	175
GWM340	GCAATCTTTTTTCTGACCACG ACGAGGCAAGAACACACATG	$(GA)_{26}$	60	3B	145
GWM547	GTTGTCCCTATGAGAAGGAACG TTCTGCTGCTGTTTTCATTTAC	$(CA)_{12}$	60	3B	180
BARC77	GCGTATTCTCCCTCGTTTCCAAG GTGGGAATTTCTTGGGAGTCTGT	$(ATCT)_6$ +18	55	3B	190

[a] Fragment size of solid stem parental alleles.

Table 6. Marker class means, parental means and regression analysis between microsatellite markers (*Xgwm247*, *Xgwm340*, and *Xgwm547*) and agronomic traits using 93 DH lines derived from a Rampart X Jerry winter wheat cross over all locations in 2002.

Location	Entry	Stem Solidness Score	Yield (Kg ha^{-1})	Test Weight (Kg m^{-3})	Grain Protein %	Emergence %	Winter Survival %	Head Date (J days)	Height (cm)	Lodging %
All	Population	10.7	3939.95	738.30	15.7	77.20	74.13	174.84	99.92	27.00
	Range	5.7 - 20.2	3043.7 - 4508.5	684.7 – 827.5	14.3 - 17.6	65.0 - 85.0	63.6 - 83.7	170.8 - 178.2	68.7 - 124.2	-0.04 - 0.66
	S allele	14.4	3970.93	737.45	15.7	77.00	73.70	175.00	98.00	26.00
	H allele	7.2	3910.46	738.74	15.8	77.00	74.60	174.70	101.70	28.00
	R^2	0.76**	0.01	0.00	0.01	0.00	0.02	0.01	0.03	0.00
	Jerry	6.3	3993.07	728.63	15.3	73.70	80.74	175.38	99.80	19.00
	Rampart	20.3	3991.50	736.68	16.3	66.10	71.51	175.24	111.18	32.00
	LSD	2.8	415.91	19.95	0.6	1.03	8.32	1.29	10.73	0.16

** Regression of phenotypic value on marker class means significant at the 0.01 probability level.

stem solidness among the DH lines (Table 6). An R^2 value of 0.136 was derived for *Xbarc77* suggesting that it is either linked to an additional QTL contributing 13.6% of the total variation for stem solidness, or it is located further from *Qss.msub-3BL* than *Xgwm247, Xgwm340,* and *Xgwm547*. We suspect that *Xbarc77* is associated with *Qss.msub-3BL* rather than a different QTL, because the degree of association between the marker and stem solidness decreases as the distance from *Xgwm247, Xgwm340,* and *Xgwm547* increases. The high percentage of total stem solidness variation attributed to *Qss.msub-3BL,* indicates *Xgwm247, Xgwm340,* and *Xgwm547* are linked to the primary gene controlling development of stem solidness identified by McNeal (1956) and McKenzie (1965).

Verification of Microsatellite Linkage to *Qss.msub-3BL*

To test whether the linkage between *Xgwm247, Xgwm340, Xgwm547,* and *Xbarc77* to *Qss.msub-3BL* is present in other cultivars, several hollow and solid stem winter and spring wheat cultivars from diverse genetic backgrounds were screened. All cultivars contained an allelic profile that corresponded to their stem solidness phenotype, however the allelic profiles of *Xgwm247* and *Xgwm340* varied between the winter and spring wheat cultivars (Fig 4). Variation appeared to only occur among the hollow stem cultivars, but it was decided to confirm linkage of *Xgwm247* and *Xgwm340* to *Qss.msub-3BL* in a spring wheat population.

To verify linkage of *Xgwm247* and *Xgwm340* to *Qss.msub-3BL*, a spring wheat population derived from a 'McNeal' (hollow stem) X 'MT 9929' (solid stem) cross

consisting of 444 F4 lines was analyzed. All lines were measured for stem solidness and 61 solid lines and 97 hollow lines were characterized for association between the markers and *Qss.msub-3BL*. A total of 157 of the 158 selected lines were amplified, and only two lines had a parental allele that did not correspond with the phenotypic data. Results indicated the markers were strongly associated with *Qss.msub-3BL* in spring wheat.

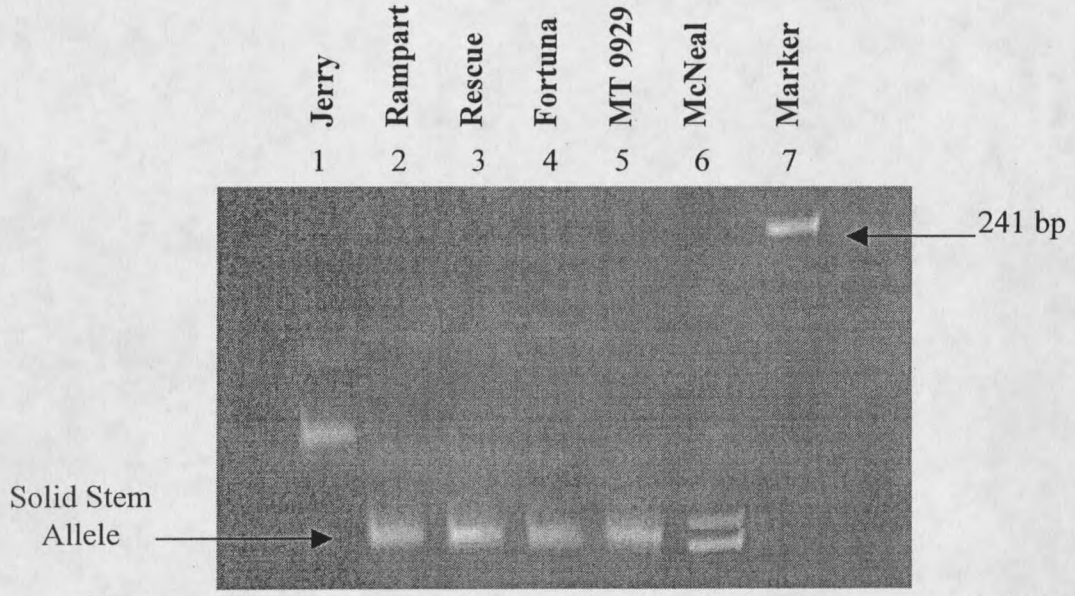

Fig. 4 PCR amplified fragments from amplification of wheat genotypes with GWM 340. Lanes 1 and 2 are winter wheat genotypes; 3 – 6 are spring wheat genotypes; 7 is a pUC19/*Rsa* DNA ladder. Jerry and McNeal are hollow stemmed; Rampart, Rescue, Fortuna, and MT 9929 are solid stem genotypes.

Physical mapping of *Xgwm247, Xgwm340, Xgwm547* and *Xbarc77*

Roder et al. (1998) mapped the *Xgwm* microsatellites by integrating them into a framework RFLP map of all wheat chromosomes. If the markers did not exceed a LOD score of 2.5, they were not directly placed into the RFLP framework. Rather, the markers

were assigned to the most likely RFLP interval in which they might reside. Microsatellite loci *Xgwm247, Xgwm340,* and *Xgwm547* were mapped to an interval located on the distal end of chromosome 3BL (Roder et al. 1998). The *Xbarc* microsatellites were mapped similarly, and *Xbarc77* was also mapped to the distal end of chromosome 3BL (USDA – ARS and U.S. Wheat and Barley Scab Initiative, http://www.scabusa.org/research_bio.html). We confirmed the microsatellites approximate map location by screening the markers with a 'Chinese Spring' nulli-tetrasomic line that was nullisomic for chromosome 3B. No amplified products from the four markers were detected, indicating the markers reside on chromosome 3B.

Further mapping, using deletion lines developed by Endo and Gill (1996), was conducted to physically assign the markers to a more defined region on chromosome 3B. Two deletion lines, 3BL-7 (FL = 0.63) and 3BL-11 (FL = 0.81), derived from 'Chinese Spring' and specific to the distal end of chromosome 3BL were analyzed. No amplification products from the four markers were observed in either 3BL-7 or 3BL-11. Results indicate the markers reside in the most distal chromosomal deletion, 3BL-11, of chromosome 3BL.

Physically mapping the markers linked to *Qss.msub-3BL* to the distal end of chromosome 3BL will aid in selecting additional markers, such as expressed sequence tags (ESTs), for fine mapping of the QTL. Presently, there are more then 400,000 ESTs that have been isolated from the wheat genomes (NCBI, 2003). ESTs are being assigned to specific chromosomal regions using the wheat deletion lines. Markers potentially

linked to *Qss.msub-3BL* can be selected from those ESTs that reside in chromosome deletion 3BL-11.

Association of *Qss.msub-3BL* to additional agronomic traits

To determine whether an association exists between *Qss.msub-3BL* and grain yield, a linear regression analysis was conducted using yield data obtained from Bozeman and Moccasin, MT in 2002. Only *Xgwm247, Xgwm340,* and *Xgwm547* were used in the regression, because they are the most closely associated markers to *Qss.msub-3BL.* Results within and across locations showed the markers were not significantly associated with yield and explained almost no variation in yield (Tables 6, 7, 8).

Linear regressions were also used to determine if a relationship existed between the markers and any of the other important agronomic traits: test weight, grain protein, plant emergence, winter survival, heading date, height, and lodging. Data obtained in 2002 from Bozeman and Moccasin, MT and Williston, ND was used in the analysis. Results showed no significant associations were present between the markers and traits (Tables 6, 7, 8, 9). The lack of association between *Qss.msub-3BL* and important agronomic traits indicates the QTL can be incorporated into cultivars without potentially adverse effects.

Use of markers associated to *Qss.msub-3BL* for MAS

Since *Xgwm247, Xgwm340,* and *Xgwm547* are more tightly linked to *Qss.msub-3BL* than *Xbarc77,* these three *Xgwm* markers would be more useful for selecting

Table 7. Marker class means, parental means and regression analysis between microsatellite markers (*Xgwm247*, *Xgwm340*, and *Xgwm547*) and agronomic traits using 93 DH lines derived from a Rampart X Jerry winter wheat cross at Bozeman, MT in 2002.

Location	Entry	Stem Solidness Score	Yield (Kg ha^{-1})	Test Wt. (Kg m^{-3})	Grain Protein %	Emergence %	Winter Survival %	Head Date (J days)	Height (cm)	Lodging %
Bozeman	Population	8.6	5004.15	720.96	16.0	80.00	100.00	175.40	113.21	27.00
	Range	5.3 - 17.0	4125.5 - 5993.4	664.1 - 770.9	14.4 - 17.7	73.0 - 90.0	100.0 -100.0	170.0 - 179.0	77.6 - 140.0	-0.04 - 0.66
	S allele	10.9	5052.69	719.43	15.9	80.00	100.00	175.50	110.90	26.00
	H allele	6.5	4958.62	723.29	16.0	80.00	100.00	175.30	115.40	28.00
	R^2	0.55**	0.01	0.01	0.02	0.01	0.00	0.00	0.03	0.00
	Jerry	5.6	5035.43	708.58	15.7	80.08	100.00	176.02	115.01	19.00
	Rampart	16.8	5176.11	722.52	16.6	83.20	100.00	175.86	116.83	32.00
	LSD	1.4	520.05	16.34	0.5	1.02	-	1.43	4.86	0.16

** Regression of phenotypic value on marker class means significant at the 0.01 probability level.

Table 8. Marker class means, parental means and regression analysis between microsatellite markers (*Xgwm247*, *Xgwm340*, and *Xgwm547*) and agronomic traits using 93 DH lines derived from a Rampart X Jerry winter wheat cross at Moccasin, MT in 2002.

Location	Entry	Stem Solidness Score	Yield (Kg ha^{-1})	Test Wt. (Kg m^{-3})	Grain Protein %	Emergence %	Winter Survival %	Head Date (J days)	Height (cm)	Lodging %
Moccasin	Population	9.7	2875.74	755.64	15.5	74.36	83.19	174.27	86.63	-
	Range	4.9 - 19.5	1780.5 - 3507.3	697.6 - 888.0	14.0 - 17.7	54.0 - 89.0	70.8 - 95.1	171.6 - 177.7	59.8 - 108.5	-
	S allele	13.7	2889.17	756.76	15.4	75.00	82.70	174.40	85.10	-
	H allele	6.0	2862.29	755.47	15.5	74.00	83.60	174.10	88.10	-
	R^2	0.73**	0.00	0.00	0.01	0.00	0.01	0.02	0.03	-
	Jerry	5.1	2950.71	748.69	15.00	66.70	81.88	174.91	89.89	-
	Rampart	20.9	2806.88	750.84	16.1	49.00	80.92	174.47	84.60	-
	LSD	3.6	346.70	36.55	0.6	1.15	7.80	1.35	13.60	-

** Regression of phenotypic value on marker class means significant at the 0.01 probability level.

Table 9. Marker class means, parental means and regression analysis between microsatellite markers (Xgwm247, Xgwm340, and Xgwm547) and agronomic traits using 93 DH lines derived from a Rampart X Jerry winter wheat cross at Williston, MT in 2002.

Location	Entry	Stem Solidness Score	Yield (Kg ha^{-1})	Test Wt. (Kg m^{-3})	Grain Protein %	Emergence %	Winter Survival %	Head Date (J days)	Height (cm)	Lodging %
Williston	Population	13.8	-	-	-	-	39.21	-	-	-
	Range	6.8 - 24.1	-	-	-	-	11.7 - 60.7	-	-	-
	S allele	18.7	-	-	-	-	38.20	-	-	-
	H allele	9.3	-	-	-	-	40.10	-	-	-
	R2	0.80**	-	-	-	-	0.01	-	-	-
	Jerry	8.4	-	-	-	-	60.35	-	-	-
	Rampart	23.2	-	-	-	-	33.61	-	-	-
	LSD	2.7	-	-	-	-	10.39	-	-	

** Regression of phenotypic value on marker class means significant at the 0.01 probability level.

cultivars that have *Qss.msub-3BL*. Tight linkage between the *Xgwm* markers and *Qss.msub-3BL* reduces the likelihood of recombination events occurring between the loci. Lower recombination rates increase the probability of selecting a cultivar with *Qss.msub-3BL* using a molecular marker. In a population with heterozygous lines however, *Xgwm247* and *Xgwm340* would be the most informative markers because they are co-dominant whereas *Xgwm547* is a dominant marker. Co-dominant markers are capable of distinguishing between homozygous and heterozygous loci.

Selection for *Qss.msub-3BL* alone might not be sufficient for developing solid stem cultivars that are resistant to wheat stem sawfly. Distributions of hollow and solid stem parental alleles at individual locations and across locations shows several of the DH lines with solid stem parental alleles associated with *Qss.msub-3BL* have relatively low stem solidness scores (Fig 5, 6, 7, 8). A cultivar that has sufficient resistance to wheat stem sawfly should exhibit a stem solidness score of 20 or greater (Talbert 2003, personal communication). The DH lines grown in Bozeman, MT that contained solid stem parental alleles had a mean stem solidness score of 10.90 and a range of 6.35 to 17.00 (Table 7). DH lines grown at Moccasin, MT with the solid stem parental allele had a mean stem solidness score of 13.70 and a range of 8.23 to 19.48 (Table 8). At Williston, ND, DH lines with solid stem parental alleles had a mean stem solidness score of 18.7 and a range of 12.26 to 24.08 (Table 9). Stem solidness scores across all locations showed the DH lines that contained the solid stem parental allele had a mean score of 14.40 and a range of 9.31 to 20.15 (Table 6). Although the environment affects the level of stem solidness (Platt 1941, Platt et al. 1948, Holmes et al. 1960), as observed in the range of stem

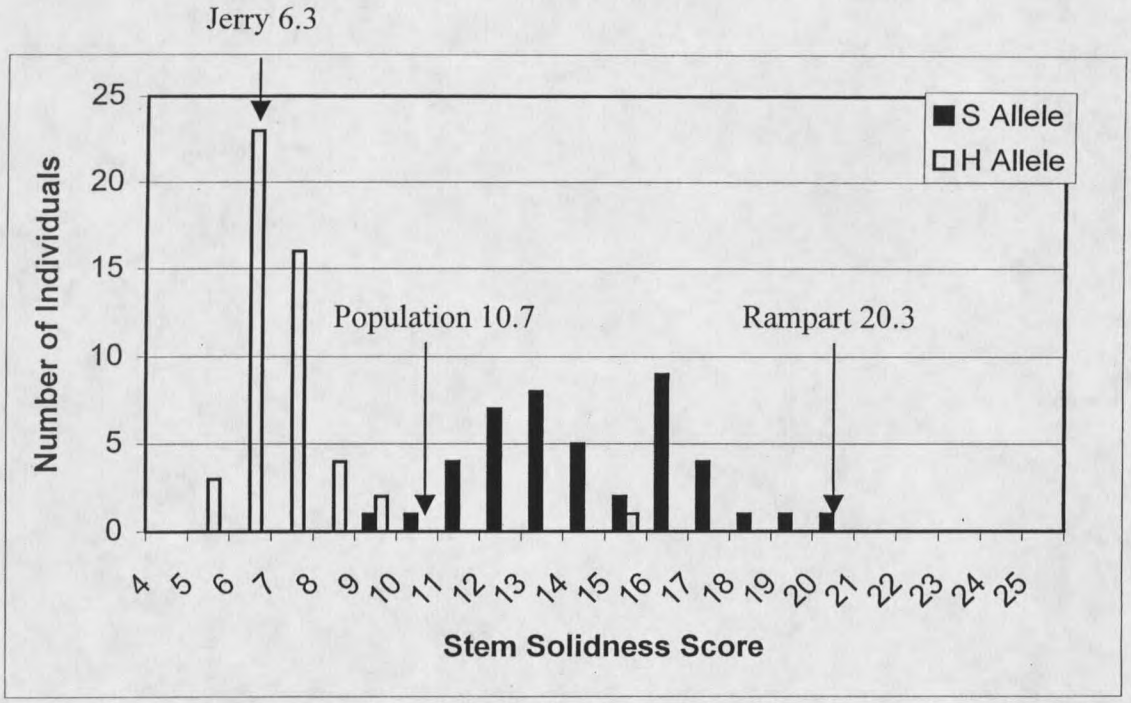

Fig. 5 Histogram of stem solidness score distribution of solid or hollow parental alleles, of *Xgwm247*, *Xgwm340*, and *Xgwm547*, associated to *Qss.msub-3BL*. 2002 stem solidness scores are combined means across experimental locations.

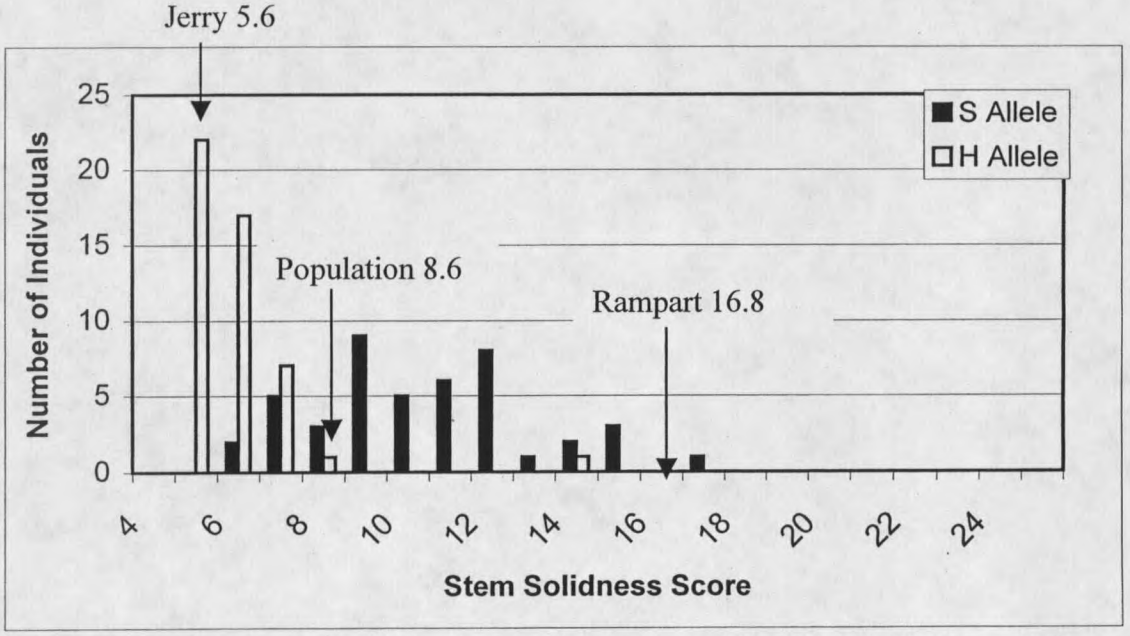

Fig. 6 Histogram of stem solidness score distribution with solid or hollow parental alleles, derived from screening *Xgwm247, Xgwm340, and Xgwm547*, associated with *Qss.msub-3BL*. 2002 stem solidness score means from Bozeman, MT.

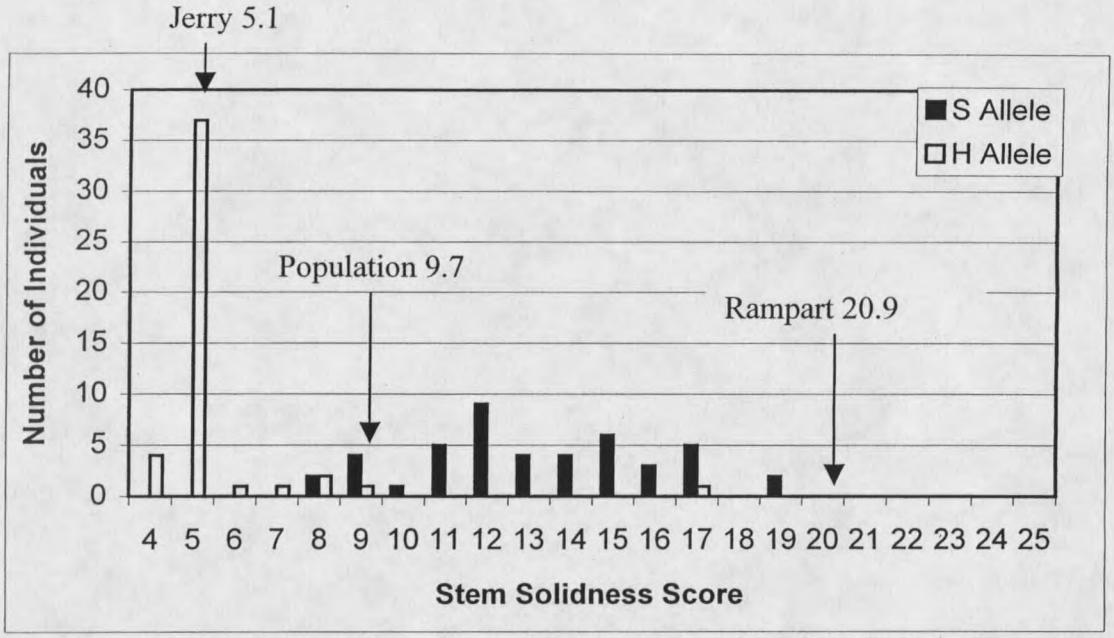

Fig. 7 Histogram of stem solidness score distribution of solid or hollow parental alleles, of *Xgwm247*, *Xgwm340*, and *Xgwm547*, associated with *Qss.msub-3BL*. 2002 stem solidness score means from Moccasin, MT.

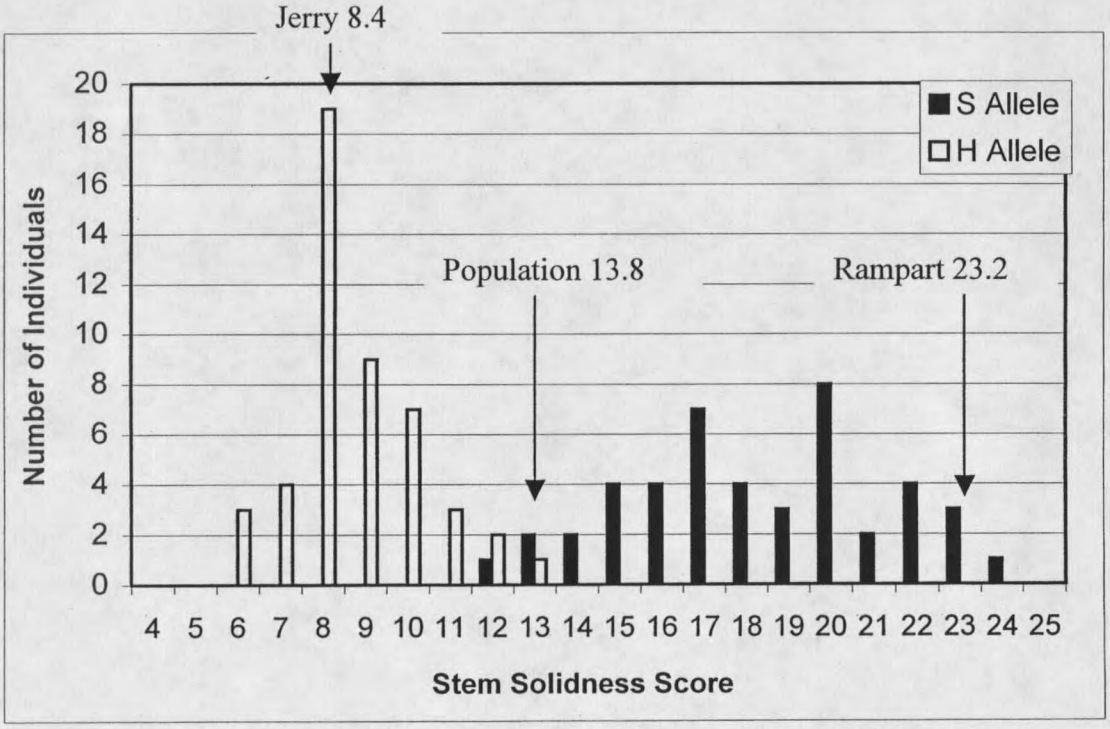

Fig. 8 Histogram of stem solidness score distribution of solid or hollow parental alleles, of *Xgwm247*, *Xgwm340*, and *Xgwm547*, associated to *Qss.msub-3BL*. 2002 stem solidness score means from Williston, ND.

solidness scores across, these results show that MAS for *Qss.msub-3BL* only will not be sufficient in selecting cultivars with the requisite stem solidness levels for wheat stem sawfly resistance.

Identifying additional genes for solid stems

The broad range of stem solidness within DH lines with the solid stem alleles linked to *Qss.msub-3BL* indicates other genetic factors contribute to expression of stem solidness (Fig. 5, 6, 7, 8). Several previous studies have noted that multiple genes control solid stem development (Larson 1952, McNeal 1956, Larson 1959a, Larson and MacDonald 1962, McKenzie 1965). McNeal (1956) and McKenzie (1965) conducted heratibility studies on solid stem wheat and surmised several genes control solid stem development. Larson (1952, 1959a) cytogenetically analyzed 'S-615' and concluded that there were genetic factors on chromosomes 3B, 3D, 5A, 5B, and 5D affecting stem solidness expression. To obtain wheat cultivars with sufficient stem solidness to provide wheat stem sawfly resistance using MAS, it would be necessary to identify markers linked to the other modifying genes.

The QTL, *Qss.msub-3BL,* identified in the DH mapping population used in this study contributes such a high level of the variation for stem solidness that the variation contributed by the minor genes is not detectable. To identify markers linked to the less significant genes would require the development of a new mapping population. Such a population would derive from a cross between a moderately solid stem parent that only contains *Qss.msub-3BL* and a parent with very high solid stem expression levels, which

would indicate the parent contains both *Qss.msub-3BL* and several modifying genes which influence expression of stem solidness. The markers linked to *Qss.msub-3BL* ensure both parents have *Qss.msub-3BL* thereby fixing *Qss.msub-3BL* in the progeny. With *Qss.msub-3BL* fixed, the variation of the other genetic factors controlling solid stem expression could be detected. This would allow the identification of markers linked to these modifying factors.

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APPENDICES

APPENDIX A

PHENOTYPIC MEANS

2002 Rampart X Jerry DH mapping population: combined phenotypic data.

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
1	401	6.76	4145.89	748.56	15.59	80.15	73.81	172.94	91.02	-1.16
2	403	6.40	3493.48	745.79	17.57	82.01	74.55	172.83	115.49	64.74
3	404	6.77	3877.57	750.82	15.72	77.31	80.16	176.62	117.09	36.33
4	405	14.56	4068.74	744.98	15.72	79.65	72.93	174.67	91.29	17.88
5	407	5.82	3919.04	712.68	15.53	81.22	75.35	175.06	90.28	11.98
6	423	6.29	3875.61	730.99	16.38	77.68	76.30	174.05	111.13	23.39
7	424	16.41	3937.12	741.97	15.59	75.49	79.97	175.43	83.25	-3.86
8	425	16.03	3865.15	760.62	14.96	73.82	78.21	176.16	111.89	30.53
9	426	6.75	3879.41	740.59	15.38	73.43	74.30	173.95	118.20	43.19
10	427	13.98	3898.26	761.05	16.37	76.38	70.19	171.86	105.67	53.33
11	428	15.13	3719.90	743.56	16.34	72.14	75.29	174.92	106.40	43.58
12	429	7.64	3678.29	751.56	15.73	65.21	75.24	175.56	112.18	35.84
13	430	13.90	4092.37	726.21	15.38	71.91	76.18	174.72	104.43	10.25
14	431	20.16	3666.52	685.21	16.92	77.31	71.80	175.57	68.68	1.54
15	432	13.40	3578.54	765.56	15.90	68.30	73.91	175.86	109.74	32.75
16	433	16.68	4031.60	738.57	16.02	78.35	74.17	176.57	98.37	11.60
17	434	17.50	4124.26	766.87	15.57	79.59	74.85	174.58	105.69	13.33
18	435	6.18	3530.75	715.25	16.18	75.17	75.98	174.48	115.32	58.84
19	436	13.45	3802.67	729.26	15.28	79.33	69.76	175.39	112.58	46.28
20	437	11.28	4145.58	709.17	14.97	81.31	63.63	176.79	99.59	28.79
21	439	8.26	3935.53	732.01	15.03	77.81	75.52	176.97	100.05	13.91
22	440	6.10	3890.19	727.59	15.98	75.79	75.97	177.62	93.18	6.47
23	441	12.94	3813.21	754.80	16.25	77.66	73.93	175.12	105.71	31.60

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
24	442	14.74	3837.73	750.27	15.79	67.98	76.63	174.95	93.79	12.37
25	443	8.50	3044.45	762.29	16.96	79.85	69.58	176.49	107.76	52.37
26	444	7.15	4446.08	730.34	15.35	73.82	78.31	174.67	95.79	15.26
27	445	13.24	3824.95	731.52	15.64	73.09	76.57	176.87	83.53	2.12
28	446	15.08	3445.16	696.54	16.09	74.03	72.09	175.81	88.45	20.39
29	447	6.55	3834.65	717.98	15.56	68.26	76.47	173.50	99.30	42.51
30	448	7.05	4205.81	774.48	15.50	70.15	75.53	175.52	99.91	20.19
31	449	17.21	3649.75	725.21	16.23	69.50	77.71	175.17	88.77	7.05
32	450	13.71	4144.95	741.95	15.30	75.79	78.94	176.36	88.24	8.02
33	451	9.30	4509.34	710.45	14.38	74.80	65.30	172.92	85.16	7.63
34	452	18.40	4457.26	759.39	14.85	79.95	72.93	174.27	92.45	7.44
35	453	11.57	4324.90	768.32	15.18	80.48	74.53	173.47	115.07	33.53
36	454	6.65	3997.94	765.66	16.00	84.12	78.20	175.56	98.09	12.95
37	455	8.40	4224.04	770.99	15.56	84.63	75.85	174.09	106.21	35.18
38	456	7.03	3555.65	718.47	15.97	84.03	71.94	174.00	110.14	55.37
39	457	14.10	4205.17	827.94	15.99	80.75	75.86	174.87	115.41	30.44
40	458	10.62	4085.41	739.52	16.28	79.81	77.28	172.55	105.97	14.88
41	459	7.97	4031.48	716.50	15.94	79.48	73.25	172.80	86.05	6.67
42	460	6.92	4076.54	718.52	15.38	79.80	72.54	173.92	88.02	6.28
43	461	6.90	3993.94	736.31	15.45	82.30	78.90	175.44	112.08	35.84
44	462	12.66	3933.87	711.44	15.85	81.72	74.52	175.97	106.85	26.47
45	463	13.80	4213.93	758.24	15.21	79.50	73.13	175.17	95.88	15.07
46	464	6.96	3993.32	756.25	15.15	80.56	73.46	177.19	112.18	35.95
47	465	16.93	3739.90	718.55	15.88	71.44	70.56	177.29	86.25	2.32

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
48	466	11.40	4293.56	745.41	14.95	74.27	76.09	175.03	91.82	21.54
49	467	6.87	3897.02	721.32	15.54	72.21	73.17	174.74	103.87	37.68
50	468	6.75	4229.75	729.70	15.42	69.40	70.14	175.62	90.57	14.11
51	469	15.52	3638.15	775.11	16.97	76.33	75.22	173.51	112.20	49.47
52	470	19.43	4088.06	741.20	15.21	79.85	73.83	174.97	81.72	0.58
53	471	6.99	3667.16	753.02	16.53	78.30	71.13	174.74	124.32	39.61
54	472	12.59	3944.37	745.26	15.41	83.35	75.45	173.48	116.11	41.93
55	473	17.24	3824.74	744.42	15.93	83.35	73.51	175.27	103.30	42.32
56	474	5.84	4118.67	717.79	15.61	80.86	73.27	173.36	103.27	50.82
57	475	9.63	4236.33	746.91	15.47	77.68	75.18	178.20	105.59	14.11
58	476	6.24	3674.23	715.54	16.19	79.87	74.91	176.70	88.58	1.16
59	477	7.47	3480.91	767.13	15.81	75.26	70.80	176.92	115.63	25.89
60	478	12.14	3966.21	714.70	15.52	75.41	74.75	175.23	99.70	48.98
61	479	6.64	4099.75	725.18	15.49	77.49	68.52	174.55	93.42	8.02
62	480	12.07	3757.45	713.41	15.60	84.35	76.42	174.67	83.32	48.60
63	481	7.48	4001.39	727.82	15.58	79.48	71.04	175.40	82.76	6.47
64	482	13.06	3738.83	697.88	15.60	80.79	75.77	175.52	94.15	12.56
65	484	12.40	3566.90	753.13	16.00	71.91	68.49	174.15	106.94	66.47
66	485	7.74	3538.51	740.90	16.06	75.71	77.36	175.77	111.04	48.21
67	486	16.84	3917.82	689.01	15.95	66.98	71.37	174.29	102.97	60.39
68	487	9.60	3850.71	736.24	16.06	73.01	77.74	174.43	101.59	23.28
69	488	11.74	4346.86	737.51	15.86	77.32	74.40	173.71	96.21	35.26
70	489	7.44	4029.68	745.25	15.86	78.03	76.07	174.65	105.95	35.65
71	490	7.66	4003.72	744.94	15.44	77.88	74.99	174.97	106.92	42.89

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
72	491	7.73	3409.57	740.75	17.13	74.18	74.24	174.63	115.66	19.04
73	492	14.33	3939.28	742.97	15.36	74.42	74.60	176.20	93.43	19.42
74	493	7.19	4301.13	732.86	15.34	77.29	70.40	170.78	85.01	17.88
75	494	7.22	3773.59	715.00	15.80	70.86	70.31	175.71	86.58	8.40
76	495	6.44	4081.18	773.44	15.89	79.18	76.76	173.89	101.52	14.30
77	496	16.09	3745.75	727.96	16.02	75.94	71.60	175.58	105.48	39.81
78	497	6.86	4013.81	766.48	15.51	71.76	73.65	172.45	84.24	0.19
79	498	12.76	4179.18	719.01	15.48	78.00	73.93	173.58	85.61	32.37
80	499	6.66	3600.95	721.19	15.57	77.14	74.56	176.13	108.15	33.33
81	500	7.28	4455.22	739.63	14.98	76.89	72.03	172.13	87.75	21.54
82	501	6.39	3295.11	742.14	16.09	75.45	83.54	176.20	98.04	30.91
83	503	7.39	3870.62	702.95	15.26	74.87	77.57	173.84	90.08	33.72
84	504	5.70	4329.13	725.09	14.34	76.44	75.96	174.17	96.43	32.37
85	505	7.28	4327.78	739.69	15.44	77.87	75.84	175.13	83.86	1.93
86	506	6.54	3511.88	718.92	17.37	79.63	76.04	173.67	108.47	50.82
87	507	16.17	4062.92	729.03	15.38	81.24	69.64	175.09	98.24	21.16
88	508	9.72	4087.37	734.16	15.34	80.47	74.03	173.90	87.44	12.95
89	509	14.33	3953.94	725.34	15.23	77.81	74.81	174.43	87.79	15.84
90	510	7.02	4163.09	743.49	15.67	80.82	75.26	173.84	111.90	20.00
91	511	6.73	4193.79	772.48	15.50	78.80	73.76	174.29	109.36	50.44
92	512	7.25	4082.76	743.23	15.72	78.65	76.26	174.69	98.45	20.19
93	513	16.46	3689.55	740.32	16.08	81.16	68.42	175.63	104.79	41.35
94	515	16.22	4062.18	728.96	15.89	80.99	71.37	174.02	110.98	60.39
95	516	17.81	4313.99	748.25	15.38	84.20	74.52	174.92	86.37	49.56

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
96	519	8.99	3811.82	747.26	15.86	81.85	72.58	171.84	105.24	18.84
97	JUDITH	6.56	4026.90	703.87	15.34	73.73	74.33	175.38	95.29	18.65
98	ND 9258	6.34	3993.07	728.61	16.32	66.16	80.74	175.27	103.35	31.40
99	NORSTAR	6.57	3361.85	759.50	15.08	69.03	84.50	178.69	111.18	29.18
100	RAMPART	20.26	3994.72	737.18	15.22	78.33	71.52	173.83	99.80	13.91
	LSD	2.76	415.91	19.95	0.59	1.03	8.32	1.29	10.73	0.16
	C.V.%	14.90	9.20	2.70	3.70	13.51	5.42	0.50	6.60	43.19
	F-Test Lines	4.10	7.20	13.40	6.40	1.49	3.28	13.40	16.50	7.37

2002 Rampart X Jerry DH mapping population: Bozeman, MT phenotypic data.

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
1	401	6.00	5340.87	727.33	15.85	79.40	100.00	173.61	97.54	-1.16
2	403	5.93	4632.81	731.54	17.46	81.73	100.00	173.24	129.39	64.74
3	404	5.87	4893.27	744.13	16.01	75.21	100.00	177.18	137.13	36.33
4	405	13.87	5378.04	720.01	15.77	83.82	100.00	175.37	99.00	17.88
5	407	5.54	4833.93	681.68	15.84	84.72	100.00	176.03	104.08	11.98
6	423	5.75	4718.89	714.89	16.83	80.53	100.00	175.14	124.58	23.39
7	424	14.94	4970.80	723.43	15.73	75.66	100.00	176.04	90.73	-3.86
8	425	12.14	4466.12	742.93	15.64	75.65	100.00	176.18	126.39	30.53
9	426	5.69	4706.79	721.67	15.81	78.35	100.00	174.81	132.75	43.19
10	427	8.86	5372.68	755.13	16.04	78.54	100.00	171.51	122.26	53.33
11	428	14.68	4843.70	729.43	16.47	79.55	100.00	176.05	121.84	43.58
12	429	7.24	4500.35	731.32	16.38	76.48	100.00	175.91	127.47	35.84
13	430	9.09	4839.37	697.93	15.97	81.39	100.00	176.16	123.49	10.25
14	431	17.00	4868.10	664.54	17.16	84.01	100.00	176.33	77.57	1.54
15	432	11.57	4616.36	754.17	16.05	72.62	100.00	176.56	130.18	32.75
16	433	12.46	4997.64	729.64	16.69	86.11	100.00	176.77	109.37	11.60
17	434	12.34	5484.86	758.75	15.35	80.53	100.00	175.74	122.50	13.33
18	435	5.87	4419.14	695.10	16.61	81.09	100.00	175.52	137.65	58.84
19	436	9.74	4735.10	711.64	15.24	78.35	100.00	176.16	128.19	46.28
20	437	7.62	5434.48	690.16	15.49	84.26	100.00	176.57	114.20	28.79
21	439	7.20	4793.15	720.81	15.48	77.60	100.00	178.35	114.32	13.91
22	440	5.67	4932.44	714.78	16.48	80.34	100.00	177.99	105.70	6.47
23	441	8.48	4919.51	736.64	16.44	75.77	100.00	175.49	123.27	31.60

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
24	442	11.55	4895.38	726.32	15.90	79.14	100.00	175.85	104.15	12.37
25	443	7.13	4307.54	754.59	16.88	81.98	100.00	176.39	125.30	52.37
26	444	6.47	5643.24	721.62	15.52	80.04	100.00	175.72	106.88	15.26
27	445	9.92	4797.47	711.47	16.09	74.67	100.00	177.45	93.93	2.12
28	446	11.52	4306.29	675.33	16.07	77.16	100.00	176.45	102.81	20.39
29	447	5.91	4651.41	694.13	15.89	73.82	100.00	173.93	111.79	42.51
30	448	6.97	5436.94	770.54	15.79	83.78	100.00	175.96	118.01	20.19
31	449	12.22	4475.20	687.94	16.75	82.03	100.00	176.54	94.89	7.05
32	450	11.30	5258.48	722.97	15.73	82.32	100.00	177.37	97.41	8.02
33	451	6.35	5995.73	691.81	14.40	89.29	100.00	171.67	92.92	7.63
34	452	15.07	5646.50	742.52	15.64	82.18	100.00	174.77	100.38	7.44
35	453	10.33	5481.09	754.99	15.35	77.78	100.00	173.95	128.09	33.53
36	454	6.47	5075.72	748.67	16.27	81.42	100.00	176.54	104.79	12.95
37	455	7.55	5434.52	766.06	15.43	82.44	100.00	174.82	120.89	35.18
38	456	6.20	4659.42	705.68	16.22	78.95	100.00	174.26	125.71	55.37
39	457	9.84	5450.46	767.87	16.03	76.96	100.00	175.59	129.99	30.44
40	458	7.17	5181.46	725.68	16.37	81.61	100.00	172.48	114.94	14.88
41	459	6.27	5094.31	690.83	16.53	77.01	100.00	172.08	92.03	6.67
42	460	6.15	5218.18	687.40	15.62	87.16	100.00	174.50	98.69	6.28
43	461	5.87	5052.11	718.94	15.73	80.97	100.00	175.98	132.27	35.84
44	462	9.08	5069.94	706.30	16.00	77.87	100.00	175.86	124.75	26.47
45	463	10.55	5259.12	744.47	15.33	75.96	100.00	175.88	106.22	15.07
46	464	6.58	5029.12	750.51	15.62	78.54	100.00	177.60	134.21	35.95
47	465	12.97	4554.14	696.30	16.46	74.86	100.00	178.16	96.27	2.32

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
48	466	8.00	5596.95	722.23	15.17	82.77	100.00	176.04	105.66	21.54
49	467	6.47	4828.52	701.82	16.02	77.75	100.00	175.98	122.04	37.68
50	468	6.01	4951.08	705.51	16.24	81.24	100.00	176.35	97.34	14.11
51	469	11.81	4925.30	769.03	16.62	75.66	100.00	174.10	127.92	49.47
52	470	15.84	5116.91	716.17	15.67	72.73	100.00	176.07	83.97	0.58
53	471	6.33	4535.38	747.07	16.70	78.99	100.00	175.54	139.89	39.61
54	472	9.30	4879.79	731.07	15.70	78.80	100.00	173.63	130.69	41.93
55	473	14.02	4744.04	731.12	16.18	78.98	100.00	175.86	118.33	42.32
56	474	5.69	5235.78	690.40	15.74	82.47	100.00	173.59	113.82	50.82
57	475	6.84	5415.35	745.16	15.93	75.96	100.00	178.68	125.72	14.11
58	476	5.81	4570.57	691.24	16.78	83.67	100.00	177.06	96.50	1.16
59	477	5.96	4323.12	759.53	15.92	77.49	100.00	177.31	134.77	25.89
60	478	7.59	5059.23	698.54	15.74	74.45	100.00	175.33	118.46	48.98
61	479	5.69	5293.31	706.16	15.64	75.58	100.00	175.15	103.44	8.02
62	480	9.76	4836.73	692.59	15.88	90.19	100.00	176.04	103.53	48.60
63	481	6.74	5045.12	705.80	16.00	84.27	100.00	176.51	92.04	6.47
64	482	10.80	4487.77	669.80	15.81	81.73	100.00	176.62	103.77	12.56
65	484	7.96	4650.13	738.50	16.01	73.82	100.00	174.21	127.68	66.47
66	485	6.19	4429.24	714.12	16.06	83.85	100.00	175.82	125.91	48.21
67	486	12.23	4955.34	680.67	16.48	73.81	100.00	174.40	117.00	60.39
68	487	8.75	4702.49	719.15	16.22	79.06	100.00	174.99	110.74	23.28
69	488	10.15	5341.92	711.67	16.26	79.66	100.00	174.24	101.84	35.26
70	489	6.24	5106.00	731.84	16.00	78.05	100.00	175.95	123.09	35.65
71	490	6.89	5123.46	729.30	15.93	78.65	100.00	175.90	121.99	42.89

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
72	491	7.25	4331.52	733.26	17.37	78.35	100.00	175.55	129.58	19.04
73	492	8.66	5186.32	729.68	15.57	78.39	100.00	176.46	107.83	19.42
74	493	5.90	5546.68	715.53	15.20	80.49	100.00	169.96	96.32	17.88
75	494	6.58	4811.11	693.54	16.41	79.14	100.00	176.23	98.37	8.40
76	495	5.97	5470.78	761.34	15.65	83.97	100.00	174.85	110.27	14.30
77	496	11.36	4789.17	706.70	16.19	77.79	100.00	176.03	119.00	39.81
78	497	7.24	5136.80	747.14	15.50	80.34	100.00	173.03	91.34	0.19
79	498	9.73	5169.18	698.21	15.86	84.31	100.00	174.60	91.82	32.37
80	499	5.81	4344.94	705.42	16.09	83.22	100.00	176.01	127.36	33.33
81	500	6.40	5807.10	716.30	15.04	82.44	100.00	172.06	98.58	21.54
82	501	5.28	4128.78	723.35	15.92	89.50	100.00	176.89	109.86	30.91
83	503	6.58	4991.85	683.33	15.08	84.91	100.00	174.52	103.56	33.72
84	504	5.33	5519.68	695.73	14.40	76.67	100.00	175.23	108.58	32.37
85	505	7.07	5402.12	718.21	15.61	78.76	100.00	176.44	91.98	1.93
86	506	5.50	4557.68	709.74	17.73	78.20	100.00	174.25	128.05	50.82
87	507	12.49	5158.69	709.32	15.98	82.03	100.00	175.94	112.13	21.16
88	508	8.23	5156.81	719.20	15.42	79.89	100.00	174.45	95.97	12.95
89	509	9.25	5016.99	699.99	15.48	83.37	100.00	175.49	97.50	15.84
90	510	5.85	5422.00	733.79	15.70	86.03	100.00	174.52	129.29	20.00
91	511	5.78	5500.74	770.02	15.62	76.22	100.00	175.30	126.31	50.44
92	512	6.56	5067.85	719.85	16.08	80.49	100.00	175.91	111.06	20.19
93	513	10.55	4668.97	714.20	16.08	81.87	100.00	176.14	121.09	41.35
94	515	12.91	5144.79	711.41	16.09	81.84	100.00	173.99	125.27	60.39
95	516	15.05	5657.18	733.50	15.37	84.76	100.00	174.57	95.60	49.56

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
96	519	6.99	5154.06	748.71	15.75	84.75	100.00	170.95	124.55	18.84
97	JUDITH	5.92	4866.16	678.08	15.71	80.79	100.00	175.85	105.31	18.65
98	ND 9258	5.56	5035.39	708.58	16.54	83.22	100.00	176.05	116.82	31.40
99	NORSTAR	6.29	3961.82	741.45	15.87	77.01	100.00	179.99	133.21	29.18
100	RAMPART	16.76	5182.57	722.85	15.79	78.65	100.00	174.48	114.97	13.91
	Average	8.62	5004.15	720.96	15.98	80.00	100.00	175.40	113.21	27.00
	LSD (0.05)	1.42	520.05	16.34	0.51	1.02	-	1.43	4.86	0.16
	C.V.%	10.40	8.90	2.10	3.10	16.10	0.00	0.50	3.00	43.19
	F-Test Lines	35.30	2.60	7.90	4.00	0.94	-	9.10	52.60	7.37

2002 Rampart X Jerry DH mapping population: Moccasin, MT phenotypic data.

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
1	401	5.55	2950.91	769.80	15.33	80.90	78.33	172.27	84.51	-
2	403	5.05	2354.15	760.04	17.67	82.29	84.69	172.42	101.59	-
3	404	5.57	2861.86	757.51	15.43	79.40	83.08	176.06	97.06	-
4	405	14.00	2759.45	769.95	15.66	75.47	84.81	173.97	83.58	-
5	407	5.07	3004.16	743.68	15.22	77.72	87.09	174.10	76.47	-
6	423	5.72	3032.33	747.10	15.92	74.83	80.61	172.97	97.68	-
7	424	13.69	2903.43	760.51	15.45	75.32	78.88	174.82	75.77	-
8	425	15.19	3264.17	778.32	14.27	71.99	81.54	176.15	97.38	-
9	426	5.44	3052.03	759.51	14.95	68.50	87.89	173.09	103.66	-
10	427	13.53	2423.83	766.96	16.71	74.23	82.29	172.21	89.08	-
11	428	17.63	2596.10	757.69	16.22	64.72	87.59	173.79	90.95	-
12	429	5.45	2856.23	771.79	15.08	53.93	87.79	175.20	96.89	-
13	430	14.66	3345.36	754.49	14.78	62.44	84.56	173.28	85.37	-
14	431	19.39	2464.96	705.88	16.67	70.60	79.37	174.81	59.78	-
15	432	15.15	2540.73	776.95	15.75	63.97	80.01	175.16	89.30	-
16	433	15.46	3065.56	747.49	15.35	70.60	75.80	176.36	87.37	-
17	434	17.10	2763.65	775.00	15.79	78.65	86.62	173.43	88.89	-
18	435	5.24	2642.36	735.39	15.75	69.25	92.60	173.43	93.00	-
19	436	12.40	2870.24	746.89	15.33	80.30	76.53	174.63	96.96	-
20	437	9.00	2856.68	728.18	14.44	78.35	78.63	177.02	84.97	-
21	439	5.41	3077.92	743.21	14.58	78.01	78.27	175.59	85.78	-
22	440	5.44	2847.95	740.40	15.49	71.24	82.23	177.25	80.67	-
23	441	12.61	2706.90	772.96	16.05	79.55	81.11	174.75	88.14	-

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
24	442	12.42	2780.09	774.23	15.67	56.82	85.93	174.05	83.42	-
25	443	5.73	1781.35	769.99	17.04	77.72	75.31	176.59	90.23	-
26	444	5.57	3248.92	739.05	15.18	67.60	86.31	173.61	84.70	-
27	445	12.71	2852.44	751.58	15.19	71.50	82.65	176.29	73.13	-
28	446	12.89	2584.02	717.75	16.12	70.90	85.92	175.17	74.09	-
29	447	4.85	3017.90	741.82	15.24	62.70	87.15	173.07	86.80	-
30	448	5.84	2974.68	778.42	15.22	56.52	82.29	175.09	81.82	-
31	449	16.09	2824.30	762.48	15.71	56.97	86.74	173.80	82.66	-
32	450	11.80	3031.42	760.93	14.88	69.25	83.20	175.35	79.06	-
33	451	8.23	3022.97	729.09	14.36	60.30	84.19	174.17	77.39	-
34	452	17.20	3268.01	776.26	14.05	77.72	82.59	173.77	84.51	-
35	453	10.29	3168.71	781.66	15.00	83.18	85.30	172.99	102.05	-
36	454	5.45	2920.15	782.66	15.74	86.82	82.36	174.59	91.40	-
37	455	6.35	3013.56	775.92	15.69	86.82	83.58	173.35	91.53	-
38	456	4.97	2451.89	731.26	15.73	89.10	89.59	173.73	94.57	-
39	457	14.41	2959.89	888.01	15.96	84.53	85.00	174.16	100.83	-
40	458	9.36	2989.36	753.35	16.19	78.01	82.53	172.62	97.01	-
41	459	9.03	2968.66	742.17	15.35	81.95	86.31	173.53	80.07	-
42	460	5.93	2934.90	749.64	15.14	72.44	85.20	173.34	77.35	-
43	461	5.89	2935.77	753.69	15.17	83.63	87.72	174.90	91.90	-
44	462	12.41	2797.80	716.59	15.69	85.58	78.57	176.08	88.95	-
45	463	14.90	3168.73	772.01	15.10	83.03	77.33	174.45	85.54	-
46	464	5.83	2957.52	761.98	14.68	82.59	82.97	176.78	90.15	-
47	465	17.21	2925.64	740.80	15.30	68.01	76.67	176.42	76.24	-

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
72	491	5.38	2487.62	748.24	16.90	70.00	83.40	173.71	101.75	-
73	492	13.90	2692.22	756.26	15.15	70.45	87.42	175.94	79.03	-
74	493	5.25	3055.58	750.19	15.47	74.08	85.50	171.61	73.70	-
75	494	5.99	2736.08	736.46	15.19	62.59	70.77	175.18	74.79	-
76	495	5.12	2691.57	785.55	16.14	74.38	83.21	172.93	92.77	-
77	496	16.00	2702.33	749.23	15.85	74.08	82.21	175.13	91.96	-
78	497	5.16	2890.82	785.82	15.53	63.18	81.98	171.87	77.13	-
79	498	11.32	3189.19	739.81	15.11	71.69	84.89	172.56	79.40	-
80	499	5.64	2856.97	736.96	15.06	71.05	83.32	176.25	88.95	-
81	500	5.16	3103.34	762.95	14.92	71.35	88.51	172.20	76.91	-
82	501	5.07	2461.44	760.93	16.26	61.39	95.12	175.50	86.21	-
83	503	5.77	2749.39	722.57	15.43	64.83	90.30	173.17	76.60	-
84	504	4.98	3138.59	754.46	14.28	76.22	84.94	173.10	84.28	-
85	505	5.53	3253.44	761.17	15.27	76.97	86.49	173.82	75.73	-
86	506	5.38	2466.08	728.11	17.01	81.05	82.79	173.08	88.89	-
87	507	12.71	2967.15	748.74	14.77	80.45	79.62	174.24	84.36	-
88	508	8.37	3017.92	749.13	15.26	81.05	82.77	173.34	78.91	-
89	509	14.59	2890.90	750.69	14.98	72.25	84.44	173.38	78.08	-
90	510	5.33	2904.19	753.18	15.65	75.62	88.03	173.16	94.51	-
91	511	5.23	2886.85	774.93	15.38	81.39	84.20	173.27	92.41	-
92	512	5.45	3097.67	766.61	15.35	76.82	87.40	173.46	85.84	-
93	513	17.28	2710.14	766.45	16.07	80.45	76.98	175.12	88.48	-
94	515	17.14	2979.57	746.50	15.69	80.15	83.07	174.05	96.68	-
95	516	15.51	2970.81	763.00	15.39	83.63	83.89	175.26	77.14	-

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
72	491	5.38	2487.62	748.24	16.90	70.00	83.40	173.71	101.75	-
73	492	13.90	2692.22	756.26	15.15	70.45	87.42	175.94	79.03	-
74	493	5.25	3055.58	750.19	15.47	74.08	85.50	171.61	73.70	-
75	494	5.99	2736.08	736.46	15.19	62.59	70.77	175.18	74.79	-
76	495	5.12	2691.57	785.55	16.14	74.38	83.21	172.93	92.77	-
77	496	16.00	2702.33	749.23	15.85	74.08	82.21	175.13	91.96	-
78	497	5.16	2890.82	785.82	15.53	63.18	81.98	171.87	77.13	-
79	498	11.32	3189.19	739.81	15.11	71.69	84.89	172.56	79.40	-
80	499	5.64	2856.97	736.96	15.06	71.05	83.32	176.25	88.95	-
81	500	5.16	3103.34	762.95	14.92	71.35	88.51	172.20	76.91	-
82	501	5.07	2461.44	760.93	16.26	61.39	95.12	175.50	86.21	-
83	503	5.77	2749.39	722.57	15.43	64.83	90.30	173.17	76.60	-
84	504	4.98	3138.59	754.46	14.28	76.22	84.94	173.10	84.28	-
85	505	5.53	3253.44	761.17	15.27	76.97	86.49	173.82	75.73	-
86	506	5.38	2466.08	728.11	17.01	81.05	82.79	173.08	88.89	-
87	507	12.71	2967.15	748.74	14.77	80.45	79.62	174.24	84.36	-
88	508	8.37	3017.92	749.13	15.26	81.05	82.77	173.34	78.91	-
89	509	14.59	2890.90	750.69	14.98	72.25	84.44	173.38	78.08	-
90	510	5.33	2904.19	753.18	15.65	75.62	88.03	173.16	94.51	-
91	511	5.23	2886.85	774.93	15.38	81.39	84.20	173.27	92.41	-
92	512	5.45	3097.67	766.61	15.35	76.82	87.40	173.46	85.84	-
93	513	17.28	2710.14	766.45	16.07	80.45	76.98	175.12	88.48	-
94	515	17.14	2979.57	746.50	15.69	80.15	83.07	174.05	96.68	-
95	516	15.51	2970.81	763.00	15.39	83.63	83.89	175.26	77.14	-

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
96	519	8.99	2469.58	745.81	15.96	78.95	78.08	172.74	85.93	-
97	JUDITH	5.19	3187.63	729.67	14.96	66.67	85.05	174.90	85.28	-
98	ND 9258	5.09	2950.75	748.63	16.09	49.10	81.87	174.48	89.88	-
99	NORSTAR	4.86	2761.88	777.55	14.28	61.05	86.79	177.38	89.15	-
100	RAMPART	20.87	2806.88	751.50	14.65	78.01	80.92	173.19	84.62	-
	Average	9.70	2875.74	755.64	15.48	74.36	83.19	174.27	86.63	-
	LSD (0.05)	3.15	346.70	36.55	0.63	1.15	7.80	1.35	13.6	-
	C.V.%	19.90	8.90	2.10	4.30	9.61	5.88	0.60	10.10	-
	F-Test Lines	18.00	2.60	7.90	3.90	4.20	2.07	6.30	3.00	-

2002 Rampart X Jerry DH mapping population: Williston, ND phenotypic data.

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
1	401	8.74	-	-	-	-	43.10	-	-	-
2	403	8.21	-	-	-	-	38.97	-	-	-
3	404	8.87	-	-	-	-	57.41	-	-	-
4	405	15.80	-	-	-	-	33.97	-	-	-
5	407	6.86	-	-	-	-	38.97	-	-	-
6	423	7.38	-	-	-	-	48.28	-	-	-
7	424	20.61	-	-	-	-	61.03	-	-	-
8	425	20.77	-	-	-	-	53.10	-	-	-
9	426	9.11	-	-	-	-	35.00	-	-	-
10	427	19.56	-	-	-	-	28.28	-	-	-
11	428	13.08	-	-	-	-	38.28	-	-	-
12	429	10.23	-	-	-	-	37.94	-	-	-
13	430	17.97	-	-	-	-	43.97	-	-	-
14	431	24.08	-	-	-	-	36.03	-	-	-
15	432	13.49	-	-	-	-	41.72	-	-	-
16	433	22.13	-	-	-	-	46.72	-	-	-
17	434	23.04	-	-	-	-	37.94	-	-	-
18	435	7.42	-	-	-	-	35.34	-	-	-
19	436	18.19	-	-	-	-	32.75	-	-	-
20	437	17.22	-	-	-	-	12.25	-	-	-
21	439	12.16	-	-	-	-	48.28	-	-	-
22	440	7.18	-	-	-	-	45.69	-	-	-

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
23	441	17.72	-	-	-	-	40.69	-	-	-
24	442	20.25	-	-	-	-	43.97	-	-	-
25	443	12.66	-	-	-	-	33.44	-	-	-
26	444	9.40	-	-	-	-	48.62	-	-	-
27	445	17.09	-	-	-	-	47.06	-	-	-
28	446	20.84	-	-	-	-	30.34	-	-	-
29	447	8.87	-	-	-	-	42.25	-	-	-
30	448	8.34	-	-	-	-	44.31	-	-	-
31	449	23.33	-	-	-	-	46.38	-	-	-
32	450	18.05	-	-	-	-	53.62	-	-	-
33	451	13.33	-	-	-	-	11.72	-	-	-
34	452	22.93	-	-	-	-	36.22	-	-	-
35	453	14.10	-	-	-	-	38.28	-	-	-
36	454	8.02	-	-	-	-	52.25	-	-	-
37	455	11.29	-	-	-	-	43.97	-	-	-
38	456	9.91	-	-	-	-	26.22	-	-	-
39	457	18.03	-	-	-	-	42.59	-	-	-
40	458	15.34	-	-	-	-	49.31	-	-	-
41	459	8.62	-	-	-	-	33.44	-	-	-
42	460	8.67	-	-	-	-	32.41	-	-	-
43	461	8.95	-	-	-	-	48.97	-	-	-
44	462	16.49	-	-	-	-	45.00	-	-	-
45	463	15.96	-	-	-	-	42.06	-	-	-
46	464	8.47	-	-	-	-	37.41	-	-	-

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
47	465	20.61	-	-	-	-	35.00	-	-	-
48	466	16.79	-	-	-	-	38.62	-	-	-
49	467	8.60	-	-	-	-	33.28	-	-	-
50	468	8.88	-	-	-	-	30.00	-	-	-
51	469	19.40	-	-	-	-	40.00	-	-	-
52	470	22.96	-	-	-	-	39.31	-	-	-
53	471	9.49	-	-	-	-	33.28	-	-	-
54	472	16.09	-	-	-	-	44.31	-	-	-
55	473	20.80	-	-	-	-	38.62	-	-	-
56	474	6.96	-	-	-	-	40.00	-	-	-
57	475	12.26	-	-	-	-	44.31	-	-	-
58	476	7.88	-	-	-	-	47.06	-	-	-
59	477	11.09	-	-	-	-	39.13	-	-	-
60	478	17.15	-	-	-	-	35.34	-	-	-
61	479	8.90	-	-	-	-	23.97	-	-	-
62	480	14.17	-	-	-	-	39.31	-	-	-
63	481	10.07	-	-	-	-	32.06	-	-	-
64	482	16.56	-	-	-	-	43.97	-	-	-
65	484	17.22	-	-	-	-	24.66	-	-	-
66	485	11.93	-	-	-	-	37.94	-	-	-
67	486	21.46	-	-	-	-	29.66	-	-	-
68	487	11.53	-	-	-	-	46.38	-	-	-
69	488	15.27	-	-	-	-	40.34	-	-	-
70	489	8.84	-	-	-	-	51.38	-	-	-

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
71	490	10.84	-	-	-	-	37.25	-	-	-
72	491	10.56	-	-	-	-	39.31	-	-	-
73	492	20.45	-	-	-	-	36.38	-	-	-
74	493	10.42	-	-	-	-	25.69	-	-	-
75	494	9.10	-	-	-	-	40.16	-	-	-
76	495	8.22	-	-	-	-	47.06	-	-	-
77	496	20.90	-	-	-	-	32.59	-	-	-
78	497	8.19	-	-	-	-	38.97	-	-	-
79	498	17.22	-	-	-	-	36.90	-	-	-
80	499	8.53	-	-	-	-	40.34	-	-	-
81	500	10.29	-	-	-	-	27.59	-	-	-
82	501	8.83	-	-	-	-	55.50	-	-	-
83	503	9.80	-	-	-	-	42.41	-	-	-
84	504	6.80	-	-	-	-	42.94	-	-	-
85	505	9.25	-	-	-	-	41.03	-	-	-
86	506	8.74	-	-	-	-	45.34	-	-	-
87	507	23.32	-	-	-	-	29.31	-	-	-
88	508	12.56	-	-	-	-	39.31	-	-	-
89	509	19.16	-	-	-	-	40.00	-	-	-
90	510	9.88	-	-	-	-	37.75	-	-	-
91	511	9.17	-	-	-	-	37.06	-	-	-
92	512	9.76	-	-	-	-	41.38	-	-	-
93	513	21.55	-	-	-	-	28.28	-	-	-
94	515	18.62	-	-	-	-	31.03	-	-	-

Entry	ID	Solid Stem Score	Yield (Kg ha-1)	Test Wt. (Kg m-3)	Protein %	Emergence %	Winter Survival %	Heading Date	Height (cm)	Lodging %
95	516	22.85	-	-	-	-	39.66	-	-	-
96	519	10.98	-	-	-	-	39.66	-	-	-
97	JUDITH	8.56	-	-	-	-	37.94	-	-	-
98	ND 9258	8.35	-	-	-	-	60.34	-	-	-
99	NORSTAR	8.56	-	-	-	-	66.72	-	-	-
100	RAMPART	23.15	-	-	-	-	33.62	-	-	-
	Average	13.82	-	-	-	-	39.21	-	-	-
	LSD (0.05)	2.68	-	-	-	-	10.39	-	-	-
	C.V.%	12.1	-	-	-	-	16.52	-	-	-
	F-Test Lines	20.7	-	-	-	-	3.58	-	-	-

APPENDIX B

GENOTYPIC DATA

Genotypic data from a Rampart X Jerry DH mapping population using microsatellite markers.

ID	Solid Stem Score	GWM247	GWM340	GWM547	BARC77	ID	Solid Stem Score	GWM247	GWM340	GWM547	BARC77
476	5.9	H	H	H	H	503	10.6	H	H	H	S
404	6.2	H	H	H	H	481	10.7	H	H	H	H
504	6.6	H	H	H	H	447	10.8	H	H	H	S
489	6.9	H	H	H	H	455	10.8	H	H	H	S
512	7.4	H	H	H	S	471	10.9	H	H	H	H
501	7.5	H	H	H	S	474	10.9	H	H	H	H
477	7.6	H	H	H	H	435	11.0	H	H	H	H
493	7.6	H	H	H	H	497	11.1	H	H	H	S
511	7.9	H	H	H	H	444	11.2	H	H	H	S
518	7.9	H	H	H	H	456	11.3	H	H	H	S
510	8.0	H	H	H	H	459	11.4	H	H	H	H
490	8.5	H	H	H	S	461	11.5	H	H	H	H
499	8.6	H	H	H	H	443	11.9	H	H	H	S
491	8.7	H	H	H	H	439	12.1	H	H	H	H
495	8.9	H	H	H	H	460	12.1	H	H	H	H
519	9.1	H	H	H	H	401	12.3	H	H	H	S
506	9.4	H	H	H	H	485	12.3	H	H	H	S
500	9.5	H	H	H	S	454	12.5	H	H	H	S
479	9.8	H	H	H	H	508	12.5	H	H	H	H
429	9.9	H	H	H	H	440	13.0	H	H	H	S
505	9.9	H	H	H	H	423	13.3	H	H	H	H
403	10.3	H	H	H	H	426	13.6	H	H	H	H
407	10.3	H	H	H	H	468	13.7	H	H	H	S
494	10.3	H	H	H	H	488	14.0	S	S	S	H

ID	Solid Stem Score	GWM247	GWM340	GWM547	BARC77
448	14.2	H	H	H	H
464	14.8	H	H	H	H
467	14.8	H	H	H	H
480	15.2	S	S	S	S
509	15.4	S	S	S	S
451	15.9	S	S	S	S
458	15.9	S	S	S	S
487	16.1	H	H	H	H
482	16.2	S	S	S	N
445	16.5	S	S	S	S
475	16.6	S	S	S	S
405	16.7	S	S	S	S
472	16.8	S	S	S	H
457	16.9	S	S	S	H
430	17.2	S	S	S	S
498	17.3	S	S	S	H
441	17.6	S	S	S	S
484	17.6	S	S	S	S
466	17.8	S	S	S	H
478	17.9	S	S	S	S
450	18.0	S	S	S	H
453	18.1	S	S	S	S
462	18.3	S	S	S	H
492	18.5	S	S	S	S
442	18.6	S	S	S	S
427	18.9	S	S	S	S
436	19.1	S	S	S	S
437	19.2	S	S	S	H
463	19.4	S	S	S	H
516	19.5	S	S	S	S
432	19.6	S	S	S	?
469	20.0	S	S	S	S
486	20.0	S	S	S	S
496	20.2	S	S	S	S
513	20.3	S	S	S	S
434	20.6	S	S	S	S
515	21.8	S	S	S	S
473	22.1	S	S	S	H
465	22.3	S	S	S	S
433	22.5	S	S	S	H
428	22.7	S	S	S	H
449	22.7	S	S	S	?
507	23.0	S	S	S	S
425	23.2	S	S	S	H
446	23.2	S	S	S	S
431	23.5	S	S	S	S
424	23.7	S	S	S	H
452	23.7	S	S	S	S
470	24.7	S	S	S	S