AN ABSTRACT OF THE THESIS OF

Vamsi J. Nalam for the degree of Master of Science in Crop Science presented on December 17, 2004

Title: Localization and Genetic Mapping of Some Factors Influencing Rachis Brittleness and Free-threshing Habit in Wheat

Abstract approved:

Redacted for Privacy

Oscar Riera-Lizarazu

Domesticated forms of wheat exhibit traits that have increased their adaptation to cultivation by humans. Some of the most critical adaptive differences involve changes to morphological features that make the crop easier to harvest like ear rachis stiffness (brittle to non-brittle rachis) and the ease with which the seed is released from its enclosing leaf-like structures (non-free-threshing to free-threshing). The brittle rachis trait is primarily controlled by genes on homoeologous group 3 chromosomes (3A, 3B and 3D) while the free-threshing phenotype is controlled by genes on group 2 chromosomes (2A, 2B, and 2D) in tetraploid and hexaploid wheat (*Triticum turgidum* L. and *T. aestivum* L., respectively). In order to broaden our understanding of the genetic basis of these domestication traits, this research was undertaken to more precisely localize some of these factors. Two populations of recombinant inbred

chromosome lines for chromosome 3A and chromosome 3B (RICL-3A and RICL-3B) were used to localize *brittle rachis 2* (*Br-A2*) and *brittle rachis 3* (*Br-A3*), two major loci that control the brittle rachis character. Using the RICL-3A population, *Br-A2* was localized to a 10.9-cM region between *Xgwm2* and *Xbarc19* on chromosome 3A. Another factor in the RICL-3B population, *Br-A3* was localized to a 44.9-cM region between *Xbarc218* and *Xwmc540* on chromosome 3B. With respect to the free-threshing habit, a recombinant inbred line (RIL) population developed by the International Triticeae Mapping Initiative (ITMI) and F_2 progeny (CS/CS2D F_2) of a cross between Chinese Spring and a 2D2 substitution line [Chinese Spring (*tauschii* 2D)] were used. Quantitative trait mapping revealed that two QTL on chromosome 2D affected both threshability and glume tenacity in the ITMI population. The locus underlying one QTL was *tenacious glumes 1* (*Tg1*) that was localized to a 23-cM region flanked by *Xwmc25* and *Xgdm107*. The other QTL was localized near *Xgwm455* and the factor responsible for it, designated *tenacious glumes 3* (*Tg3*) was subsequently localized to an 11.3-cM interval between *Xbcd102* and *Xgwm455*. Two QTL also affected glume tenacity in the CS/CS2D F_2 population. One QTL corresponded to the QTL identified in the ITMI population. This QTL also represented the action of *Tg1* and was localized to a 20.8-cM interval between the markers *Xwmc503* and *Xbarc168*. The other QTL designated *Q.Gt.orst-2D.3* was near *Xgwm157*. The identities of *Br-A2*, *Br-A3*, *Tg1*, *Tg3*, and *Q.Gt.orst-2D.3* are not known but their localization on linkage maps represents a first step towards their eventual isolation and characterization.

Localization and Genetic Mapping of Some Factors Influencing Rachis Brittleness and Free-threshing Habit in Wheat

by

Vamsi J. Nalam

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented December 17, 2004
Commencement June 2005

Master of Science thesis of Vamsi J. Nalam presented on

December 17, 2004

APPROVED:

Redacted for Privacy

Major Professor, representing Crop Science

Redacted for Privacy

Head of the Department of Crop and Soil Science

Redacted for Privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Vamsi J. Nalam, Author

ACKNOWLEDGEMENTS

I would like to acknowledge the contributions of the following groups and individuals to the development of my thesis

I would like to express my deep gratitude to my major professor, Dr. Oscar Riera-Lizarazu for his efforts of guidance and aiding in the development of this research. I also wish to thank him for the inspiration and encouragement during my entire stay at Oregon State University.

I would also like to thank Christy Watson for all the assistance she provided and for the hours of editing. I also extend my thanks to my committee members Dr. Shawn Mehlenbacher, Dr. Pat Hayes and Dr. Jeffery McDonnell.

Additionally, I would like to thank my fellow graduate students Melanie Edwards, Jason Nunes, Harish Gandhi and Pradeep Tempalli for assisting me with my work and also for their good humor that helped me during the most challenging times. Acknowledgment is due to the College of Agricultural Sciences and Oregon State University for the facilities.

Finally, I would like to thank my family and Punya, who have been a pillar of support in my life. I would also to thank my friends who have been a source of constant encouragement and have supported me throughout this entire process.

CONTRIBUTION OF AUTHORS

Dr. Oscar Riera-Lizarazu proposed the subject of this research and was involved in all genetic, statistical analysis and writing each manuscript. Dr. Isabel Vales worked in the development and exploration of the subject and also helped in the genetic, statistical analysis and provided lab support and useful discussion. Christy J. W. Watson provided lab support, writing assistance and also useful discussion. Dr. Shahryar F. Kianian is a collaborator on the group 3 consortium and provided RFLP marker genotype data for the RICL-3A population.

TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LOCALIZATION AND GENETIC MAPPING OF SOME FACTORS INFLUENCING RACHIS BRITTLENESS AND FREE-THRESHING HABIT IN WHEAT

Chapter 1

Introduction

Wheat (*Triticum aestivum* L.), a cereal plant of the Triticeae tribe (family Gramineae), was domesticated in the Neolithic period (Harris, 1998; Zohary and Hopf, 1993) and is today a major food source and an important commodity on the world grain market. Wheat is grown in a wide range of environments around the world (208 million hectares) with an annual production exceeding 550 million metric tons (FAO Statistical Databases; http://apps.fao.org/). Approximately two-thirds of the wheat produced in the world is used for human food and about one-sixth is used for livestock feed. Industrial uses, seed requirements, and post-harvest losses account for the remaining withdrawals from the world's wheat granaries. Worldwide there are more foods made with wheat than any other cereal grain, and wheat contributes between 10-20% of the daily calorie intake of people in over 60 countries.

Besides being a highly important crop, wheat has also been the subject of intensive scientific research as a polyploid model (Heyne, 1987). The availability of substantial genetic, cytogenetic, and genomic resources including collections of aneuploid and chromosome deletion stocks (Sears, 1954, 1966; Nishikawa et al., 1992; Endo and Gill, 1996), chromosome substitution lines (Joppa, 1993), DNA-based

markers, genetic and physical maps (Hart, 2001), large DNA insert libraries (Lijavetzky et al., 1999; Liu et al., 2000), a large collection (63,000) of sequences for expressed genes (U.S. Wheat Genome Project - http://wheat.pw.usda.gov/NSF/), and a transcriptome array (http://affymetrix.com/products/arrays/specific/wheat.affx) have made wheat an ideal system to study the genetic basis of crop domestication.

Wheat Evolution

Cultivated wheats constitute an allopolyploid series with diploid ($2n$ = 14), tetraploid ($2n$ = 28), and hexaploid ($2n$ = 42) species. The general pathway of evolution of hexaploid wheat (*T. aestivum* L.) is understood (Figure 1.1, Kimber and Feldman, 1987 a, b). The first step was the hybridization between *Triticum urartu* Thumanjan ex Gandilian ($2n$ = 14, A^uA^u genome) and a species related to *Aegilops speltoides* Tausch ($2n$ = 14, SS genome). The donor of the B genome to durum wheat, *T. turgidum,* and hexaploid wheat, *T. aestivum,* has not been firmly established but various sources of evidence suggest *Ae. speltoides* is closely related to this species (Riley et al., 1958; Friebe and Gill, 1996; Kerby and Kuspira, 1988; Johnson, 1972; Witcombe, 1983; Dvorak and Zhang, 1990). In addition, plasmon analysis showed a close relationship between the cytoplasm of *Ae. speltoides* and polyploid wheats, suggesting that the B-genome donor served as the female parent in the formation of wheat (Tsunewaki and Ogihara, 1983). Whether a single species is the sole source of the B genome or the genome resulted from an introgression of several parental species remains uncertain.

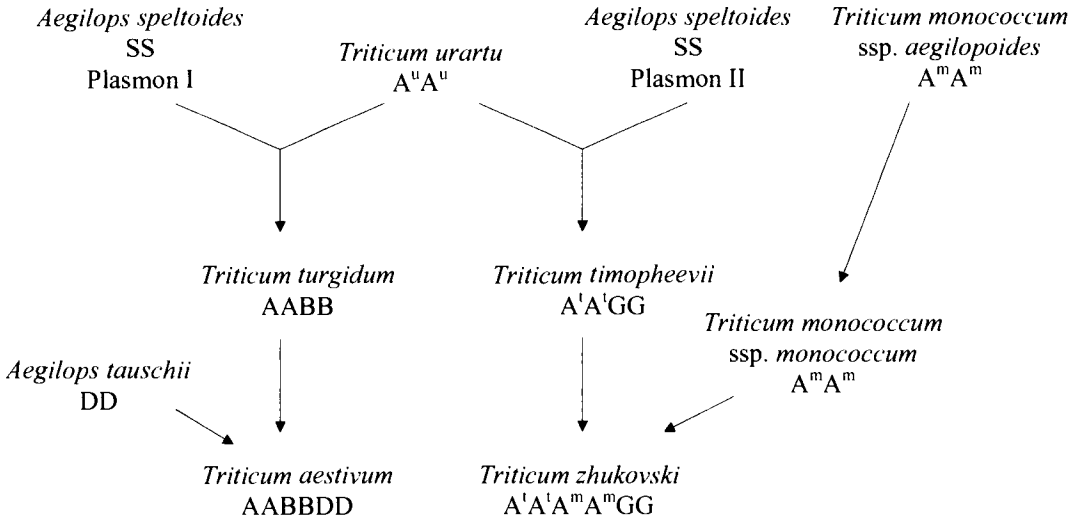


Figure 1.1 General pathway of evolution of hexaploid wheats (Kimber and Feldman, 1987 a, b).

The hybridization between *T. urartu* and a B-genome donor resulted in the formation of *T. turgidum* ssp. *dicoccoides* Koern. ($2n$ = 28, AABB genomes) 500,000 years ago (Figure 1.1). Several varietal groups are recognized in this species, of which a cultivated form, *T. turgidum* ssp. *dicoccum* Schuebl. is believed to be the most primitive type. This variety in turn gave rise to several cultivated forms. These groups are all inter-fertile and their differentiation is based on traits controlled by one or a few major genes (Mac Key, 1966; Morris and Sears, 1967). Shortly after the emergence of agriculture (~8,000 years ago), hexaploid wheat ($2n$ = 42) arose. Hexaploid wheat resulted from the hybridization between a cultivated tetraploid progenitor, probably *T. turgidum* ssp. *dicoccum,* and a diploid goatgrass, *Aegilops tauschii* Cosson ($2n$ = 14, DD genome), the source for the D genome (Kihara, 1944; McFadden and Sears, 1946). The initial product of this hybridization has been hypothesized to be what today is known as *T. aestivum* ssp. *spelta* or other hulled types of wheat, such as ssp. *macha* or

ssp. *vavilovii* (McFadden and Sears, 1946; Kerber and Rowland, 1974). Free-threshing forms, such as *T. aestivum* ssp. *aestivum*, ssp. *sphaerococcum,* and ssp. *compactum* are believed to be derived from the hulled or non-free-threshing wheats (Feldman, 2001).

Triticum monococcum L. ssp. *aegilopoides* ($2n = 14$, A^mA^m genome) is a close relative of *T. urartu* and the domesticated form is known as einkorn, *T. monococcum* ssp. *monococcum* (Feldman, 2001). There are two other cultivated species in the *Triticum* complex, tetraploid *T. timopheevii* Zhuk. (A^tA^tGG genomes) and hexaploid *T. zhukovskyi* Menabde & Ericz. ($A^tA^tA^mA^mGG$ genomes), that also formed through interspecific hybridization (Figure 1.1). These species are only grown in the Caucasus. Only *T. timopheevii* has a wild form that grows fairly widely in Southeast Asia (Kimber and Sears, 1987b).

Wheat Domestication

Wheats were among the first cereal crops to be domesticated. Based on archaeological studies, humans were cultivating wild emmer (*T. turgidum* ssp. *dicoccoides*) and wild einkorn (*T. monococcum* ssp. *aegilopoides*) 10,300 to 9,500 years ago. Domesticated forms (with non-brittle spikes) appeared between 9,500 to 7,500 years ago (Harris, 1998).

Domestication is the process where human intervention (selection) transforms wild forms into varieties that are more efficiently reared and utilized. Even primitive domesticated forms of wheat show the effect of domestication-driven selection. The most critical adaptive differences between wild and domesticated forms involve

changes in three principal morphological features that made wheat easier to harvest. These are seed size, ear rachis stiffness, and the ease with which the seed is released from its enclosing leaf-like structures (Davies and Hillman, 1992).

Seed Size

Wild forms (*T. monococcum* ssp. *aegilopoides* and *T. turgidum* ssp. *dicoccoides*) have significantly smaller seeds than their cultivated counterparts (*T. monococcum* ssp. *monococcum* and *T. turgidum* ssp. *durum*). Although research on the genetic basis of seed size differences between wild and domesticated wheats has been limited, an analysis of *T. turgidum* ssp. *dicoccoides* (wild) chromosome substitution lines in *T. turgidum* ssp. *durum* (cultivated) showed that kernel size was under polygenic control (Elias et al., 1996; Cantrell and Joppa, 1991). Genes that affected seed size were present on chromosomes 1A, 2A, 3A, 4A, 7A, 5B, and 7B.

Rachis Fragility

In wild wheats, the mature rachis disarticulates between each of the fertile spikelets, thereby allowing them to be shed spontaneously. By contrast, in some domesticated wheats, the rachis fails to disarticulate spontaneously, and the ear remains intact until harvested and threshed. The mechanism of rachis disarticulation involves the development of an abscission layer at the joint of articulation of the spikelet and rachis. This abscission layer collapses at maturity permitting the seed unit to fall (Harlan, 1992). Disarticulation occurs from the top of the ear downwards. The arrow-like morphology of the spikelets ensures that they quickly penetrate surface

litter and wedge themselves in cracks in the ground where at least a proportion of them are relatively safe from birds and rodents. In domesticated races, the formation of abscission layers in the rachis is suppressed or collapse of the rachis is delayed until harvest, resulting in spikes that fail to disarticulate spontaneously. If sown in the wild, domesticated forms cannot be perpetuated, as their spikelets are not efficiently disseminated and protected from predation (Davies and Hillman, 1992).

The first wheat to be successfully cultivated was einkorn, *T. monococcum* ssp. *monococcum* L. ($2n$ = 14, A^mA^m genome). Einkorn was domesticated from wild einkorn (*T. monococcum* ssp. *aegilopoides*). Einkorn differs from its wild progenitor mainly with respect to seed size and ear traits such as stiffness of the rachis. Wild diploid wheat has a brittle rachis, whereas einkorn has a tougher non-brittle rachis which prevents disarticulation of the spikelets. Rachis brittleness, in diploid wheats, was shown to be controlled by two genes in the F_2 progeny of crosses between *T. monococcum* ssp. *monococcum* × *T. monococcum* ssp. *aegilopoides* (Sharma and Waines, 1980).

Archaeological evidence suggests that the first tetraploid wheats that were cultivated had brittle spikes and were grown for several hundred years until mutants with tough rachis and non-brittle spikes appeared (Kislev, 1984). *T. turgidum* ssp. *dicoccum*, a primitive cultivated emmer was derived from wild tetraploid wheat, *T. turgidum* ssp. *dicoccoides*, after selection for the non-brittle rachis trait. Thus, the rachis of ssp. *dicoccum* is tougher and does not disarticulate, whereas the rachis of ssp. *dicoccoides* will disarticulate prior to harvest. The fragile rachis of *T. turgidum* ssp.

dicoccoides is controlled by two dominant genes (*Br-A2* and *Br-A3*) on chromosomes 3A and 3B, respectively (Watanabe and Ikebata, 2000).

There are no wild forms of hexaploid *T. aestivum*. Thus, varieties of *T. aestivum* are found only in cultivated fields. Hexaploid wheat contains several subspecies, which have distinct morphological characters. Of these, *T. aestivum* ssp. *sphaerococcum*, ssp. *compactum* and ssp. *vulgare* (common wheat) have a tough rachis and are free-threshing (Sears, 1946; Unrau, 1950), while ssp. *spelta*, ssp. *vavilovii* and ssp. *macha* have a fragile rachis and are not free-threshing (Kabarity, 1966). The pattern of disarticulation of the rachis of ssp. *spelta* wheat is different from that of ssp. *macha* and ssp. *vavilovii* wheat. The spikes of ssp. *spelta* disarticulate below the junction of the rachis and rachilla (barrel-type of disarticulation) and those of ssp. *macha* and ssp. *vavilovii* disarticulate above the junction of the rachis and the rachilla (wedge-type of disarticulation). The semi-wild wheat (SWW) discovered in Tibet (Shao et al., 1983), has a particularly fragile rachis and a wedge-type of disarticulation.

The brittle rachis phenotype of spelt wheat was initially thought to be tightly linked to the *Q* locus on chromosome 5A (Kuckuck, 1964). However, later studies suggest that this association might have been due to the segregation of multiple loci and pseudo-linkage (Luo et al., 2002). Based on numerous studies, the brittle rachis trait has been shown to be principally controlled by loci on group 3 homoeologous chromosomes. Cao et al. (1997) reported that rachis fragility in the SWW is controlled by a single dominant gene, *Br-A1*, located on the short arm of chromosome 3D (Chen et al., 1998). Similarly, the brittle rachis trait in tetraploid wheat is controlled by two

dominant genes, *Br-A2* and *Br-A3*, located on the short arms of chromosomes 3A and 3B (Watanabe and Ikebata, 2000; Watanabe et al., 2002).

The genetic basis of disarticulation-type (barrel versus wedge) was addressed in one study (Chen, 2001). In crosses between spelt, SWW, and common wheat, Chen (2001) determined that disarticulation type was governed by three dominant barrel modifying genes (*Bm*) and six dominant complementary wedge modifying genes (*Wm*) separate from the brittle rachis genes on homoeologous group 3 chromosomes. Thus, genotypes that have brittle rachis may exhibit various types of disarticulation based on the interactions between wedge- and barrel-type modifiers.

Free-threshing habit

Spikelets in wheat consist of florets which are surrounded by protective bracts called glumes. A floret is composed of two bracts (lemma and palea) that enclose three stamens and a carpel. After fertilization and seed development, each grain in a floret is surrounded by the lemma and the palea. The lemma, palea, and the outer glumes provide protection to the mature grain and allow its storage. When mature, wheat is harvested and threshed in order to separate the grain from these protective bracts (or chaff). The condition of the bracts after threshing defines the two major groups of wheats - hulled or non-free-threshing wheats and free-threshing wheats. In hulled wheats, spikelets separate from the spikes at threshing but their glumes and other bracts remain firmly attached. Additional mechanical action is required to release the grain from the chaff. This is in contrast to free-threshing wheats, where glumes and

other bracts surrounding the grains are loosely attached at maturity allowing the separation of seed from the chaff in one operation.

Free-threshing einkorn varieties are uncommon; however, a free-threshing einkorn line was discovered in 1970 in a collection of the botanist Petr M. Zhukovskii (Szabó and Hammer, 1995). This line was used to show that the free-threshing trait was inherited as a recessive allele of the *soft glumes* (*Sog*) locus. Taenzler et al. (2002) mapped this locus to chromosome 2A in a genomic position that has conserved synteny with the *tenacious glumes* (*Tg*) loci of polyploid wheats (*Tg1* on chromosome 2D; Jantasuriyarat et al., 2004 and *Tg2* on chromosome 2B; Simonetti et al., 1999).

Durum wheat, *T. turgidum* ssp. *durum* (Desf.) Husn., the principal tetraploid wheat cultivated today, has large grains and is free-threshing. Three major morphological characters that distinguish it from the primitive cultivated tetraploid, *T. turgidum* ssp. *dicoccum*, are nakedness or free-threshability, ear compactness and grain size. Earlier studies have shown that some of these differences are the result of pleiotropic effects of the *Q* locus on chromosome 5AL (Muramatsu, 1986). For instance, the dominant *Q* allele present in *T. turgidum* ssp. *durum* affects the free-threshing phenotypes by decreasing glume tenacity and spike morphology by increasing ear compactness (Muramatsu, 1986). When the free-threshing habit was studied in a *T. turgidum* ssp. *durum* × *T. turgidum* ssp. *dicoccoides* cross, four quantitative trait loci (QTL) on chromosomes 2B, 5A, and 6A were identified (Simonetti et al., 1999). The free-threshing character was predominantly affected by a QTL on chromosome 2BS, that corresponded to the *tenacious glumes 2* (*Tg2*) gene, and a QTL on chromosome 5AS (*Qft.mbg-5A*). The QTL on chromosome 5AL

(corresponding to *Q*) and 6AS (*Qft.mbg-6A*) were secondary. Thus, the control of the free-threshing trait was clearly polygenic involving known major genes (*Tg2* and *Q*) as well as factors not previously described.

Hexaploid wheat, *T. aestivum*, originated after the domestication of diploid and tetraploid wheats. Since there is no wild hexaploid progenitor to cultivated wheats, hexaploid wheat is thought to have formed by hybridization of a cultivated form of tetraploid wheat (*T. turgidum* ssp. *dicoccum* or ssp. *durum*) and *Ae. tauschii*. *T. aestivum* has been subdivided into several subspecies, some of which are non-free-threshing and some that are free-threshing. Other major differences among the major hexaploid taxa are mainly due to a few genes that affect gross morphology (Table 1.1). Non-free-threshing hexaploids are considered to be the predecessors of the free-threshing types like *T. aestivum* ssp. *aestivum*. Similar to the situation with tetraploid wheats, the free-threshing trait is controlled by multiple factors including *Q* on chromosome 5AL (McFadden and Sears, 1946). Kerber and Rowland (1974) found that the *tenacious glumes 1*, *Tg1*, gene on chromosome 2D also controlled the free-threshing phenotype in hexaploid wheats. Synthetic hexaploids that were produced by crossing free-threshing tetraploids with non-free threshing *Ae. tauschii* were non-free-threshing despite being homozygous for the *Q* allele. The suppression of the free-threshing character was attributed to a partially dominant *Tg1* allele on chromosome 2D of *Ae. tauschii*. Kerber and Rowland (1974) concluded that a dominant *Tg1* allele counteracted or inhibited the effect of the dominant *Q* allele leading to spikes with tenacious glumes and a non-free-threshing phenotype. Thus, the development of free-threshing hexaploid wheats also required a mutation from *Tg1* to *tg1* which is

presumed to have occurred at the hexaploid level. Sears' (1954) analysis of Chinese Spring aneuploids also suggested the presence of factors that affected glume tenacity on homoeologous group 2 chromosomes. Sears (1954) noted that plants missing (nullisomics) chromosomes 2A, 2B, or 2D had papery glumes while plants that were tetrasomic for these chromosomes had glumes that were stiffer than normal disomic plants.

Table 1.1 Genetic and phenotypic characterization of the various subspecies of hexaploid wheat, *T. aestivum*, for important domestication related traits.

Sub-Species	**Genotype***	**Phenotype**
spelta	*Tg1Tg1qqccSS*	Non-free-threshing; Normal (or Lax) spikes and grain
vavilovii	*Tg1Tg1QQccSS*	
macha	*tg1tg1qqccSS*	
aestivum	*tg1tg1QQccSS*	Free-threshing; normal spike and grains
compactum	*tg1tg1QQCCSS*	Free-threshing; compact spike and normal grains
sphaerococcum	*tg1tg1QQccss*	Free-threshing; normal spike and spherical grains

**Tg1* (Tenacious glumes) on chromosome 2DS (Kerber and Rowland, 1974); *Q* (free-threshing) on 5AL (Sears, 1954); *C* (compact spike) on 2DL (Rao, 1972); and *S* (spherical grain) on 3DS (Rao, 1977). Plants homozygous recessive at *Tg1* and homozygous dominant at *Q* are free-threhsing.

When the free-threshing habit was studied in a recombinant inbred line population developed from a cross between a spring wheat, Opata-85, and a synthetic hexaploid wheat, W-7984, two major QTL on chromosomes 2DS and 5AL were identified (Jantasuriyarat et al., 2004). QTL on chromosome 2DS was believed to

represent the effect of *Tg1* and the QTL on chromosome 5AL corresponded to *Q*. Free-threshing habit was found to be predominantly affected by *Tg1* and to a lesser extent by *Q*. Other QTL that were significantly associated with free-threshing habit were also localized on chromosomes 2A, 2B, 6A, 6D and 7B. Although, the exact sequence of events leading to the development of free-threshing hexaploid wheats is not known, the free-threshing phenotype of hexaploid wheat has been found to result from interactions between several genetic systems - the two major ones being *Q* on chromosome 5AL and the *tenacious glumes* loci on chromosomes 2A, 2B and 2D.

Objectives

The study of wheat domestication is not only of historical interest, but is also important as changing human needs and availability of non-renewable resources drive continuing investigation into new strategies to improve agronomic traits. New genomic tools applied in conjunction with other approaches will accelerate and streamline the identification of specific genes. In turn, characterization of genes involved in domestication and an understanding of their function may permit the development of strategies to enhance the striking changes in plant development that permitted the development of wheat into a crop.

As discussed earlier, modern wheats differ from their wild progenitors in a number of ways. Among the most important are differences in rachis fragility and seed dissemination. Wild wheats are characterized by brittle spikes that disarticulate upon maturity into arrow-shaped spikelets. Collection of these spikelets from the ground

would have proven to be difficult for early farmers. Therefore, types with non-brittle heads were unconsciously or consciously selected. Wild wheats also have non-threshable hulled grains. Thus, mutations that increased threshability were selected during wheat domestication. Today, free-threshing wheats with tough rachises represent the overwhelming majority of the wheat that is grown today.

Genetic mapping has contributed greatly to an understanding the mechanisms of domestication. The notion of using discrete traits as 'genetic markers' to determine the number, chromosomal locations, and phenotypic effects of genes that determine either simple or complex traits is nearly a century old (Sax, 1923). However, outside of a few favorable models, the comprehensive 'molecular dissection' of the genetic control of phenotypes only became feasible with the advent of DNA-based genetic markers in the late 1970s (Botstein et al., 1980). Application of such methods to plants has resulted in the development of detailed molecular maps for most of the world's major crops as well as selected wild relatives (Phillips and Vasil, 1994). In the early 1990s, scientists began constructing genetic linkage maps of the wheat genome using DNA markers (Nelson et al., 1995a, b, c; Röder et al., 1998). These molecular maps have provided detailed information regarding the structure of the wheat genome and have allowed researchers to determine positions of genes along chromosomes. For the study of domestication, a genome mapping approach is particularly efficient in crosses between the crop and a wild relative since the progeny will segregate for traits involved in domestication as well as for a large number of DNA polymorphisms. This has led to the genetic analysis of some traits that distinguish modern cultivated varieties from their wild ancestors (Salamini et al., 2002).

The brittle rachis trait is primarily controlled by genes (*Br-A1*, *Br-A2* and *Br-A3*) on homoeologous group 3 chromosomes (3A, 3B and 3D) (Chen et al., 1998; Watanabe and Ikebata, 2000; Watanabe 2002) but they have not been assigned a precise location on current linkage maps. The free-threshing phenotype of hexaploid wheat results from interactions between two genetic systems – *Q* on chromosome 5AL and the *tenacious glumes* loci on group 2 chromosomes (Sears, 1954; Simonetti et al., 1999; Taenzler et al., 2002; Jantasuriyarat et al., 2004). *Q* has been the subject of intense research leading to its precise localization and isolation (Faris et al., 2003). On the other hand, studies on the tenacious glumes loci that primarily affect glume tenacity and threshability have been sparse. In an effort to apply map-based methods to fill some of the gaps in our understanding on the genetic basis of these domestication related traits in wheat, the following objectives were addressed in this thesis:

i. To develop linkage maps of chromosomes 3A and 3B of tetraploid wheat,
ii. To genetically map genes for the brittle rachis character, *Br-A2* and *Br-A3*,
iii. To generate microsatellite-based linkage maps of the short arm of chromosome 2D of hexaploid wheat, and
iv. To localize factors affecting glume tenacity and free-threshing character on chromosome 2D.

Objectives (i.) and (ii.) are addressed in chapter 2 of this thesis, while objectives (iii.) and (iv.) are present in chapter 3. Conclusions are presented in chapter 4.

Chapter 2

GENETIC LOCALIZATION OF GENES AFFECTING THE BRITTLE RACHIS CHARACTER IN TETRAPLOID WHEAT (*Triticum turgidum* L.)

Vamsi J. Nalam, Christy J.W. Watson, M. Isabel Vales, Shahryar F. Kianian and Oscar Riera-Lizarazu

Abstract

Domesticated plants are characterized by a set of traits that confer adaptation to an agricultural environment. The transition from wild to domesticated forms of tetraploid wheat entailed changes in ear rachis stiffness that made the crop easier to harvest. In wild wheats, the mature rachis disarticulates between each of the fertile spikelets, thereby allowing them to be shed spontaneously. By contrast, in domesticated wheats, the rachis fails to disarticulate spontaneously, and the ear remains intact until harvested and threshed. The brittle rachis trait in tetraploid wheat (*Triticum turgidum* L.) is primarily affected by two genes, *brittle rachis 2* (*Br-A2*) and *brittle rachis 3* (*Br-A3*) on chromosomes 3A and 3B, respectively. In this study, two populations of recombinant inbred chromosome lines (RICL), derived from crosses between Langdon and disomic *T. dicoccoides* 3A [Langdon (Dic-3A)] and *T. dicoccoides* 3B [Langdon (Dic-3B)] chromosome substitution lines were used to localize *Br-A2* and *Br-A3* on microsatellite-marker based linkage maps of chromosomes 3A and 3B. *Br-A2* was localized to a 10.9-cM region between *Xgwm2* and *Xbarc19* on chromosome 3A while *Br-A3* was localized to a 44.9-cM region between *Xbarc218* and *Xwmc540* on chromosome 3B. Deletion bin mapping and comparative analyses indicated that *Br-A2* and *Br-A3* were located on a chromosomal segment with an estimated frequency of recombination of 580 Kb/cM. These estimates indicate that cloning *Br-A2* and *Br-A3* using map-based methods will be extremely challenging.

Introduction

The genus *Triticum* has species of various ploidy and chromosome numbers namely, diploid ($2n = 14$), tetraploid ($2n = 28$) and hexaploid ($2n = 42$). Wheats of all ploidy levels have been domesticated. Wild as well as domesticated forms occur in both the diploid and tetraploid groups, whereas only domesticated types occur in the hexaploid group. Even the most primitive domesticated forms of wheat differ from their wild progenitors in a number of polygenically determined characters. The most notable adaptive differences involve changes in three principal morphological features that make the crop easier to harvest: ear rachis stiffness, and the ease with which the seed is released from its enclosing leaf-like structures (Davies and Hillman, 1992).

In wild wheats, the mature rachis disarticulates between each of the fertile spikelets, thereby allowing them to be shed spontaneously. By contrast, in some domesticated wheats, the rachis fails to disarticulate at maturity, and the ear remains intact until harvested and threshed. In grasses, the mechanism of rachis disarticulation involves the development of an abscission layer at the joint of articulation of the spikelet and rachis followed by a collapse at maturity permitting the seed unit (diaspore) to fall. In domesticated races, the formation of an abscission layer is suppressed or collapse is delayed until harvest (Harlan, 1992).

The first wheat to be cultivated successfully was einkorn (*Triticum monococcum* L, $2n = 14$, A^mA^m genome), a diploid species. Diploid wheats *T. monococcum*, and its wild relatives, *T. monococcum* ssp. *aegilopoides* and *T. urartu* Thumanjan ex Gandilian, have brittle rachises. The rachis of *T. monococcum* can be

less brittle (semi-fragile) than other wild species and may not disarticulate prior to harvest, but when fully ripe it will disarticulate. Sharma and Waines (1980) showed that two dominant genes determined the brittle rachis character in *T. monococcum*. A similar situation to that of diploid wheats exists in tetraploid wheats. Wild emmer (*T. turgidum* ssp. *dicoccoides* Koern.) has a brittle rachis and cultivated emmer (*T. turgidum* ssp. *dicoccum* Scheubl.) has a tougher or non-brittle rachis. Watanabe and Ikebata (2000) reported that brittle or fragile rachis in ssp. *dicoccoides* is controlled by two dominant genes, *Br-A2* and *Br-A3*, located on chromosomes 3A and 3B, respectively.

Hexaploid wheat contains several subspecies, which have distinct morphological characters. Of these, *T. aestivum* ssp. *sphaerococcum*, ssp. *compactum* and ssp. *vulgare* or common wheat have a tough rachis and are free-threshing (Sears, 1946; Unrau, 1950), while ssp. *spelta*, ssp. *vavilovii* and ssp. *macha* wheat have a fragile rachis and are not free-threshing (Kabarity, 1966). The pattern of disarticulation of the rachis of spelt (ssp. s*pelta*) wheat is different from that of ssp. *macha* and ssp. *vavilovii*. The spikes of spelt wheat disarticulate below the junction of the rachis and rachilla (barrel-type of disarticulation) and those of ssp. *macha* and ssp. *vavilovii* disarticulate above the junction of the rachis and the rachilla (wedge-type of disarticulation). Another hexaploid, the semi-wild wheat (SWW) discovered in Tibet (Shao et al., 1983) has a particularly fragile rachis which exhibits a wedge type of disarticulation (Chen, 2001).

The brittle rachis phenotype of spelt wheat was initially thought to be due to a locus tightly linked to the *Q* locus on chromosome 5A (Kuckuck, 1964). However,

later studies suggest that the association between the brittle rachis trait and *Q* may be due to multiplicate gene segregation (Luo et al., 2002). A number of studies have shown that brittle rachis is primarily controlled by loci on group 3 homoeologous chromosomes (Cao et al., 1997; Watanabe and Ikebata, 2000). Cao et al. (1997) reported that rachis fragility in the SWW is controlled by a single dominant gene, *Br-A1*, located on the short arm of chromosome 3D (Chen et al., 1998). Furthermore, genetic studies of crosses between spelt, SWW, and common wheat indicated that disarticulation type was governed by several modifying genes (wedge modifying genes, *Wm*, and barrel modifying genes, *Bm*) separate from the brittle rachis gene, *Br-A1* (Chen, 2001). Therefore, genotypes with a brittle rachis may exhibit various types of disarticulation depending on interactions between modifying genes and the genetic background.

Watanabe et al. (2002), using comparative telosomic mapping, localized *Br-A1*, *Br-A2,* and *Br-A3* to the short arms of chromosomes 3D, 3A and 3B, respectively. Still, the precise location of these loci with respect to DNA-based markers in current linkage maps has not been determined. Thus, the objectives of this study were (i.) to develop microsatellite marker-based linkage maps of chromosomes 3A and 3B, and (ii.) to genetically map the brittle rachis genes, *Br-A2* and *Br-A3*, as a first step towards their map-based isolation and characterization.

Materials and Methods

Plant Material

The localization of *Br-A2* and *Br-A3* was performed using two mapping populations. The first mapping population (RICL-3A) consisted of 83 recombinant inbred chromosome lines (RICL) from a cross between Langdon-16 (LDN) and a substitution line, Langdon (*dicoccoides* 3A) [LDN(Dic-3A)] (Joppa 1993). The second mapping population (RICL-3B) consisted of 91 RICL lines developed from a cross between LDN and Langdon (*dicoccoides* 3B) [LDN (Dic-3B)] substitution line (Joppa 1993). The seeds for the parents and the RICL populations were kindly provided by Dr. Justin Faris (USDA-ARS, Fargo, North Dakota). Both populations were planted at West Greenhouse, Oregon State University, in 2004.

Group 3 cytogenetic stocks were used to assign markers to chromosomes and chromosome segments. These stocks included Chinese Spring nullisomic-tetrasomics (N3AT3B, N3AT3D, N3BT3D, N3DT3A and N3DT3B), ditelosomics (Dt3AS, Dt3AL, Dt3BS and Dt3AL), four deletion lines for chromosome 3A (3AS-2, 3AS-4, 3AL-3 and 3AL-5) and four deletion lines for chromosome 3B (3BS-1, 3BS-8, 3BS-9 and 3BL-1). The deletion lines used for chromosome 3A divided the short and the long arm into three distinct bins each. The deletion lines used for chromosome 3B divided the short arm into four bins and the long arm into two bins. The chromosome karyotypes detailing the breakpoints of chromosome 3A and 3B deletion lines can be found at http://wheat.pw.usda.gov/west/binmaps/wheat3_rice.html (date verified: 16th

November, 2004). The Chinese Spring aneuploids were obtained from Dr. B. S. Gill (Kansas State University, Manhattan, Kansas).

DNA isolation and microsatellite marker analysis

About 30 to 50 mg of leaf tissue from lines from the RICL-3A and RICL-3B populations and the parental lines were used for DNA extraction. DNA was extracted using a Qiagen/Retsch MM300 mixer mill (Qiagen Inc, Valencia, CA) as described by Riera-Lizarazu et al. (2000). Microsatellite markers mapped on chromosome 3A and chromosome 3B from different research groups (Nelson et al., 1995; Röder et al., 1998; Pestova et al., 2000; Somers et al., 2004) were used in this study. Of 52 microsatellites that were screened on LDN and LDN(Dic-3A), 22 were found to be polymorphic and were used to genotype the RICL-3A population. RFLP marker genotypes previously used in this population were also used (Otto et al., 2002). Of the 86 microsatellites screened on LDN and LDN(Dic-3B), 33 were polymorphic and used to genotype the RICL-3B population.

Polymerase chain reaction (PCR) amplification of the microsatellite markers was performed in a volume of 10 μL in a MWG Thermalcycler (Primus 96 Plus). The reaction mixture contained 0.5 μM of each primer, 0.2 mM of each deoxynuleotide, 0.03 U/μL Taq DNA Polymerase (Qiagen), 1X Taq buffer from Qiagen, 2% sucrose in cresol red and 50 ng of template DNA. After 5 min at 94°C, 45 cycles were performed with 30s at 94°C, 30s at 50, 55 or 60°C (depending on the individual primer set) and 30s at 72°C, followed by a final extension step of 10 min at 72°C. Products were screened on 4% agarose gels and visualized after staining with ethidium bromide.

Phenotypic Assessment

Before evaluation, mature spikes of plants from both RICL populations and their parents were dried at 54°C for three days. Subsequently, spikes with good seed fill were dropped from a height of 1.5 m. Spikes that disarticulated on impact were classified as brittle and spikes that failed to disarticulate were classified as having a tough rachis. Three observers independently assessed rachis fragility of different spikes in the same populations. Chi-square (χ^2) analyses were used to test genotype frequencies.

Map Construction

Linkage maps were constructed using Mapmaker/Exp 3.0 (Lander et al., 1987). Genotypic data for the RICL populations were encoded as F_2 backcross populations since Mapmaker/Exp 3.0 has not been implemented to analyze doubled-haploid or RICL-type populations. The group command with a minimum LOD of 3.0 and a maximum distance of 50-cM was used to identify linked markers. Subsequently, the order command (LOD 3.0) was used to build maps. Finally, the ripple command was used to verify map orders. Recombination fractions were converted into map distances (cM) using the Kosambi mapping function.

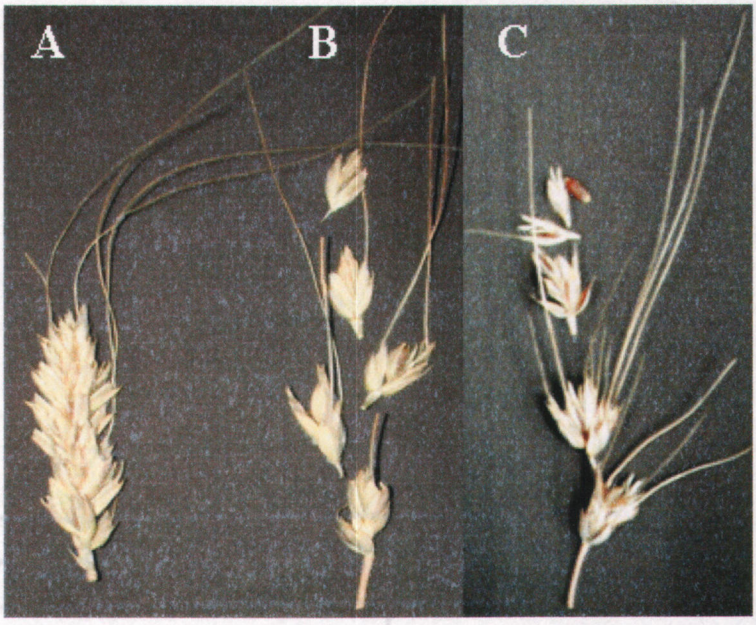


Figure 2.1 Pattern of spike disarticulation observed in Langdon 16 (A), Langdon (Dic-3A) (B) and Langdon (Dic-3B) (C).

Results

The rachis of LDN was non-brittle and did not disarticulate even after application of mechanical pressure. The spikes of LDN(Dic-3A) and LDN(Dic-3B), on the other hand, were brittle and disarticulated with the slightest mechanical action (Figure 2.1 A). The spikes of LDN(Dic-3A) and LDN(Dic-3B), disarticulated at the node above the insertion point of the spikelet, thus creating a wedge-shaped spikelet attached to a subtending rachis internode (Figure 2.1 B and C). The pattern of disarticulation observed in LDN(Dic-3A) and LDN(Dic-3B) were similar to that observed in wild emmer, *T. turgidum* ssp. *dicoccoides*. The segregation ratios of brittle to non-brittle rachis in both RICL populations differed significantly from a 1:1 ratio (Table 2.1). There was an excess of individuals with a tough rachis in both populations.

Table 2.1 Segregation for brittle rachis in the RICL populations

RICL Population	Number of Plants		N[a]	χ^2 (1:1)[b]
	Tough	Brittle		
RICL-3A	51	30	81	5.44 *
RICL-3B	57	27	84	10.71 **

[a] N = Population Size (2 missing data points in RICL-3A and 7 missing data points in RICL-3B)
[b] Chi-square values testing for a 1:1 segregation of tough vs. brittle rachis.
* significant at p = 0.05
** significant at p = 0.01.

Localization of the Br-A2 and Br-A3

Genetic linkage analysis of the RICL-3A population resulted in a genetic map for chromosome 3A that was 179.5 cM in length (Figure 2.2 A). The segregation data for the microsatellite markers along with the classification of individuals as tough or brittle are provided in Appendix 1. The average distance between markers in the linkage map for chromosome 3A was 5.98 cM. The largest interval in the map was 21.5 cM between *Xbarc294* and the linked markers *Xbarc310* and *Xbarc12*, at the telomeric end of chromosome 3AS. *Br-A2* was mapped to a 10.9-cM interval between *Xgwm2* and *Xbarc19* (linked to *Xgwm666.1*) (Figure 2.2 A).

The linkage map of chromosome 3B was constructed using 33 loci. The length of the map was 218.1 cM with an average distance of 6.6 cM between markers (Figure 2.3 A). The largest interval was 30.8 cM between *Xbarc218* and *Br-A3*. *Br-A3* was localized to a 44.9-cM interval between *Xbarc218* and *Xwmc540*. The length of the

linkage maps of chromosomes 3A and 3B and marker orders were comparable to other published maps (Nelson et al., 1995; Somers et al., 2004).

Group 3 cytogenetic stocks were used to place markers into bins on the short arm of chromosome 3A (Figure 2.2 B). Markers associated with *Br-A2* were placed in the most distal bin 3AS4-0.45-1.00. Markers associated with *Br-A3* (*Xbarc218* and *Xwmc540*) were placed into deletion bin 3BS-9 0.57-0.78 (Figure 2.3 B). Comparative mapping analyses with the consensus map of homoeologous group 3 chromosomes described by Erayman et al. (2004) indicate that *Br-A2* and *Br-A3* are localized in the gene rich region (GRR) termed 3S0.8 which is delimited by the deletions 3AS-3(0.71) – 3BS-3(0.87).

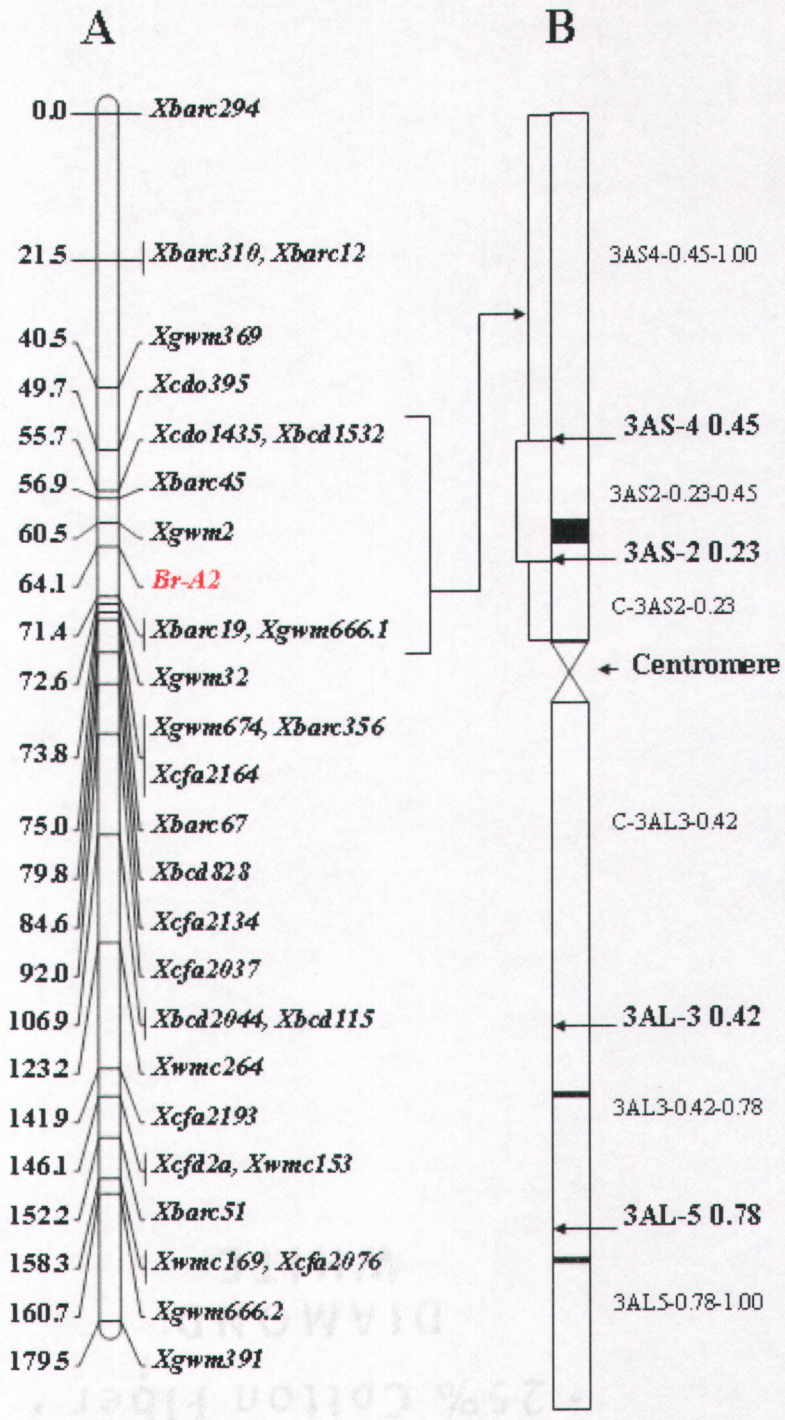


Figure 2.2 **A.** Genetic linkage map of chromosome 3A, developed using the RICL-3A population, showing the location of *Br-A2*. Genetic distances are given in Kosambi cM. **B.** Deletion bin map of chromosome 3A. Deletion breakpoints are indicated by arrows. Dark bands on the chromosome indicate the pattern of C-banding (Gill et al., 1991).

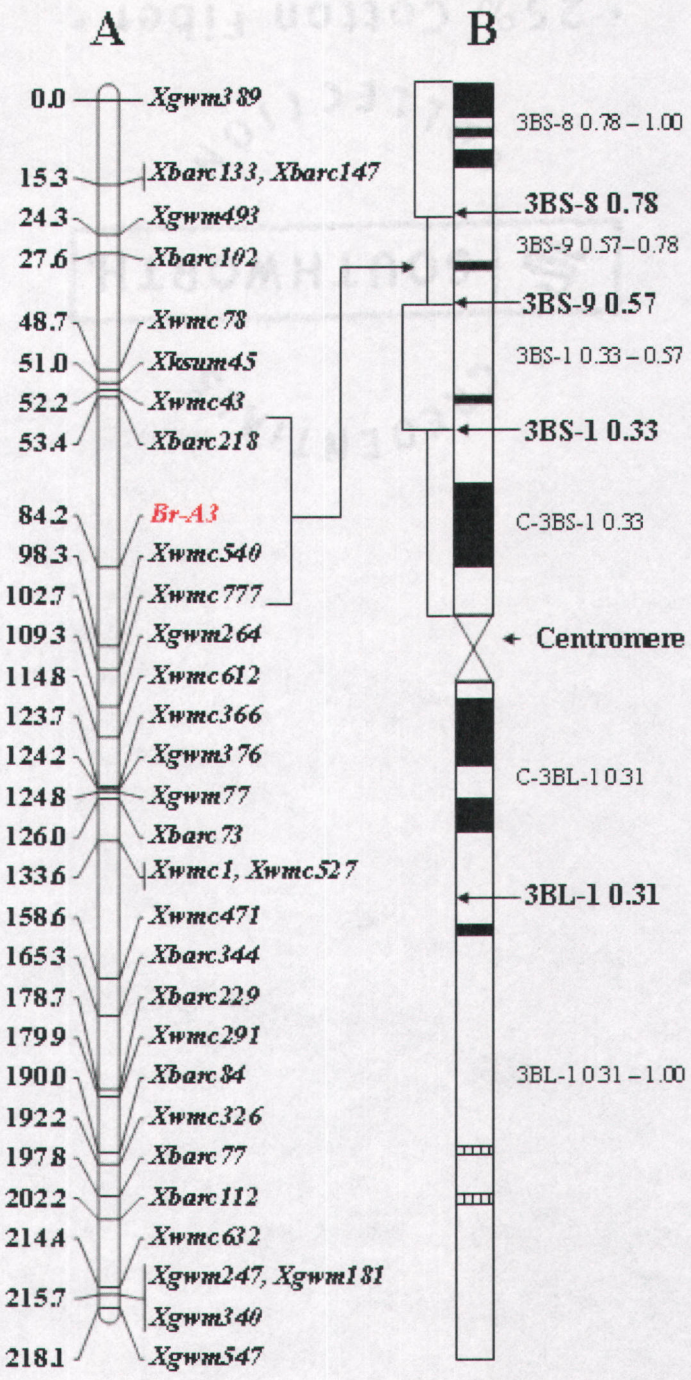


Figure 2.3 **A.** Genetic linkage map of chromosome 3B, developed using the RICL-3B population, showing the location of *Br-A3*. Genetic distances are given in Kosambi cM. **B.** Deletion bin map of chromosome 3B. Deletion breakpoints are indicated by arrows. Dark and stippled bands on the chromosome indicate the pattern of C-banding (Gill et al., 1991).

Discussion

Two mapping populations (RICL-3A and RICL-3B) were used to localize *Br-A2* and *Br-A3* on chromosome 3A and 3B of tetraploid wheat (*T. turgidum* L). The *Br-A2* locus was localized between *Xgwm2* and *Xbarc19* on chromosome 3A and *Br*-A3 was mapped to the *Xbarc218* - *Xwmc540* interval on the short arm of chromosome 3B. These results are consistent with the reported chromosomal locations of these genes (Watanabe et al., 2002).

In barley, the brittle rachis character is controlled by two complementary genes at two tightly linked loci, *btr1* and *btr2*, located on chromosome 3HS (Takahasi and Hayashi, 1964) with brittle rachis being dominant to non-brittle rachis. Komatsuda et al. (2002) mapped *btr1* on linkage maps of barley chromosome 3H. Chromosome 3H of barley has been shown to be syntenic with group 3 chromosomes of wheat (Smilde et al., 2001). Map comparisons suggest that *btr1* is homoeologous to the brittle rachis loci in wheat (Figure 2.4). On chromosome 3A, *Br-A2* is flanked by the RFLP markers *Xcdo1435* and *Xbcd828*. The distance between these markers is 11.7 cM. These two markers span a 23-cM interval in the barley consensus map for chromosome 3H (Qi et al., 1996). Map comparisons suggested that *btr1* is located in bin 5 or bin 6 in the bin map of barley (Figure 2.4 E).

Several other reports conclusively show that brittle rachis is controlled by genes on homoeologous group 3 chromosomes of wheat (Chen et al., 1998; Chen 2001, Watanabe and Ikebata, 2000; Watanabe, 2002), as well as that of other *Triticeae* such as chromosome $3S^b$ of *Aegilops bicornis* (Riley et al., 1966; Urbano et al., 1988), 3V

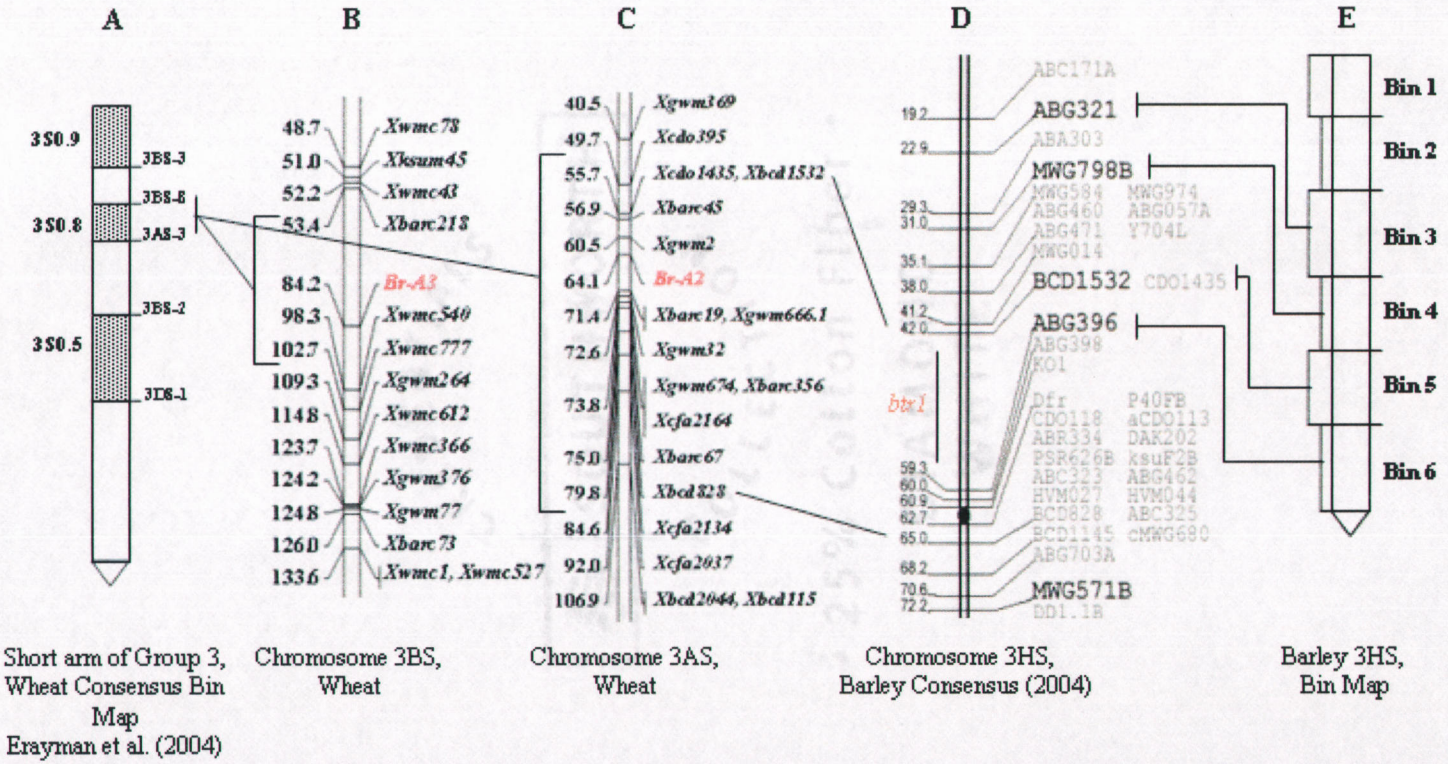


Figure 2.4 Homoeologous genomic regions affecting brittle rachis character in wheat and barley. Marker synteny is conserved in the region where the genes for brittle rachis in wheat, *Br-A3, Br-A2* and the genes for brittle rachis in barley, *btr1* are present. **A.** Consensus bin map for homoeologous group 3 chromosomes of wheat with the names of the GRRs on the left and the deletion lines flanking them on the left of the chromosome. **B.** Genetic linkage map of the short arm of chromosome 3B containing *Br-A3*. **C.** Genetic linkage map of the short arm of chromosome 3A containing *Br-A2*. **D.** Consensus genetic linkage map of barley chromosome 3HS containing the gene for brittle rachis, *btr1* (Komatsuda et al., 2002; Qi et al., 1996). **E.** Physical Bin map of barley chromosome 3HS (http://barleygenomics.wsu.edu/databases/databases.html).

in *Dasypyrum villosum*, $3S^l$ of *Ae. sharonensis* and $3S^l$ of *Ae. longissima* (Urbano et al., 1988). Miller et al. (1995) demonstrated that chromosome 3N of *Ae. uniaristata* induced brittle rachis in the CS/3N addition lines as well as substitution lines in which 3N replaces wheat chromosome 3A, 3B or 3D. King et al. (1997) found that the genes responsible for brittle rachis in x *Tritipyrum* were located on chromosome $3E^b$ of *Thinopyrum bessarabicum*. Furthermore, homoeologous group 3 chromosomes of wheat and chromosome 3H of barley have been shown to have conserved synteny with rice chromosome 1 (Smilde et al., 2001; Sorrells et al., 2003; Munkvold et al., 2004), also known to contain genes/factors for shattering (Cai and Morishima, 2000) and other genes/factors controlling domestication related traits (Xiong et al., 1999).

In stark contrast with the above discussion, a recent study by Peng et al. (2003) presented evidence that the brittle rachis trait in a F_2 mapping population from a cross between *T. turgidum* ssp. *durum* × *T. turgidum* ssp. *dicoccoides* was controlled by a factor localized to a terminal location of the linkage map of the long arm of chromosome 2A. We suspect that this inconsistency with other findings and ours is due to the fact that individuals were classified into discrete classes (brittle vs. non-brittle) and these scores were then used for linkage analysis. If the brittle rachis is controlled by two unlinked dominant genes (on chromosomes 3A and 3B) in *T. turgidum* ssp. *dicoccoides*, as the literature suggests, it would be impossible to find the true location for these factors if qualitative scores were used for mapping. Thus, it seems plausible that the location on chromosome 2A is a result of duplicate gene segregation coupled with pseudo-linkage reported in this population.

Deletion mapping placed *Br-A2* and *Br-A3* into the bins 3AS4-0.45-1.00 and 3BS-9 0.57-0.78, respectively. Comparative mapping analyses with the consensus physical maps of homoeologous group 3 chromosomes described by Erayman et al. (2004) indicate that *Br-A2* and *Br-A3* are localized in a minor gene rich region (GRR) termed 3S0.8 (Figure 2.4 A). This GRR is delimited by the deletions 3AS-3(0.71) – 3BS-3(0.87). GRR 3S0.8 has a physical size of 25 Mb and contains ~31% of the genes present the short arm of this chromosome. GRR 3S0.8 also accounts for 39% of recombination and the estimated frequency of recombination is estimated to be 580 Kb/cM. These estimates indicate that cloning *Br-A2* and *Br-A3* using map-based methods will be extremely challenging.

References

Cai H W and Morishima H (2000) Genomic regions affecting seed shattering and seed dormancy in rice. Theor Appl Genet 100:840-846.

Cao W, Scoles G J and Hucl P (1997) The genetics of rachis fragility and glume tenacity in semi-wild wheat. Euphytica 94:119-124.

Chen Q-F (2001) Inheritance of disarticulation derived from some hexaploid brittle rachis wheat. Genet Res Crop Evol 48:21-25.

Chen Q-F, Yen C and Yang J-L (1998) Chromosome location of the gene for brittle rachis in the Tibetan weedrace of common wheat. Genet Res Crop Evol 45: 21-25.

Davies M S and Hillman G C (1992) Domestication of cereals. In Chapman G C (ed) Grass evolution and domestication, University press, Cambridge, pp 199-224.

Erayman M, Sandhu D, Sidhu D, Dilbirligi M, Baenziger P S and Gill K S (2004) Demarcating gene-rich regions of the wheat genome. Nuc Acid Res 32(12): 3546-3565.

Gill B S, Friebe B and Endo T R (1991) Standard karyotype and nomenclature system for description of chromosome bands and aberrations in wheat (*Triticum aestivum*). Genome 34: 830-839.

Harlan J R (1992) Origins and processes of domestication. In: Chapman G P (Ed), Grass evolution and domestication. University Press, Cambridge, pp 159-175.

Joppa L R (1993) Chromosome engineering in tetraploid wheat. Crop Sci 33: 908-913.

Kabarity A (1966) On the origin of the new cultivated wheat II. Cytogenetical studies on the karyotypes of some *Triticum macha* varieties. Beitr Biol Pflanzen 42:339-346.

King I P, Law C N, Cant K A, Orford S E, Reader S M and Miller T E (1997) *Tritipyrum* a potential new salt-tolerant cereal. Plant Breed 116:127-132.

Komatsuda T and Mano Y (2002). Molecular mapping of the intermedium spike-c (*int-c*) and non-brittle rachis 1 (*btr1*) loci in barley (*Hordeum vulgare* L.). Theor Appl Genet 105: 85-90.

Kuckuck H (1964) Experimentelle Untersuchungen zur Entstehung der Kulturweizen. Z Pflanzenzuchtg 51:97-140.

Lander E S, Green P, Abrahamson J, Barlow A, Daly M J, Lincoln S E, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic maps of experimental and natural populations. Genomics 1: 174-181.

Luo M C, Yang Z L and Dvorak J (2000) The *Q* locus of Iranian and European spelt wheat. Theor Appl Genet 100:601-606.

Miller T E, Reader S M, Mahmood A, Purdie K A and King I P (1995) Chromosome 3N of *Aegilops uniristata* – a source of tolerance to high levels of aluminum for wheat. In: Proc. 8th Int. Wheat Genet Symp 1993, Z S Li and Z Y Xin (Eds), China Agricultural Scientech Press, Beijing, China: 1037-1042.

Munkvold J D, Greene R A, Bermudez-Kandianis C E, La Rota C M, Edwards H, Sorrells S F, Dake T, Benscher D, Kantety R, Linkiewicz A M, Dubcovsky J, Akhunov ED, Dvorak J, Miftahudin, Gustafson J P, Pathan M S, Nguyen H T, Matthews DE, Chao S, Lazo G R, Hummel D D, Anderson O D, Anderson J A, Gonzalez-Hernandez J L, Peng J H, Lapitan N, Qi L L, Echalier B, Gill B S, Hossain K G, Kalavacharla V, Kianian S F, Sandhu D, Erayman M, Gill K S, McGuire P E, Qualset C O, Sorrells M E (2004) Group 3 chromosome bin maps of wheat and their relationship to rice chromosome 1. Genetics 168:639-650.

Nelson J C, Van Deynze A E, Autrique E, Sorrells M E, Lu Y H, Negre S, Bernard M, Leroy P (1995) Molecular mapping of wheat homoeologous group 3. Genome 38:525-533.

Otto C D, Kianian S F, Elias E M, Stack R W and Joppa L R (2002) Genetic dissection of a major Fusarium head blight QTL in tetraploid wheat. Plant Mol. Biol. 48:625-632.

Peng J, Ronin Y, Fhima T, Röder M S, Li Y, Nevo E and Korol A (2003) Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. Proc Natl Acad Sci 100: 2489-2494.

Pestsova E, Ganal M W, Röder M S (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689-697.

Qi X, Stam P, Lindhout P (1996) Comparison and integration of four barley genetic maps. Genome 39: 379-394.

Riera-Lizarazu O, Vales M I, Ananiev E V, Rines H W and Phillips R L (2000) Production and characterization of maize chromosome 9 radiation hybrids derived from an oat-maize addition line. Genetics 156:327-339.

Riley R G, Kumber G and Law C N (1966) Correspondence between wheat and alien chromosomes. Ann Rep Plant Breed Inst 1964-65:108-109.

Röder M S, Korzun V, Wendehake K, Plaschke J, Tixier M H, Leroy P, Ganal M W (1998) A microsatellite map of wheat. Genetics 149:2007-2023.

Sears E R (1946) The *sphaerococcum* gene in wheat. Rec Genet Soc Amer 15:65-66.

Shao Q, Li C and Basang C (1983) Semi-wild wheat from Xizang (Tibet). Proc 6th Intern Wheat Genet Symp Kyoto, Japan 111-114.

Sharma, H C and Waines, J G (1980) Inheritance of tough rachis in crosses of *Triticum monococcum* and *T. boeticum.* J. Hered. 71: 214 - 216.

Smilde W D, Haluśkova J, Sasaki T and Graner (2001) New evidence for the synteny of rice chromosome 1 and barley chromosome 3H from rice expressed sequence tags. Genome 44:361-467.

Somers D J, Issac P and Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109: 1105-1114.

Sorrells M E, La Rota M, Bermudez-Kandianis C E, Greene R A, Kantety R, Munkvold J D, Miftahudin, Mahmoud A, Ma X, Gustafson P J, Qi L L, Echalier B, Gill BS, Matthews DE, Lazo GR, Chao S, Anderson OD, Edwards H, Linkiewicz A M, Dubcovsky J, Akhunov E D, Dvorak J, Zhang D, Nguyen HT, Peng J, Lapitan N L, Gonzalez-Hernandez J L, Anderson J A, Hossain K, Kalavacharla V, Kianian S F, Choi D W, Close T J, Dilbirligi M, Gill KS, Steber C, Walker-Simmons M K, McGuire P E, Qualset CO (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818-1827.

Takahashi R and Hayashi J (1964) Linkage study of two complementary genes for brittle rachis in barley. Ber Ohara Inst Landw Biol, Okayama Univ. 12:99-105.

Unrau J (1950) The use of monosomic and nullisomics in cytogenetic studies of common wheat. Sci Agr 30:66-89

Urbano M, Rest P, Benedettelli S and Blanco A (1988) A *Dasypyrum villosum* (L.) Candargy chromosome related to homoeologous group 3 of wheat. In. T E Miller and R M D Koebner 9Eds), Proc 7th Internatl Wheat Genet Symp: 169-173. IPSR Cambridge Lab, Cambridge, UK.

Watanabe N, Sugiyama K, Yamagashi Y and Sakata Y (2002) Comparative telosomic mapping of homoeologous genes for brittle rachis in tetraploid and hexaploid wheats. Hereditas 137:180-185.

Watanabe N and Ikebata, N (2000) The effects of homoeologous group 3 chromosomes on grain color dependent seed dormancy and brittle rachis in tetraploid wheat. Euphytica 115: 215-220.

Xiong L Z, Liu K D, Dai X K, Xu C G and Zhang Q (1999) Identification of genetic factors controlling domestication-related traits of rice using a F_2 population of a cross between *Oryza sativa* and *O. rufipogon*. Theor Appl Genet 98:243-251.

Chapter 3

LOCALIZATION OF FACTORS AFFECTING GLUME TENACITY AND THE FREE-THRESHING CHARACTER ON CHROMOSOME 2D OF COMMON WHEAT (*Triticum aestivum* L.)

Vamsi J. Nalam, M. Isabel Vales, Christy J.W. Watson, and Oscar Riera-Lizarazu

Abstract

During the domestication of bread wheat (*Triticum aestivum* L.), modifications in seed dispersal occurred that enhanced its adaptability to agricultural conditions. Of these modifications, one that has been investigated because of its evolutionary significance and its importance in the practical utilization of the wheat grain is the free-threshing habit. In this study, we mapped and characterized quantitative trait loci (QTL) affecting the free-threshing habit on chromosome 2D in a recombinant inbred line (RIL) population developed by the International Triticeae Mapping Initiative (ITMI) and F_2 progeny (CS/CS2D F_2) of a cross between Chinese Spring and the 2D2 substitution line [Chinese Spring (*tauschii* 2D)]. Two QTL affected both threshability and glume tenacity in the ITMI population. One QTL corresponded to *tenacious glumes 1* (*Tg1*), a previously described gene. We localized *Tg1* to a 23-cM region flanked by *Xwmc25* and *Xgdm107*. The other QTL, which also affected both glume tenacity and percent threshability, was near *Xgwm455*. The factor underlying this QTL was localized to an 11.3-cM interval between *Xbcd102* and *Xgwm455*. We designated this factor *tenacious glumes 3* or *Tg3*. In the CS/CS2D F_2 population, glume tenacity was found to be controlled by two QTL. One QTL corresponded to the action of *Tg1* and was localized to a 20.8-cM interval between the markers *Xwmc503* and *Xbarc168*. This interval corresponds to the genetic location of *Tg1* in the ITMI population. The other QTL was near *Xgwm157*. Deletion bin mapping and comparative analyses indicated that *Tg1* and *Tg3* were located on a chromosomal segment with an estimated frequency of recombination of 215 Kb/cM. On the other hand, *QGT.orst-2D.3* was located on a chromosomal region with suppressed recombination where the estimated

frequency of recombination was 1.5 Mb/cM. These estimates indicate that cloning *Tg1* and *Tg3* using map-based methods will be feasible but cloning *QGt.orst-2D.3* will be extremely challenging if not impossible.

Introduction

Hexaploid wheat (*Triticum aestivum* L., $2n = 42$, AABBDD genomes) arose ~8,000 years ago from a spontaneous hybridization between tetraploid wheat (*T. turgidum* L. $2n = 28$, AABB) and the weedy diploid goatgrass *Aegilops tauschii* Cosson ($2n = 14$, DD) (Huang et al., 2002). During the domestication of wheat, modifications in seed dispersal occurred that enhanced its adaptability to agricultural conditions. These changes include modifications of rachis fragility, spikelet disarticulation, awn development, pubescence, grain size, glume tenacity, and threshability. Of these modifications, one that has been investigated, because of its evolutionary significance and its importance in the practical utilization of the wheat grain, is the free-threshing habit. Genotypes with soft glumes that require limited mechanical action during the de-hulling process are considered free-threshing (FT) while genotypes with tough, tenacious glumes that are not readily detached with mechanical pressure and vigorous rubbing characterize non-free-threshing (NFT) wheats. Hexaploid wheat has been historically subdivided into several subspecies based on rachis fragility and the free-threshing trait (Kimber and Sears, 1983). Free-threshing forms with a tough rachis include *T. aestivum* ssp. *aestivum*, ssp. *sphaerococcum,* and ssp. *compactum* while ssp. *spelta,* ssp. *macha*, and ssp. *vavilovii* have fragile rachises and are hulled and non-free threshing (Leighty and Boshnakian, 1921; Sears, 1947; Unrau, 1950; Kabarity, 1966; Feldman, 2001).

According to Mac Key (1966), a polygenic system scattered throughout the wheat genome regulates rachis brittleness and glume tenacity (threshability). Another

system regulated by the major locus *Q* on chromosome 5A has also been associated with suppression of the speltoid character, rachis brittleness, and glume adherence (Mac Key, 1954; Sears, 1954; Muramatsu, 1963; Kuckuck, 1964; Kerber and Rowland, 1974; McFadden and Sears, 1946). A more recent interpretation suggests that the dominant allele of *Q* has a direct effect on spike characteristics by suppressing the speltoid character and promoting square-headedness. On the other hand, *Q*'s effects on glume tenacity and rachis fragility are indirect and depend on interactions with other loci that control these characteristics (Luo et al., 2000). In addition to genetic background effects (Muramatsu 1986), variation in the phenotypic effects of *Q* has been attributed to allelic variation (Tsunewaki, 1966). Currently, all NFT hexaploids (except ssp. *vavilovii*) carry the recessive *q* allele while all FT forms carry the dominant *Q* allele (Feldman, 2001).

Kerber and Dyck (1969) originally reported the existence of a factor in the D genome that affected threshability or glume tenacity in hexaploid wheat. This was later confirmed by Kerber and Rowland (1974) whose studies showed that the NFT trait of synthetic hexaploids, irrespective of whether their tetraploid parent carried *Q* or *q*, was due to the *Tg1* (*tenacious glumes 1*) gene on chromosome 2DS. Because NFT hexaploids were produced when FT tetraploids were crossed with *Ae. tauschii*, the authors concluded that the dominant *Tg1* allele derived from *Ae. tauschii* interfered with or evaded the effect of *Q*.

When the free-threshing habit was studied in a recombinant inbred line population developed from a cross between a spring wheat, Opata-85 and a synthetic hexaploid wheat, W-7984, QTL on chromosomes 2A, 2B, 2D, 5A, 6A, 6D and 7B

were found to affect the free-threshing character (Jantasuriyarat et al., 2004). In this study, the QTL on the short arm of chromosomes 2D (corresponding to *Tg1*) and the long arm of chromosome 5A (corresponding to *Q*) had the largest effects on the trait. Overall, the free-threshing habit was predominantly affected by *Tg1* and to a lesser extent by *Q*. Investigations with hexaploid wheat aneuploids (Sears, 1954), tetraploid wheat (Simonetti et al., 1999), and *T. monococcum* (Taenzler et al., 2002) also suggest that genes on group 2 chromosomes primarily influence the free-threshing habit by their direct effects on glume tenacity. Thus, the free-threshing phenotype of hexaploid wheat is largely the result of interactions between *tenacious glumes* loci on group 2 chromosomes and *Q* on chromosome 5A.

Q has been the subject of intense research leading to its precise localization and isolation (Faris et al., 2003). On the other hand, there has been a scarcity of studies involving genes that affect glume tenacity like *Tg1*. Thus, the aim of our research is to fill this void by using map-based methods to ultimately localize and characterize *Tg1*. Previously, we used quantitative trait mapping to regionally localize *Tg1* on chromosome 2D (Jantasuriyarat et al., 2004). The objectives of this study are: (i) to generate microsatellite marker-based linkage maps of the short arm of chromosome 2D; and (ii) to localize factors influencing glume tenacity and threshability on chromosome 2D of hexaploid wheat.

Materials and Methods

Plant Material

The localization of factors affecting threshability and glume tenacity was studied using two mapping populations. One mapping population consisted of recombinant inbred lines (RIL) developed by a collaborative mapping project of the International Triticeae Mapping Initiative (ITMI). The RIL population was developed from a cross between a hard red spring wheat cultivar, Opata-85 and synthetic wheat, W-7984. W-7984 was derived from a cross between a durum wheat Altar 84 and *Ae. tauschii* (Nelson et al., 1995a, b, c; Marino et al., 1996; Van Deynze et al., 1995). Seed of the ITMI RIL population and the two parents were provided by Dr. C. Qualset (University of California, Davis). Opata-85, W-7984, and 110 ITMI RILs were previously grown in three sites (University East Farm, West Greenhouse, and Hyslop Farm Field Laboratory, Corvallis, Oregon) for two years (1999 and 2000) to study traits associated with the free-threshing habit (Jantasuriyarat et al., 2004). For this study, the ITMI population and its parents were again planted in un-replicated 5-m row plots at Hyslop Farm Field Laboratory in 2001.

The second mapping population (CS/CS2D) used in this study consisted of F_2 progeny from a cross between Chinese Spring and the 2D2 substitution line. The 2D2 line is a substitution line in which chromosome 2D from Chinese Spring was substituted by chromosome 2D from *Ae. tauschii* [Chinese Spring (*tauschii* 2D)]. Seed for the 2D2 substitution line was provided by Dr. Jan Dvorak (University of California,

Davis). Chinese Spring, 2D2, and 93 F_2 individuals were planted at West Greenhouse, Oregon State University, in 2003.

Homoeologous group 2 cytogenetic stocks were used to place markers to chromosomes and chromosome segments. These stocks included Chinese Spring nullisomic-tetrasomic (N2AT2B, N2BT2D, and N2DT2A), ditelosomic (Dt2DS and Dt2DL), and four deletion lines (2DS1, 2DS5, 2DL3 and 2DL9). The group 2 cytogenetic stocks were provided by Dr. B. S. Gill (Kansas State University, Manhattan). Karyotypes detailing chromosome deletion breakpoints can be found at http://wheat.pw.usda.gov/west/ binmaps/wheat2_rice.html.

DNA isolation and microsatellite marker analysis

About 30 to 50 mg of leaf tissue from lines from the ITMI and CS/CS2D populations and the parental lines were used for DNA extraction. DNA was extracted using a Qiagen/Retsch MM300 mixer mill (Qiagen Inc, Valencia, CA) as described by Riera-Lizarazu et al. (2000). Microsatellite markers previously placed on chromosome 2D by various research groups were used in this study (Nelson et al., 1995a, b, c; Röder et al., 1998; Pestova et al., 2000; Somers et al., 2004). In addition, STS markers were developed from restriction fragment length polymorphism (RFLP) loci present in the region of interest. RFLP probe sequences were obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/). The GenBank accession numbers for the RFLP probe sequences are provided in Table 3.1. The RFLP probe sequence was used in a BLAST search to identify tentative contigs (TC) in wheat at the TIGR wheat database (http://www.tigr.org/tigrscripts/tgi/Tindex.cgi?species=wheat). The TCs with the

highest BLAST hit were then used to identify rice orthologues at the TIGR rice database (http://www.tigr.org/tdb/ e2k1/osa1/). Genomic rice sequences which matched a pertinent wheat TC sequence were then used to obtain a predicted intron/exon structure using PlantGDB GeneSeqer Online (http://www.plantgdb.org /cgi-bin/PlantGDB/GeneSeqer/PlantGDBgs.cgi). Primers were designed to amplify predicted introns to increase the chances of obtaining polymorphisms. The primer sequences of the STS markers used in this study is given in Table 3.1.

Table 3.1 Primer sequences, GenBank accession numbers, and annealing temperatures for STS markers mapped on chromosome 2DS.

STS Locus	**Primers**	**Tm (°C)**	**RFLP marker**	**GenBank Accession**
Xorw2	CGTCGTTTAAACAAGACATC CATGTGGCAGTCATCGTACA	60	*Xpsr928*	AJ440662
Xorw3	TCGACCTCCAGGTCAAGGAG GTCTCAGGTATCACCCGCGC	60	*Xbcd175*	BE438756
Xorw4	TTGCCCCATCTGTAAAAAGG TTGGGAGGAGGAAAAGAGGT	60	*Xbcd1970*	BE438952

Nine microsatellite primers sets that had been previously used to map loci on chromosome 2DS were used to genotype the parents and the 110 ITMI RILs. This was necessary since only a subpopulation of 60-70 individuals had been genotyped previously with the markers of interest (Röder et al., 1998; Pestova et al., 2000). Marker data available for 13 RFLP and two microsatellite marker loci on chromosome 2D was also used in this study. Data for these markers are publicly available at GrainGenes (http://wheat.pw.usda.gov/index.shtml). Twenty three markers were used

to construct a linkage map of chromosome 2D using the CS/CS2D F_2 population. Polymerase chain reaction (PCR) amplification of microsatellite and STS loci was performed in a volume of 10 μL in a MWG Thermalcycler (Primus 96 Plus). The reaction mixture contained 0.5 μM of each primer, 0.2 mM of each deoxynuleotide, 0.03 U/μL *Taq* DNA Polymerase (Qiagen), 1X *Taq* buffer from Qiagen, 2% sucrose in cresol red and 50 ng of template DNA. After 5 min at 94°C, 45 cycles were performed with 30s at 94°C, 30s at 50, 55 or 60°C (depending on the individual primer set) and 30s at 72°C, followed by a final extension step of 10 min at 72°C. Products were screened on 4% agarose gels and visualized after staining with ethidium bromide.

For markers that did not have easily discernible polymorphisms on agarose gels, fluorescent detection of PCR amplification products was achieved using one primer labeled with either 5-carboxy-fluroscein (5-FAM) or 4,7,2',4',5',7'-hexacfloro-6-carboxyrhodamin (HEX). Amplification products were electrophoresed and detected in an ABI Prism™ 3100 DNA sequencer at the Central Services Laboratory, Center for Gene Research and Biotechnology, Oregon State University. ABI collection software version 1.1 was used for raw data collection. Microsatellite fragments were analyzed using Genescan™ analysis software version 2.1 and Genotyper ® software.

Map Construction

Linkage maps were constructed using Mapmaker/Exp 3.0 (Lander et al., 1987). The group command with a minimum LOD of 3.0 and a maximum distance of 50-cM was used to identify linked markers. Subsequently, the order command (LOD 3.0) was used to build maps. Finally, the ripple command was used to verify map orders.

Recombination fractions were converted into map distances (cM) using the Kosambi mapping function.

Phenotypic Assessment

The ITMI and the CS/CS2D population and their parental lines (grown either at Hyslop Farm Field Laboratory in 2001 or at the West Greenhouse in 2003) were evaluated for the free-threshing habit by measuring glume tenacity and percent threshability. To measure glume tenacity, a LKG-1 Hunter force gauge (AMETEK, Inc., Hatfield, PA) was used to measure the force (N=Newton) necessary to separate the glumes at their base from four randomly selected spikelets per spike (Figure 3.1). Glume tenacity was measured in four spikes per individual. Percent threshability was measured by processing eight randomly chosen mature spikes of each line through a gasoline-powered thresher and collecting both threshed and unthreshed seeds (Figure 3.2). Threshability was calculated as the percentage of completely threshed seeds out of all seeds harvested. Due to the lack of a sufficient number of spikes, percent threshability was not evaluated for the CS/CS2D F_2 population. Percent threshability and glume tenacity data are presented in Appendices 5 and 6. The ITMI RILs and the CS/CS2D F_2 individuals were also evaluated for glaucousness (waxiness/glossiness) of stems and leaves. These evaluations were used to map *Iw2,* a dominant inhibitor of glaucousness, segregating in both populations.

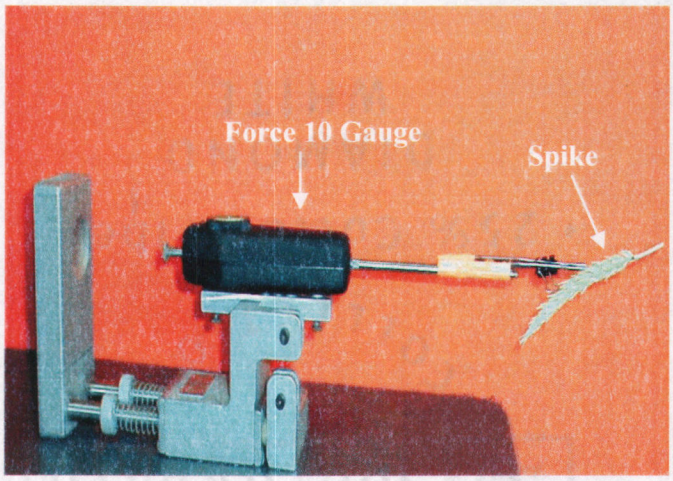
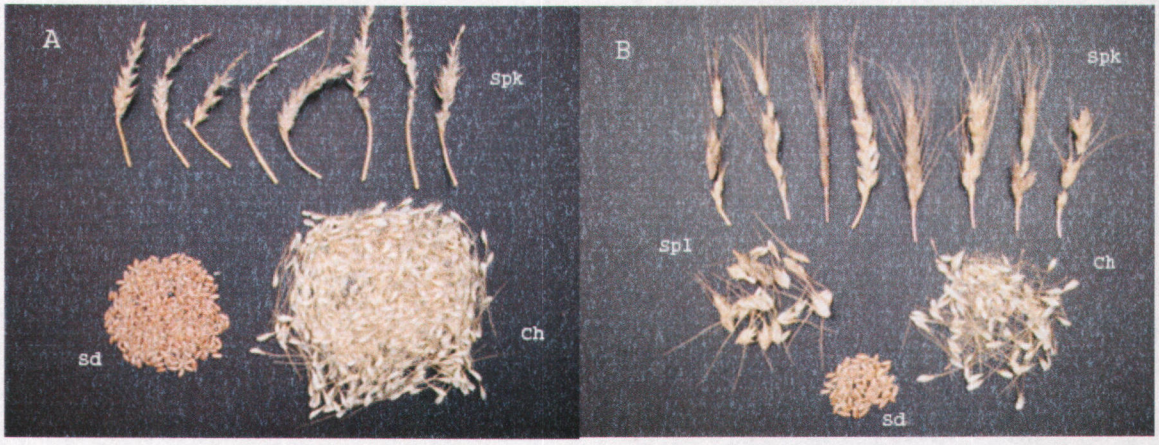


Figure 3.1 LKG-1 Hunter force 10 gauge (AMETEK, Inc., Hatfield, PA) used to measure glume tenacity.



Threshability – 100% **Threshability – 34%**

Figure 3.2 Threshed spikes and their components for Opata-85 (A) and W-7984 (B), parents of the ITMI RIL population [threshed seeds (Sd), chaff (Ch), spikelets (Spl), spike fragments (Spk)].

QTL Analysis

Percent threshability, glume tenacity measurements, and linkage maps were used for quantitative trait locus (QTL) analysis using QTL Cartographer (Basten et al. 1999). For analyses involving the ITMI population, a whole genome map with ~500 loci described by Jantasuriyarat et al. (2004) was used in conjunction with the map of chromosome 2D that was constructed for this study. Least square trait means from each environment (except for un-replicated experiments) and means across environments were analyzed. QTL were mapped using composite interval mapping (CIM) (Zeng, 1993; 1994) with a maximum of 10 co-factors selected using the forward-selection backward-elimination stepwise regression procedure. A 5-cM scan window was used for all analyses and the likelihood ratio (LR) statistic was computed every 1 cM. Based on previous work (Jantasuriyarat et al., 2004), a threshold of LOD 3.0 was deemed adequate for QTL identification. For analyses involving the CS/CS2D population, the map of chromosome 2D constructed in this study was used. QTL mapping was performed as described earlier. In addition, the multiple interval mapping (MIM: Kao et al. 1999) procedure was used to test the statistical significance of the various modes of inheritance for the QTL identified with the CS/CS2D population.

Results

Phenotypic data

Phenotypic frequency distributions for percent threshability and glume tenacity measurements of the ITMI population grown at Hyslop Farm in 2001 are shown in Figures 3.3 A and 3.3 B. The distribution of percent threshability was continuous and skewed towards the more threshable group (Figure 3.3 A). Similarly, the distribution of glume tenacity values was also continuous and skewed towards the less tenacious side (Figure 3.3 B). A phenotypic frequency distribution for glume tenacity measurements of the CS/CS2D population grown at the West greenhouse in 2003 is shown in Figure 3.3 C. The distribution was continuous but skewed towards the softer glume side (Figure 3.3 C).

The mean trait values for glume tenacity and percent threshability for the ITMI population are presented in Table 3.2. W-7984 is a synthetic wheat with highly tenacious glumes and required an average of 5.78 N of force to detach glumes from its rachis. Consequently, W-1984 was not easily threshed (33.6% threshability). Opata-85, on the other hand, required only 1.06 N of force to achieve glume detachment and was found to be very threshable (97.9% threshability). Glume tenacity measured as the force (N) necessary to separate glumes from their spike rachises ranged from 0.75 N to 7.53 N in the ITMI population. Percent threshability for the ITMI RILs ranged from 11.76% to 98.76%.

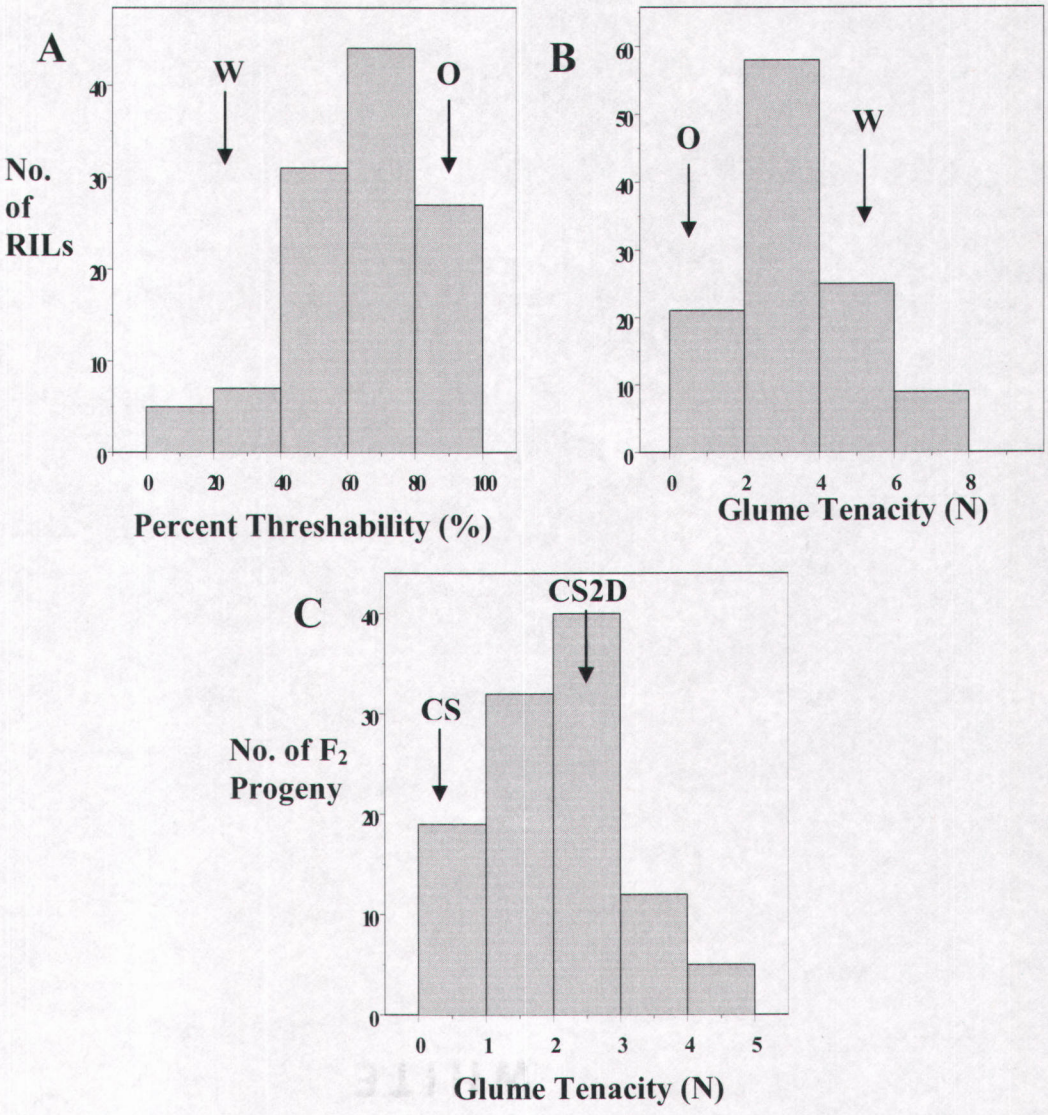


Figure 3.3 Phenotypic frequency distributions for percent threshability (A) and glume tenacity (B) for the ITMI population (Hyslop farm, 2001). Parental values for Opata-85 (O) and W-7984 (W) are indicated with arrows. Phenotypic frequency distribution for glume tenacity (C) for the CS/CS2D F_2 population (West greenhouse 2003). Parental values for Chinese Spring (CS) and 2D2 (CS2D) are indicated with arrows.

The mean trait values for glume tenacity for the CS/CS2D F_2 population are also presented in Table 3.2. The 2D2 substitution line had tough glumes requiring about 2.94 N of force to effect glume detachment. Chinese Spring, on the other hand,

had softer glumes requiring only 0.91 N of force to separate its glumes from their spikes. Glume tenacity values for the CS/CS2D F_2 population ranged from 0.50 to 4.50 N.

Table 3.2 Mean, range (Min. and Max.) and standard deviation (SD) of threshability-associated trait values of the parental lines W-7984, Opata-85 and 110 ITMI RILs and Chinese Spring, 2D2 and 93 F_2 progeny (CS/CS2D F_2s).

Environment	Lines	Glume Tenacity (N)	Threshability (%)
Hyslop Farm, 2001	W-7984	5.78	33.6
	Opata-85	1.06	97.9
	ITMI RILs		
	Mean	3.39	65.72
	Range	0.75-7.53	11.76-98.76
	SD	1.51	18.7
West greenhouse, 2003	Chinese Spring	0.91	
	2D2	2.94	
	CS/CS2D F_2s		
	Mean	2.05	
	Range	0.50– 4.50	
	SD	0.96	

Mapping

The segregation data for the microsatellites mapped on chromosome 2D using the ITMI RIL and CS/CS2D F_2 populations are provided in Appendices 3 and 4, respectively. The linkage map for chromosome 2D based on the ITMI population was composed of 26 loci (Figure 3.4 A) and spanned 168.0 cM. The average distance between the markers was 5.4 cM. The largest interval in the map was 18.7 cM (between *Xtam8* and *Xgwm349*). The linkage map of chromosome 2D based on the CS/CS2D population was composed of 23 loci (Figure 3.4 B) that spanned 162.6 cM

with an average distance of 7.1 cM between markers. The largest interval in the map was 34.4 cM between *Xcfd168* and *Xgwm349.*

QTL analysis

Glume tenacity and percent threshability, in the ITMI population, was evaluated in three environments (East Farm 2000, West Greenhouse 2000, and Hyslop Farm 2001). Two QTL, designated *QGt.orst-2D.1* and *QGt.orst-2D.2,* were detected on chromosome 2D (Table 3.3). *QGt.orst-2D.1* explained 20% to 26% of the phenotypic variance in the environments tested. In the analysis across environments, *QGt.orst-2D.1* explained 53% of the phenotypic variance. *QGt.orst-2D.2* explained 15% to 18% of the phenotypic variance. This QTL explained 44% of the phenotypic variance across environments. The loci most closely associated with *QGt.orst-2D.1* and *QGt.orst-2D.2* were *Xgwm261* and *Xgwm455*, respectively. The peaks of the two QTL were separated by a distance of 7 cM (Figure 3.4 A).

Two QTL that affected percent threshability were also identified on chromosome 2D. The QTL were designated *QFt.orst-2D.1* and *QFt.orst-2D.2* (Table 3.4). *QFt.orst-2D.1* explained from 18% to 52% of the phenotypic variance. In the analysis over three environments, *QFt.orst-2D.1* explained 44% of the phenotypic variance. *QFt.orst-2D.2* explained 33% to 46% of the phenotypic variance. A combined analysis across environments showed that *QFt.orst-2D.2* explained 36% of the phenotypic variance. The loci most closely associated with *QFt.orst-2D.1* was *Xgwm261* and with *QFt.orst-2D.2* was *Xgwm455* (Figure 3.4 A). W-7984 contributed the higher value allele for QTL that affected glume tenacity while percent threshability

increased with the Opata-85 alleles at these QTL. Markers associated with these QTL were all placed in the deletion bin 2DS5-0.47-1.00 (Figure 3.4 C).

Glume tenacity, in the CS/CS2D F_2 population, was evaluated in one environment (West Greenhouse 2003). Two QTL, designated *QGt.orst-2D.1* and *QGt.orst-2D.3*, were detected on chromosome 2D (Table 3.4). *QGt.orst-2D.1* explained 52% of the phenotypic variance. On the other hand, *QGt.orst-2D.3* only explained 14% of the phenotypic variance. The loci most closely associated with *QGt.orst-2D.1* and *QGt.orst-2D.3* were *Xwmc503* and *Xgwm157*, respectively (Figure 3.4 B). *QGt.orst-2D.1* and *QGt.orst-2D.2* showed both additive and dominance effects but an overall additive mode of inheritance had better statistical support (Table 3.4). Microsatellite markers associated with *QGt.orst-2D.1* were placed in the deletion bin 2DS5-0.47-1.0. Microsatellites associated with *QGt.orst-2D.2* were placed in the deletion bin C 2DS1-0.33 (Figure 3.4 C).

Figure 3.4 **A**. Genetic linkage map and QTL for threshability associated traits on chromosome 2D detected using the ITMI RIL population. Open bars indicate the 2-LOD interval for the QTL that affected percent threshability (*QFt.orst-2D.1* and *QFt.orst-2D.2*). Solid bars indicate the 2-LOD interval for QTL that affected glume tenacity (*QGt.orst-2D.1* and *QGt.orst-2D.2*). *QFt.orst-2D.1* and *QGt.orst-2D.1* represent the action of *Tg1* while *QFt.orst-2D.2* and *QGt.orst-2D.2* represent the action of *Tg3*. **B.** Genetic linkage map and QTL for glume tenacity on chromosome 2D detected using the CS/CS2D F_2 mapping population. A solid bar indicates the 2-LOD interval for the QTL that affects glume tenacity (*Q.Gt.orst-2D.1*) and the stippled bar indicates the other QTL that also affected glume tenacity (*Q.Gt.orst-2D.3*). The triangles mark the peaks of the respective QTL. The lines between the two linkage maps connect common markers. STS loci *Xorw2, Xorw3,* and *Xorw4* may correspond to the RFLP loci *Xpsr928, Xbcd175,* and *Xbcd1970,* respectively. *Q.Gt.orst-2D.1* represents the action of *Tg1*. **C.** Deletion bin map of chromosome 2D. Deletion breakpoints are indicated by arrows. Dark bands on the chromosome indicate the location of C-bands (Gill et al., 1991).

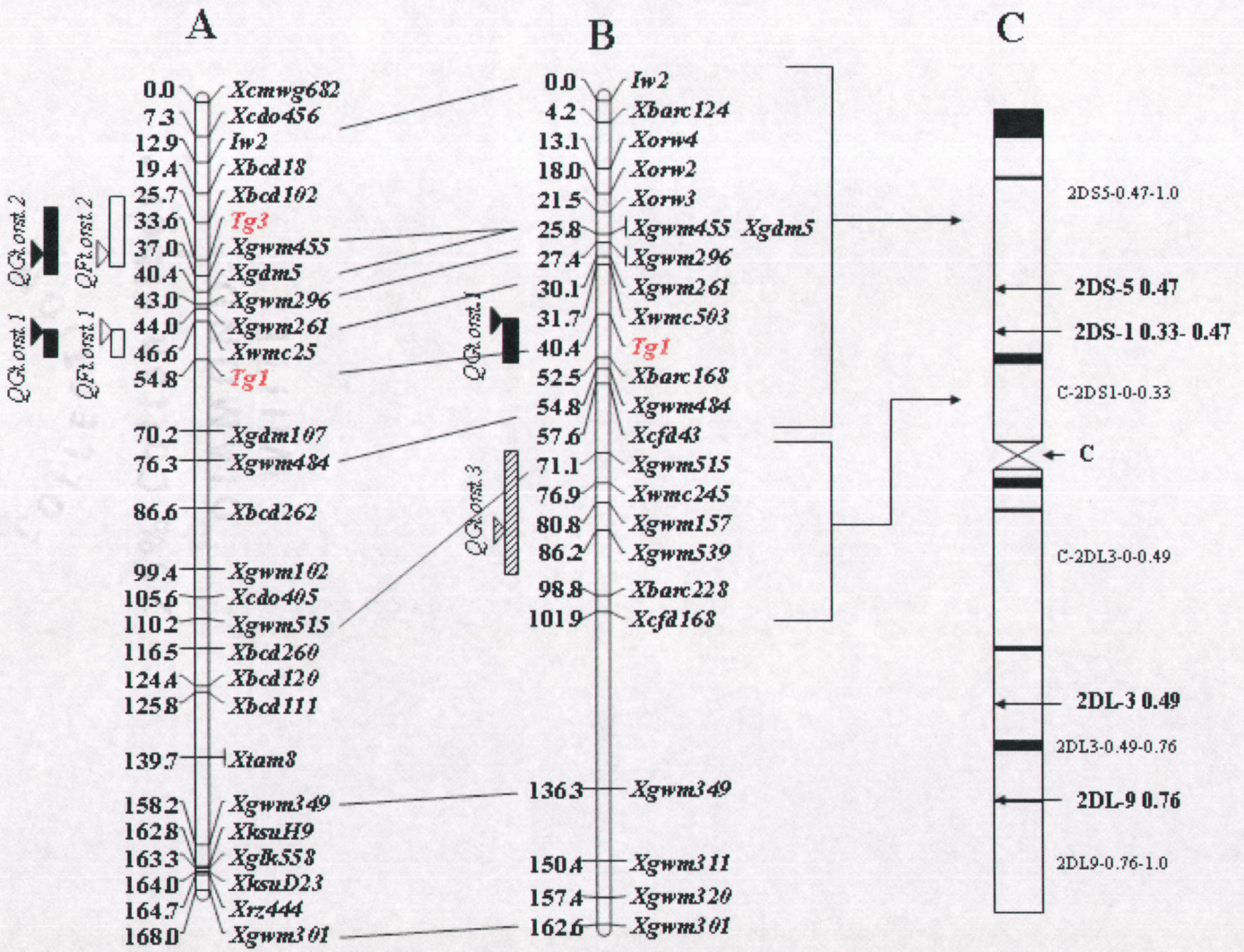

A
B
C
0.0 Xcmwg682
7.3 Xcdo456
12.9 Iw2
19.4 Xbcd18
25.7 Xbcd102
33.6 Tg3
37.0 Xgwm455
40.4 Xgdm5
43.0 Xgwm296
44.0 Xgwm261
46.6 Xwmc25
54.8 Tg1
70.2 Xgdm107
76.3 Xgwm484
86.6 Xbcd262
99.4 Xgwm102
105.6 Xcdo405
110.2 Xgwm515
116.5 Xbcd260
124.4 Xbcd120
125.8 Xbcd111
139.7 Xtam8
158.2 Xgwm349
162.8 XksuH9
163.3 Xglk558
164.0 XksuD23
164.7 Xrz444
168.0 Xgwm301
QGt.orst.2
QFt.orst.2
QGt.orst.1
QFt.orst.1
0.0 Iw2
4.2 Xbarc124
13.1 Xorw4
18.0 Xorw2
21.5 Xorw3
25.8 Xgwm455 Xgdm5
27.4 Xgwm296
30.1 Xgwm261
31.7 Xwmc503
40.4 Tg1
52.5 Xbarc168
54.8 Xgwm484
57.6 Xcfd43
71.1 Xgwm515
76.9 Xwmc245
80.8 Xgwm157
86.2 Xgwm539
98.8 Xbarc228
101.9 Xcfd168
136.3 Xgwm349
150.4 Xgwm311
157.4 Xgwm320
162.6 Xgwm301
QGt.orst.1
QGt.orst.3
2DS5-0.47-1.0
2DS-5 0.47
2DS-1 0.33- 0.47
C-2DS1-0-0.33
C
C-2DL3-0-0.49
2DL-3 0.49
2DL3-0.49-0.76
2DL-9 0.76
2DL9-0.76-1.0

Table 3.3 Threshability-associated quantitative trait loci (QTL) for the ITMI population. Location, significance, effect, and proportion of phenotypic variation explained based on composite interval mapping (CIM) analysis performed using QTL Cartographer.

Trait	QTL Symbol	Environment	QTL peak position (nearest locus)	2-LOD support limit, cM range	LR [a] statistic	R^2 [b]	Additive effect [c]
Glume Tenacity	*QGt.orst-2D.1*	East Farm 2000	41.2 (*Xgwm261*)	38.6 – 43.2 (*Xgdm5 – Xwmc25*)	55.67	0.26	0.54
		Greenhouse 2000	40.2 (*Xgwm296*)	37.6 – 43.2 (*Xgdm5 – Xwmc25*)	59.15	0.23	0.36
		Hyslop Farm 2001	41.2 (*Xgwm261*)	40.2 – 43.2 (*Xgwm296 – Xwmc25*)	62.39	0.20	0.85
		Combined	41.2 (*Xgwm261*)	41.2 – 42.2 (*Xgwm261*)	122.31	0.53	0.69
	QGt.orst-2D.2	East Farm 2000	29.7 (*Xbcd102*)	25.7 – 34.1 (*Xbcd102 – Xgwm455*)	29.31	0.18	0.44
		Greenhouse 2000	31.7 (*Xgwm455*)	25.7 – 34.1 (*Xbcd102 – Xgwm455*)	30.96	0.16	0.30
		Hyslop Farm 2001	34.7 (*Xgwm455*)	34.1 – 36.1 (*Xgwm455 – Xgdm5*)	39.9	0.15	0.81
		Combined	31.7 (*Xgwm455*)	25.7 – 34.1 (*Xbcd102 – Xgwm455*)	71.52	0.44	0.62

Table 3.3 (continued)

Trait	QTL Symbol	Environment	QTL peak position (nearest locus)	2-LOD support limit, cM range	LR [a] statistic	R^{2} [b]	Additive effect [c]
Percent Threshability	*QFt.orst-2D.1*	Hyslop Farm 1999	41.2 (*Xgwm261*)	39.6 – 43.2 (*Xgdm5 – Xwmc25*)	100.97	0.38	-12.34
		East Farm 2000	41.2 (*Xgwm261*)	40.2 – 42.2 (*Xgwm296 – Xwmc25*)	131.58	0.52	-14.02
		Hyslop Farm2001	43.8 (*Xwmc25*)	42.2 – 49.8 (*Xgwm261 – Xwmc25*)	46.27	0.18	-8.39
		Combined	42.2 (*Xgwm261*)	39.6 – 42.2 (*Xgwm261 – Xwmc25*)	121.06	0.44	-10.74
	QFt.orst-2D.2	Hyslop Farm 1999	31.7 (*Xgwm455*)	28.7 – 37.1 (*Xbcd102 – Xgwm455*)	65.73	0.33	-11.55
		East Farm 2000	31.7 (*Xgwm455*)	28.7 – 35.1 (*Xbcd102 – Xgwm455*)	76.06	0.46	-13.24
		Hyslop Farm2001	29.7 (*Xbcd102*)	27.7 – 31.7 (*Xbcd102 – Xgwm455*)	33.90	0.18	-8.07
		Combined	30.7 (*Xgwm455*)	27.1 – 33.7 (*Xbcd102 – Xgwm455*)	84.9	0.36	-9.59

[a] LR is the Likelihood ratio test statistic 2 ln (L_0/L_1), where (L_0/L_1) is the ratio of likelihoods between the hypothesis that there is no QTL in the tested interval (L_0) and the hypothesis that there is a QTL in the tested interval (L_1) (Basten et al. 1994, 1999).

[b] R^2 is the proportion of the phenotypic variance explained by the QTL after accounting for co-factors.

[c] Additive effects indicates an additive main effect of the parent contributing the higher value allele: In the ITMI population, positive values indicate that higher value alleles are from W-7984 and the negative values indicate that higher value alleles are from Opata-85.

Note: Genetic distances presented in the table represent distances calculated before the integration of *Tg1* and *Tg3* into the linkage map for chromosome 2D.

Table 3.4 Threshability-associated quantitative trait locus (QTL) for the Chinese Spring x 2D2 F_2 population. Location, significance, effect, and proportion of phenotypic variation explained based on composite interval mapping (CIM) and multiple interval mapping (MIM) analysis performed using QTL Cartographer.ng (MIM) analysis performed using QTL Cartographer.

Trait	QTL Symbol	Environment	QTL peak position (nearest locus)	2-LOD support limit, cM range	LR[1] statistic	Effects of Phenotype				MOI[6]
						a[2]	*d*[3]	*d/a*[4]	$R^{2\,5}$	
Glume Tenacity	*QGt.orst-2D.1*	Greenhouse 2003	37.71 (*Xwmc503*)	33.71 – 42.7 (*Xwmc503*)	73.50	-0.99	0.09	-0.09	0.52	A
	QGt.orst-2D.3	Greenhouse 2003	78.51 (*Xgwm157*)	59.31 – 87.91 (*Xgwm515 – Xgwm539*)	27.23	-0.40	-0.19	0.46	0.14	A

[1] LR is the Likelihood ratio test statistic 2 ln (L_0/L_1), where (L_0/L_1) is the ratio of likelihoods between the hypothesis that there is no QTL in the tested interval (L_0) and the hypothesis that there is a QTL in the tested interval (L_1) (Basten et al. 1994, 1999).

[2] Additive effects indicate an additive main effect of the parent contributing the higher value allele.

[3] Dominance effect indicates a dominance main effect of the parent contributing the higher value allele.

[4] d/a: degree of dominance

[5] R^2 is the proportion of the phenotypic variance explained by the QTL after accounting for co-factors.

[6] Mode of Inheritance (MOI) estimated based on an analysis with constrained genetics. A = additive.

Note: Genetic distances presented in the table represent distances calculated before the integration of *Tg1* into the linkage map for chromosome 2D.

Localization of discrete loci underlying QTL

Previous research and the similarity in location, effect, and behavior of QTL that affected both glume tenacity and percent threshability in this study suggested that these represented manifestations of discrete loci. These factors were localized using the following strategy. As discussed earlier, coincident QTL affecting glume tenacity (*QGt.orst-2D.1*) and percent threshability (*QFt.orst-2D.1*) were detected in the ITMI population. In order to genetically localize the factor responsible for this QTL, we first classified individuals into two groups based on their allelic configurations at *Xbcd102* and *Xgwm455*. These two markers form the 2-LOD support interval for *QGt.orst-2D.2* and *QFt.orst-2D.2* (Table 3.3, Figure 3.4 A). The first group consisted of RILs which had Opata-85 alleles at both marker loci and the second group consisted of RILs that had W-7984 alleles. Individuals that showed recombination between the *Xbcd102* and *Xgwm455* or had missing data were not used in the analysis since the QTL genotype for this group of lines was uncertain. Individuals within the first and second groups will differ from each other and their respective controls primarily because of the genetic locus underlying *QGT.orst-2D.1* or *QFt.orst-2D.1*. Using Dunnett's multiple comparisons of means (Rafter et al., 2002) the first group was compared to Opata-85 and RIL individuals that did not differ significantly from this control were considered to possess Opata-85 alleles at this locus. RILs that differed significantly from Opata-85 were considered to possess alleles from the other parent, W-7984. The second group of RILs was compared to W-7984. RILs that did not differ significantly from this control were considered to possess alleles from W-7984 and RILs that differed significantly from W-7984 were considered to possess alleles from Opata-85. By

combining glume tenacity and percent threshability data to compare the RILs in the two groups to their respective controls a consensus classification of RILs was obtained. Using this consensus classification a genetic location for the factor representing *QGt.orst-2D.1* and *QFt.orst-2D.1* was obtained. These QTL represent the action of *Tg1* that was localized to a ~23-cM interval between the markers *Xwmc25* and *Xgdm107* (Figure 3.4 A).

The detection of other coincident QTL with a significant effect on glume tenacity (*QGt.orst-2D.2*) and percent threshability (*QFt.orst-2D.2*) indicated that a second factor on chromosome 2D also affected free-threshing habit. The same strategy used to place *Tg1* was used also in this case. The 2-LOD support interval for *QGt.orst-2D.1* and *QFt.orst-2D.1* is marked by *Xgwm261* and *Xwmc25* (Table 3.3, Figure 3.4 A). The ITMI RILs were classified into groups as before and RILs within groups were compared to their respective controls using Dunnett's tests. Again, a consensus classification of RILs was achieved using glume tenacity and percent threshability data. Linkage analysis was then used to genetically localize the genetic locus underlying *QGt.orst-2D.2* and *QFt.orst-2D.2.* These QTL represent the action of a locus distinct from *Tg1* that we have named *Tg3*. *Tg3* was mapped to an 11.3-cM interval between *Xbcd102* and *Xgwm455* (Figure 3.4 A).

In the CS/CS2D F_2 mapping population, two QTL, *QGt.orst-2D.1* and *QGt.orst-2D.3,* which affected glume tenacity were identified. In order to genetically localize the genetic locus responsible for *QGt.orst-2D.1*, F_2 individuals were first classified into four groups on the basis of their allele configurations at *Xgwm157* and *Xgwm245*. These two loci are contained in the 2-LOD support interval for *QGt.orst-*

2D.3. The first group consisted of individuals that had Chinese Spring alleles at both marker loci, the second group consisted of individuals that had the 2D2 allele at both marker loci, the third group consisted of individuals heterozygous at the marker loci and the fourth group consisted of individuals which had recombinations between the two marker loci or had missing data. Using Dunnett's multiple comparisons of means, the first group was compared to Chinese Spring and F_2 individuals that did not differ significantly from this control were considered to possess Chinese Spring alleles at this locus. F_2s that differed significantly from Chinese Spring were classified as individuals that were not homozygous for alleles from Chinese Spring. The second group of F_2s were compared to 2D2. F_2s that did not differ significantly from this control were considered to possess alleles from 2D2 at this locus. F_2 individuals that differed significantly from 2D2 were classified as individuals that were not homozygous for alleles from 2D2. The third group of F_2s was compared also to Chinese Spring. Individuals that did not differ from this control were considered to possess alleles from Chinese Spring and the F_2s that differed significantly were considered to not be homozygotes for the alleles from Chinese Spring. Individuals from the fourth group were not used in this analysis. Linkage analysis using these classifications yielded a genetic location for the factor underlying *QGt.orst-2D.1*. A genetic locus was localized to a 20.8-cM interval between the markers *Xwmc503* and *Xbarc168*. Since this interval corresponded to the genetic location of *Tg1* observed in the ITMI mapping population, this locus is likely to also represent *Tg1* in this population. We were unable to unambiguously obtain qualitative classifications and to genetically localize the factor representing *QGt.orst-2D.3*.

Discussion

Two mapping populations and a quantitative mapping approach were used to localize factors affecting the free-threshing character on chromosome 2D of common wheat (*T. aestivum* L). In analyses using the ITMI RIL population, two coincident QTL that affected both glume tenacity and percent threshability were identified. Since the effects of these QTL were fairly large (Table 3.3), loci underlying these QTL were genetically localized on the linkage map of chromosome 2D. A locus corresponding to *tenacious glumes 1*, *Tg1*, was localized to a ~23-cM interval between the markers *Xwmc25* and *Xgdm107* (Figure 3.4 A). In addition, a locus distinct from *Tg1*, named *Tg3*, was mapped to an 11.3-cM interval between *Xbcd102* and *Xgwm455* (Figure 3.4 A). Similarly, analyses using the CS/CS2D mapping population allowed the identification of two QTL that affected glume tenacity. One QTL had a large effect (Table 3.4) and was localized to a discrete genetic locus. This locus was placed in an interval that corresponded to the genetic location of *Tg1* observed in the ITMI mapping population. Thus, this locus also likely represents *Tg1* (Figure 3.4 B). We also found that this locus had both additive and dominance effects but had mostly an additive mode of inheritance where heterozygotes showed an intermediate phenotype. This is also consistent with earlier descriptions of *Tg1* as a semi-dominant gene (Kerber and Rowland, 1974). Because of its relatively smaller effect (Table 3.4), we were unable to precisely localize *QGt.orst-2D.3*.

In a previous study involving the ITMI population, *Tg1* had been regionally localized on chromosome 2D but the presence of a second QTL was overlooked (Jantasuriyarat et al., 2004). It is possible that the inability to detect *Tg3* in an earlier

study was due to missing data, a more sparse coverage of the chromosomal regions in question, and the reported difficulty of separating linked QTL (Haley and Knott, 1992; Whittaker et al., 1996). Nonetheless, our results are in line with a report for the presence of an additional gene on chromosome 2D, besides *Tg1,* that also affected glume adherence in wheat (Ternowskaya and Zhirov, 1993). Whether this factor corresponds to *Tg3* or *QGt.orst-2D.3* needs to be determined.

There have been recent reports on the localization of genes that affect glume tenacity on other homoeologous group 2 chromosomes. Simonetti et al. (1999) found four QTL on chromosomes 2B, 5A, and 6A which affected threshability in a RIL population derived from a cross between a *T. turgidum* ssp. *durum* and *T. turgidum* ssp. *dicoccoides.* The free-threshing character was predominantly affected by a QTL on the short arm of chromosome 2B that represented the effect of a locus that was named *tenacious glumes 2* (*Tg2*). *Tg2* was interpreted to be a homoeologue of *Tg1* but we suggest that more comprehensive mapping of the *Tg2* region is needed in order to establish orthology especially in view of our observation that more than one locus affecting glume adherence was found in chromosome 2D using two different populations. Evidence for a possible orthologue of *Tg1* or *Tg3* in the A genome was presented by Taenzler et al. (2002) who mapped a *soft glume* (*Sog*) locus on the short arm of chromosome $2A^m$ of *T. monococcum.*

The presence of factors that affect glume tenacity on homoeologous group 2 chromosomes dates back to Sears' (1954) observations that plants that were nullisomic for chromosomes 2A, 2B, or 2D had papery glumes while plants that were tetrasomic for these chromosomes had glumes that were stiffer than normal disomic plants. With

current map-based methods and the availability of various genomic resources and the development of comprehensive genetic and cytogenetics maps (Nelson et al., 1995a, b, c; Röder et al., 1998; Pestova et al., 2000; Somers et al., 2004; Boyko et al., 2002), BAC libraries (Lijatvetzky et al., 1999; Cenci et al., 2003), physical maps (Gill et al., 1993; Kota et al., 1993; Hohmann et al., 1994; Ogihara et al., 1994, Delaney et al., 1995a; Delaney et al., 1995b, Mickelson-Young et al., 1995; Gill et al., 1996) and a large collection of mapped ESTs (U.S. Wheat Genome Project - http://wheat.pw.usda.gov/NSF/), the isolation of the genes in question may one day be possible. In order to gauge the feasibility of using map-based methods to isolate the genes in question, we mapped the pertinent microsatellite markers using chromosome 2D Chinese Spring deletion stocks. Twelve microsatellite markers associated with *Tg1* and *Tg3* were placed in deletion bin 2DS5-0.47-1.0 that spans the terminal 23% of the short arm of chromosome 2D (Figure 3.4 C). Six markers associated with *QGt.orst-2D.3* were placed in deletion bin C-2DS-0.33 which spans 14.4 % of the entire chromosome. Erayman et al. (2004) physically mapped over 3,000 loci using 334 deletion lines. This comprehensive analysis showed that the gene-containing fraction (29%) of the wheat genome was organized in 18 major and 30 minor gene-rich regions (GRRs). Comparative mapping analyses with the consensus physical maps for wheat group 2 chromosomes showed that *Tg1* and *Tg3* were located in a major GRR 2S0.8. This GRR, located in the short arm, is flanked by deletions 2BS-14(0.84) and 2BS-3(0.79). GRR 2S0.8 represents a physical segment of ~7 Mb containing ~31% of the genes in this chromosome arm. Furthermore, Erayman et al. (2004) estimated that frequency of recombination in this GRR was 215 Kb/cM. On the other hand,

QGt.orst-2D.3 was placed in GRR 2S0.5 with an estimated recombination frequency of 1.5 Mb/cM. These estimates indicate that cloning *Tg1* and *Tg3* using map-based methods is feasible while cloning *QGt.orst-2D.3* will be extremely challenging if not impossible.

References

Basten C J, Weir B S and Zeng Z B (1999) QTL Cartographer Version 1.13, A reference manual and tutorial for QTL mapping. http://www.statgen.ncsu.edu/qtlcart/cartographer.html

Boyko E, Kalendar R, Korzun V, Fellers J, Korol A, Schulman, A H and Gill B S (2002) A high-density cytogenetic map of the *Aegilops tauschii* genome incorporating retrotransposons and defense-related genes: insights into cereal chromosome structure and function. Plant Mol Biol 48:767-790.

Cenci A, Chantret N, Kong X, Gu Y, Anderson O D, Fahima T, Distfield A, Dubcovsky J (2003) Construction and characterization of a half million clone BAC library of durum wheat (*Triticum turgidum* ssp. *durum*). Theor Appl Genet 107:931-939.

Conley E J, Nduati V, Gonzalez-Hernandez J L, Mesfin A, Trudeau-Spanjers M, Chao S, Lazo G R, Hummel D D, Anderson O D, Qi LL, Gill B S, Echalier B, Linkiewicz A M, Dubcovsky J, Akhunov E D, Dvorak J, Peng J H, Lapitan NL, Pathan MS, Nguyen H T, Ma X F, Miftahudin, Gustafson J P, Greene R A, Sorrells M E, Hossain K G, Kalavacharla V, Kianian SF, Sidhu D, Dilbirligi M, Gill K S, Choi D W, Fenton R D, Close T J, McGuire P E, Qualset C O and Anderson J A (2004). A 2600-locus chromosome bin map of wheat homoeologous group 2 reveals interstitial gene-rich islands and colinearity with rice. Genetic 168: 625-637.

Cox T S (1998) Deepening the wheat gene pool. J Crop Prod 1:1-25.

Delaney D E, Nasuda S, Endo T R, Gill B S, Hulbert S H (1995a) Cytologically based physical maps of the group-2 chromosomes of wheat. Theor Appl Genet 91:568-573.

Delaney D E, Nasuda S, Endo T R, Gill B S and Hulbert S H (1995b) Cytologically based physical maps of the group-3 chromosomes of wheat. Theor Appl Genet 91:780-782.

Del Blanco I A, Rajaram S, Kronstad W E (2001) Agronomic potential of synthetic hexaploid wheat-derived populations. Crop Sci 41:670-676.

Endo T R, Gill B S (1996) The deletion stocks of common wheat. J. Hered 87: 295-307.

Erayman M, Sandhu D, Sidhu D, Dilbirligi M, Baenziger P S and Gill K S (2004) Demarcating gene-rich regions of the wheat genome. Nucleic Acid Res 32(12): 3546-3565.

Faris J D, Fellers J P, Brooks S A, Gill B S (2003) A bacterial artificial chromosome contig spanning the major domestication locus *Q* in wheat and identification of a candidate gene. Genetics 164: 311-321.

Faris J D, Anderson J A, Francl L J, Jordahl J G (1997) RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor Appl Genet 94: 98-103.

Feldman M (2001) Origin of cultivated wheat. In Bonjean A P and Angus J W (eds) The world wheat book. Lavosier Publishing, Paris, pp 3-58.

Gill K S, Gill B S, Endo T R and Boyko E V (1996) Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. Genetics 143:1001-1012.

Gill K S, Gill B S, and Endo T R (1993) A chromosome region-specific mapping strategy reveals gene-rich telomeric ends in wheat. Chromosoma 102:374-381.

Haley C S and Knott S A (1992) A simple method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315-324.

Hohmann U, Endo T R. Gill K S, Gill B S (1994) Comparison of genetic and physical maps of group 7 chromosomes from *Triticum aestivum* L. Mol Gen Genet 245:644-653.

Huang S, Sirikhachornkit A, Faris J, Gill B S, Haselkorn R, Gornicki P (2002) Phylogenetic analysis of the acetyl-CoA carboxylase and 3-phosphoglycerate kinase loci in wheat and other grasses. Plant Mol Biol 48:805-820.

Jantasuriyarat C, Vales M I, and Riera-Lizarazu O (2004) Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.). Theor Appl Genet 108:261-273.

Kabarity A (1966) On the origin of the new cultivated wheat II. Cytogenetical studies on the karyotypes of some *Triticum macha* varieties. Beitr Biol Pflanzen 42:339-346.

Kao Chen-Hung, Zeng Z and Teasdale R (1999) Multiple interval mapping for quantitative trait loci. Genetics 152: 1203-1216.

Kerber R E, Rowland G G (1974) Origin of the threshing character in hexaploid wheat. Can J Genet Cytol 16: 145-154.

Kerber R E, Dyck P L (1969) Inheritance in hexaploid wheat of leaf rust resistance and other characters derived from *Aegilops squarrosa* Can J Genet Cytol 11:639-647.

Korzun V, Röder M S, Ganal M W, Worland A J, Law CN (1998) Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.) Theor Appl Genet 96:1104–1109.

Kota R S, Gill K S, Gill B S and Endo T R (1993) A cytogenetically based physical map of chromosome 1B in common wheat. Genome 36:548-554.

Kuckuck H (1964) Experimentelle Untersuchungen zur Entstehung der Kulturweizen. Z Pflanzenzuchtg 51:97-140.

Lander E S, Green P, Abrahamson J, Barlow A, Daly M J, Lincoln S E, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic maps of experimental and natural populations. Genomics 1: 174-181.

Leighty C E and Boshnakian S (1921) Genetic behavior of the spelt form in crosses between *Triticum spelta* and *Triticum aestivum*. J Agri Res 7:335–364.

Lijavetzky D, Muzzi G,Wicker T, Keller B, Wing R, and Dubcovsky J (1999) Construction and characterization of a bacterial artificial chromosome (BAC) library for the A genome of wheat. Genome 42:1176–1182.

Luo M C, Yang Z L and Dvorak J (2000) The *Q* locus of Iranian and European spelt wheat. Theor Appl Genet 100:601-606.

Mac Key J (1966) Species relationships in *Triticum*. Proc. 2nd Intern. Wheat Genet. Symp. (Lund) 1963, Sweden. Hereditas (suppl.) 2: 237-276.

Mac Key J (1954) Neutron and X-ray experiments in wheat and a revision of speltoid problem. Hereditas 40:65-180.

Marino C L, Nelson J C, Lu Y H, Sorrels M E, Leroy P, Lopes C R, Hart G E (1996) RFLP-based linkage maps of the homoeologous group 6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell). Genome 39:359-366.

McFadden E S, Sears E R (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37:81-116.

Mickelson-Young L, Endo T R, Gill B S (1995) A cytogenetic ladder-map of the wheat homoeologous group-4 chromosomes. Theor Appl Genet 90:1007-1011.

Muramatsu M (1986) The *vulgare* super gene, *Q*: its universality in *durum* wheat and its phenotypic effects in tetraploid and hexaploid wheats. Can J Genet Cytol 28:30-41.

Muramatsu M (1963) Dosage effect of the *spelta* gene q of hexaploid wheat. Genetics 48:469–482.

Nelson J C, Autrique J E, Fuentes-D'vila G, Sorrells M E (1998) Chromosomal location of genes for resistance to karnal bunt in wheat. Crop Sci 38:231-236.

Nelson J C, Singh R P, Autrique J E, Sorrells M E (1997) Mapping genes conferring and suppressing leaf rust resistance in wheat. Crop Sci 37:1928-1935.

Nelson J C, Sorrells M E, Van Deynze A E, Lu YH, Atkinson M, Bernard M, Leroy P, Faris J D, and Anderson J A (1995a) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141:721-731.

Nelson J C, Van Deynze A E, Autrique E, Sorrells M E, Lu Y H, Merlino M, Atkinson M, Leroy P (1995b) Molecular mapping of wheat homoeologous group 2. Genome 38:516-524.

Nelson J C, Van Deynze A E, Autrique E, Sorrells M E, Lu Y H, Negre S, Bernard M, Leroy P (1995c) Molecular mapping of wheat homoeologous group 3. Genome 38:525-533.

Ogihara Y, Hasegawa K, Tsujimoto H (1994) High-resolution cytological mapping of the long arm of chromosome 5A in common wheat using a series of deletion lines induced by gametocidal (Gc) genes of *Aegilops speltoides*.. Mol Gen Genet 244:253-259.

Paterson A H, Damon S, Hewitt J D, Zamir D, Rabinowitch H D, Lincoln S E, Lander E S, Tanksley S D (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics 127:181-197.

Pestsova E, Ganal M W, Röder M S (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689-697.

Rafter A J, Abell M L, Braselton P J (2002) Multiple comparison methods for means. SIAM Rev. 40(2):259-278.

Riera-Lizarazu O, Vales M I, Ananiev E V, Rines H W and Phillips R L (2000) Production and characterization of maize chromosome 9 radiation hybrids derived from an oat-maize addition line. Genetics 156:327-339.

Röder M S, Korzun V, Wendehake K, Plaschke J, Tixier M H, Leroy P, Ganal M W (1998) A microsatellite map of wheat. Genetics 149:2007-2023.

SAS Institute, Inc. (1989) SAS/STAT Users Guide: version 6, volume 2, 4th ed. SAS Institute, Cary, North Carolina.

Sears E R (1954) The aneuploids of common wheat. Columbia, Mo., University of Missouri: 3-58.

Sears E R (1946) The *sphaerococcum* gene in wheat. Rec Genet Soc Amer 15:65-66.

Simonetti M C, Bellomo M P, Laghetti G, Perrino P, Simeone R, Blanco A (1999) Quantitative trait loci influencing free-threshing habit in tetraploid wheats. Genet Res Crop Evol 46:267-271.

Smilde W D, Haluśkova J, Sasaki T and Graner (2001) New evidence for the syntenty of rice chromosome 1 and barley chromosome 3H from rice expressed sequence tags. Genome 44:361-467.

Somers D J, Issac P and Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109: 1105-1114.

Sourdille P, Perretant M R, Charmet G, Leroy P, Gautier M F, Joudrier P, Nelson J C, Sorrells M E, Bernard M (1996) Linkage between RFLP markers and genes affecting kernel hardness in wheat. Theor Appl Genet 93(4): 580-586.

Staub J E, Serquen F C, Gupta M (1996) Genetic markers, map construction, and their application in plant breeding. HortScience 31: 729-741.

Taenzler B, Esposti R F, Vaccino P, Brandolini A, Effgen S, Heun M, Schafer-Pregl R, Borghi B, Salamini F (2002) Molecular linkage map of Einkorn wheat: mapping of storage-protein and soft-glum genes and bread-making quality QTLs. Genet Res Camb 80: 131-143.

Ternowskaya T K and Zhirov E G (1993) Bread wheat genome D. Genetic control of tender glume and depression at its base. Tsitologiya I Genetica 27:78–83.

Tsunewaki K (1966) Comparative gene analysis of common wheat and its ancestral species. II. Waxiness, growth habit and awnedness. Japan J Bot 19:175-229.

Unrau J (1950) The use of monosomic and nullisomics in cytogenetic studies of common wheat. Sci Agr 30:66-89.

Van Deynze A E, Dubcovsky J, Gill K S, Nelson J C, Sorrels M E, Dvořák J, Gill B S, Lagudah E S, McCouch S R, Appels R (1995) Molecular-genetic maps for

group 1 chromosomes of *Triticeae* species and their relation to chromosomes in rice and oat. Genome 38:45-59.

Villareal R L, Mujeeb-Kazi, Rajaram S (1996) Inheritance of threshability in synthetic hexaploid (*Triticum turgidum* x *T. tauschii*) by *T. aestivum* crosses. Plant Breeding 115:4407-4414.

Whittaker J, Thompson R and Visscher P M (1996) On the mapping of QTL by regression of phenotype on marker-type. Heredity **77**:23-32.

Zeng Z (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc Natl Acad Sci USA. 90:10972-10976.

Zeng Z (1994) Precision mapping of quantitative trait loci. Genetics 136:1457-1468.

Chapter 4

Conclusions

The goal of this thesis was to broaden our understanding of the genetic basis of domestication traits in wheat by addressing the following objectives:

i. To develop linkage maps of chromosomes 3A and 3B of tetraploid wheat;
ii. To genetically map genes for the brittle rachis character, *Br-A2* and *Br-A3*;
iii. To generate microsatellite-based linkage maps of the short arm of chromosome 2D of hexaploid wheat; and
iv. To localize factors affecting glume tenacity and free-threshing character on chromosome 2D.

Objectives (i.) and (ii.) were addressed in a study presented in chapter 2. In this study, two mapping populations (RICL-3A and RICL-3B) were used to develop linkage maps of chromosomes 3A and 3B and to localize the brittle rachis loci, *Br-A2* and *Br*-A3. The *Br-A2* locus was localized between *Xgwm2* and *Xbarc19* on chromosome 3A and *Br*-A3 was mapped to the *Xbarc218* - *Xwmc540* interval on the short arm of chromosome 3B (Figure 2.4 B and 2.4 C). These results are consistent with the reported chromosomal locations of these genes (Watanabe et al., 2002).

Deletion mapping placed *Br-A2* and *Br-A3* into deletion bins 3AS4-0.45-1.00 and 3BS-9 0.57-0.78, respectively. Comparative mapping analyses with the consensus

physical maps of homoeologous group 3 chromosomes described by Erayman et al. (2004) indicate that *Br-A2* and *Br-A3* were localized in a minor gene rich region (GRR) termed 3S0.8 (Figure 2.4 A). This GRR has a physical size of 25 Mb and accounts for 39% of recombination. The estimated frequency of recombination in GRR 3S0.8 was 580 Kb/cM. These estimates indicate that cloning *Br-A2* and *Br-A3* using map-based methods will be extremely challenging.

Objectives (iii.) and (iv.) were addressed in a study presented in chapter 3. In this study, two mapping populations and a quantitative mapping approach were used to localize factors affecting the free-threshing character on chromosome 2D of common wheat (*Triticum aestivum* L). In analyses using the ITMI RIL population, two coincident QTL that affected both glume tenacity and percent threshability were identified. Subsequently, loci underlying these QTL were genetically localized on the linkage map of chromosome 2D. A locus corresponding to *tenacious glumes 1* (*Tg1*) was localized to a ~23-cM interval between the markers *Xwmc25* and *Xgdm107* and a locus distinct from *Tg1*, named *Tg3*, was mapped to an 11.3-cM interval between *Xbcd102* and *Xgwm455* (Figure 3.4 A). Similarly, analyses using the CS/CS2D mapping population allowed the identification of two QTL that affected glume tenacity. A discrete genetic locus for one of these QTL was placed in an interval that corresponded to the genetic location of *Tg1* observed in the ITMI mapping population. Thus, this locus is likely to also represent *Tg1* (Figure 3.4 A). We also found that this locus had both additive and dominance effects but had mostly an additive mode of inheritance that was consistent with earlier descriptions of *Tg1* as a semi-dominant gene (Kerber and Rowland, 1974). We were unable to precisely localize *QGt.orst-*

2D.3, another QTL that affected glume tenacity in the CS/CS2D population (Figure 3.4 A).

Deletion mapping placed *Tg1* and *Tg3* in deletion bin 2DS5-0.47-1.0. *QGt.orst-2D.3* was placed in deletion bin C-2DS-0.33 (Figure 3.4 C). Comparative mapping analyses with the consensus physical maps of homoeologous group 2 chromosomes described by Erayman et al. (2004) indicate that *Tg1* and *Tg3* were located in GRR 2S0.8. The estimated frequency of recombination in this GRR was 215 Kb/cM. On the other hand, *QGt.orst-2D.3* was placed in GRR 2S0.5 with an estimated recombination frequency of 1.5 Mb/cM. These estimates indicate that cloning *Tg1* and *Tg3* using map-based methods is feasible while cloning *QGt.orst-2D.3* will be extremely challenging if not impossible.

In summary, this research yielded the location of five genetic loci that affected rachis brittleness (*Br-A2* and *Br-A3*) and glume tenacity (*Tg1*, *Tg3*, *QGt.orst-2D.3*) in wheat. Our analysis suggests that map-based cloning of *Br-A2, Br-A3,* and *QGt.orst-2D.3* will be extremely difficult since they reside in chromosomal areas with suppressed recombination. On the other hand, the isolation of *Tg1* and *Tg3* using map-based methods will be feasible since they are located in a chromosomal region with a moderate level of recombination. Information from model species such as *Arabidopsis thaliana*, *Oryza sativa*, and *Zea mays* may help identify candidate genes that underlie *Br-A2* and *Br-A3* since these regulate the formation of abscission zones, a process that is ubiquitous in plants and which may have conserved regulation (Paterson et al., 2001). Similarly, a candidate gene approach may help the identification of *Tg1*, *Tg3,* and *QGt.orst-2D.3* since genetic analysis suggest these genes should directly or

indirectly interact with *Q*, an AP2-like homeotic transcription factor involved in flower development (Faris et al., 2003).

Bibliography

Basten C J, Weir B S and Zeng Z B (1999) QTL Cartographer Version 1.13, A reference manual and tutorial for QTL mapping. http://www.statgen.ncsu.edu/qtlcart/cartographer.html

Botstein D, White R, Skolnick M, Davis R (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314-331.

Boyko E, Kalendar R, Korzun V, Fellers J, Korol A, Schulman, A H and Gill B S (2002) A high-density cytogenetic map of the *Aegilops tauschii* genome incorporating retrotransposons and defense-related genes: insights into cereal chromosome structure and function. Plant Mol Biol 48:767-790.

Cai H W and Morishima H (2000) Genomic regions affecting seed shattering and seed dormancy in rice. Theor Appl Genet 100:840-846.

Cantrell R G and Joppa L R (1991) Genetic analysis of quantitative traits in wild emmer (*Triticum turgidum* L. var. *dicoccoides*). Crop Sci 31:645–649.

Cao W, Scoles G J and Hucl P (1997) The genetics of rachis fragility and glume tenacity in semi-wild wheat. Euphytica 94:119-124.

Cenci A, Chantret N, Kong X, Gu Y, Anderson O D, Fahima T, Distfield A, Dubcovsky J (2003) Construction and characterization of a half million clone BAC library of durum wheat (*Triticum turgidum* ssp. *durum*). Theor Appl Genet 107:931-939.

Chen Q-F (2001) Inheritance of disarticulation derived from some hexaploid brittle rachis wheat. Genet Res Crop Evol 48:21-25.

Chen Q-F, Yen C and Yang J-L (1998) Chromosome location of the gene for brittle rachis in the Tibetan weedrace of common wheat. Genet Res Crop Evol 45: 21-25.

Conley EJ , Nduati V, Gonzalez-Hernandez J L, Mesfin A, Trudeau-Spanjers M, Chao S, Lazo G R, Hummel D D, Anderson O D, Qi LL, Gill B S, Echalier B, Linkiewicz A M, Dubcovsky J, Akhunov E D, Dvorak J, Peng J H, Lapitan NL, Pathan MS, Nguyen H T, Ma X F, Miftahudin, Gustafson J P, Greene R A, Sorrells M E, Hossain K G, Kalavacharla V, Kianian SF, Sidhu D, Dilbirligi M, Gill K S, Choi D W, Fenton R D, Close T J, McGuire P E, Qualset C O and Anderson J A (2004). A 2600-locus chromosome bin map of wheat homoeologous group 2 reveals interstitial gene-rich islands and colinearity with rice. Genetics 168: 625-637.

Cox T S (1998) Deepening the wheat gene pool. J Crop Prod 1:1-25.

Delaney D E, Nasuda S, Endo T R, Gill B S, Hulbert S H (1995a) Cytologically based physical maps of the group-2 chromosomes of wheat. Theor Appl Genet 91:568-573.

Delaney D E, Nasuda S, Endo T R, Gill B S and Hulbert S H (1995b) Cytologically based physical maps of the group-3 chromosomes of wheat. Theor Appl Genet 91:780-782.

Del Blanco I A, Rajaram S, Kronstad W E (2001) Agronomic potential of synthetic hexaploid wheat-derived populations. Crop Sci 41:670-676.

Davies M S and Hillman G C (1992) Domestication of cereals. In Chapman G C (ed) Grass evolution and domestication, University press, Cambridge, pp 199-224.

Dvorak J, Luo M C, Yang, Z L, and Zhang, H B (1998) The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor. Appl. Genet. 67:657-670.

Dvorak J and Zhang H B (1990) Variation in repeated nucleotide sequences sheds light on the origin of the wheat B and G genomes. Proc Natl Acad Sci 87:9640-9644.

Dvorak J, DiTerlizzi P, Zhang H B and Resta P (1993) The evolution of polyploid wheat: Identification of the A genome donor species. Genome, 36:21-31.

Endo T R, Gill B S (1996) The deletion stocks of common wheat. J Hered 87: 295-307.

Elias E M, Steiger D K and Cantrell R G (1996) Evaluation of lines derived from wild emmer chromosome substitutions. II. Agronomic traits. Crop Sci 36:228–233.

Erayman M, Sandhu D, Sidhu D, Dilbirligi M, Baenziger P S and Gill K S (2004) Demarcating gene-rich regions of the wheat genome. Nucleic Acid Res 32(12):3546-3565.

Faris J D, Fellers J P, Brooks S A, Gill B S (2003) A bacterial artificial chromosome contig spanning the major domestication locus *Q* in wheat and identification of a candidate gene. Genetics 164:311-321.

Faris J D, Anderson J A, Francl L J, Jordahl J G (1997) RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor Appl Genet 94:98-103.

Feldman M (2001) Origin of cultivated wheat. In Bonjean A P and Angus J W (Eds) The world wheat book. Lavosier Publishing, Paris, pp 3-58.

Friebe B. and Gill B S (1996). Chromosome banding and genome analysis in diploid and cultivated polyploid wheats. In Methods of genome analysis in plants, Jauhar, P. P (ed), CRC Press, Boca Raton, Fla, pp. 39-60.

Gill B S, Friebe B and Endo T R (1991) Standard karyotype and nomenclature system for description of chromosome bands and aberrations in wheat (*Triticum aestivum*). Genome 34: 830-839.

Harlan, J R and Zohary, D (1966) Distribution of wild wheats and barley. Science 153: 1074–1080.

Harlan J R (1992) Origins and processes of domestication. In: Chapman G P (Ed), Grass evolution and domestication. University Press, Cambridge, pp 159-175.

Harris D R (1998) The origins of agriculture in southwest Asia. Rev Archaeol 19:5-11.

Hart G E (2001) Molecular-marker maps of the cultivated wheats and other *Triticum* species. in: Phillips R L and Vasil I K (Eds) DNA-based markers in plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp.421-442.

Haley C S and Knott S A (1992) A simple method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315-324.

Heyne E G (1987) The development of wheat in Kansas. In: Ham G E and. Higham R (Eds.), The rise of the wheat state, pp. 41–56. Sunflower University Press, Manhattan, KS.

Hohmann U, Endo T R. Gill K S, Gill B S (1994) Comparison of genetic and physical maps of group 7 chromosomes from *Triticum aestivum* L. Mol Gen Genet 245:644-653.

Huang S, Sirikhachornkit A, Faris J, Gill B S, Haselkorn R, Gornicki P (2002) Phylogenetic analysis of the acetyl-CoA carboxylase and 3-phosphoglycerate kinase loci in wheat and other grasses. Plant Mol Biol 48:805-820.

Jantasuriyarat C, Vales M I, and Riera-Lizarazu O (2004) Identification and mapping of Genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.). Theor Appl Genet 108:261-273.

Johnson B L (1972) Protein electrophoretic profiles and the origin of the B genome of wheat. Proc Natl Acad Sci 69: 1398-1402.

Joppa L R (1993) Chromosome engineering in tetraploid wheat. Crop Sci 33: 908-913.

Kabarity A (1966) On the origin of the new cultivated wheat II. Cytogenetical studies on the karyotypes of some *Triticum macha* varieties. Beitr Biol Pflanzen 42:339-346.

Kao C-H, Zeng Z and Teasdale R (1999) Multiple interval mapping for quantitative trait loci. Genetics 152: 1203-1216.

Kerber R E and Rowland G G (1974) Origin of the threshing character in hexaploid wheat. Can J Genet Cytol 16: 145-154.

Kerber R E, Dyck P L (1969) Inheritance in hexaploid wheat of leaf rust resistance and other characters derived from *Aegilops squarrosa* Can J Genet Cytol 11:639-647.

Kerby K. and Kuspira J (1988) Cytological evidence bearing on the origin of the B genome in polyploid wheats. Genome 30: 36-43.

Kihara H (1944) Discovery of the DD-analyzer, one of the ancestors of vulgare wheats. Ag Hort., (Tokyo) 19: 889-890.

Kimber G and Feldman M (1987a) Wild wheat. University of Missouri Press, Columbia, Mo.

Kimber G and Sears E R (1987b) Evolution in the genus *Triticum* and the origin of cultivated wheat. In Heyne G (Ed) Wheat and Wheat Improvement, Agronomy 13:154-164.

King I P, Law C N, Cant K A, Orford S E, Reader S M and Miller T E (1997) *Tritipyrum* a potential new salt-tolerant cereal. Plant Breed 116:127-132.

Kislev M E (1984) Emergence of wheat agriculture. Palaeorient 10/2:61-70.

Komatsuda T and Mano Y (2002). Molecular mapping of the intermedium spike-c (*int-c*) and non-brittle rachis 1 (*btr1*) loci in barley (*Hordeum vulgare* L.). Theor Appl Genet 105: 85-90.

Korzun V, Röder M S, Ganal M W, Worland A J, Law CN (1998) Genetic Analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.) Theor Appl Genet 96:1104–1109.

Kota R S, Gill K S, Gill B S and Endo T R (1993) A cytogenetically based physical map of chromosome 1B in common wheat. Genome 36:548-554.

Kuckuck H (1964) Experimentelle Untersuchungen zur Entstehung der Kulturweizen. Z Pflanzenzuchtg 51:97-140.

Lander E S, Green P, Abrahamson J, Barlow A, Daly M J, Lincoln S E and Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic maps of experimental and natural populations. Genomics 1: 174-181.

Leighty C E and Boshnakian S (1921) Genetic behavior of the spelt form in crosses between *Triticum spelta* and *Triticum aestivum*. J Agri Res 7:335–364.

Lijavetzky D, Muzzi G,Wicker T, Keller B, Wing R, and Dubcovsky J (1999) Construction and characterization of a bacterial artificial chromosome (BAC) library for the A genome of wheat. Genome 42:1176–1182.

Liu Y-G, Nagaki K, Fujita M, Kawara K, Uozumi M and Ogihara Y (2000) Development of an efficient maintenance and screening system for large-insert genomic DNA libraries of hexaploid wheat in a transformation-competent artificial chromosome (TAC) vector. The Plant Journal 23:687-695.

Luo M C, Yang Z L and Dvorak J (2000) The *Q* locus of Iranian and European spelt wheat. Theor Appl Genet 100:601-606.

Mac Key J (1966) Species relationships in Triticum. Proc. 2nd Intern. Wheat Genet. Symp. (Lund) 1963, Sweden. Hereditas (suppl.) 2: 237-276.

Mac Key J (1954) Neutron and X-ray experiments in wheat and a revision of the speltoid problem. Hereditas 40:65-180.

Marino C L, Nelson J C, Lu Y H, Sorrels M E, Leroy P, Lopes C R, Hart G E (1996) RFLP-based linkage maps of the homoeologous group 6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell). Genome 39:359-366.

McFadden E S and Sears E R (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37:81-116.

Mickelson-Young L, Endo T R, Gill B S (1995) A cytogenetic ladder-map of the wheat homoeologous group-4 chromosomes. Theor Appl Genet 90:1007-1011.

Miller T E, Reader S M, Mahmood A, Purdie K A and King I P (1995) Chromosome 3N of *Ageilops uniristata* – a source of tolerance to high levels of aluminum for wheat. In Z S Li and Z Y Xin (Eds), Proc. 8th Int. Wheat Genet. Symp. 1993, China Agricultural Scientech Press, Beijing, China: 1037-1042.

Morris R and Sears E R (1967) The cytogenetics of wheat and its relatives. In: K S Quisenberry, L P (Ed), Wheat and Wheat Improvement, American Society of Agronomoy, Madison, WI, USA. 19-87.

Munkvold J D, Greene R A, Bermudez-Kandianis C E, La Rota C M, Edwards H, Sorrells S F, Dake T, Benscher D, Kantety R, Linkiewicz A M, Dubcovsky J, Akhunov ED, Dvorak J, Miftahudin, Gustafson J P, Pathan M S, Nguyen H T, Matthews DE, Chao S, Lazo G R, Hummel D D, Anderson O D, Anderson J A, Gonzalez-Hernandez J L, Peng J H, Lapitan N, Qi L L, Echalier B, Gill B S, Hossain K G, Kalavacharla V, Kianian S F, Sandhu D, Erayman M, Gill K S, McGuire P E, Qualset C O, Sorrells M E. Group 3 chromosome bin maps of wheat and their relationship to rice chromosome 1. Genetics 168:639-650.

Muramatsu M (1986) The vulgare super gene, *Q*: its universality in durum wheat and its phenotypic effects in tetraploid and hexaploid wheats. Can J Genet Cytol 28:30-41.

Muramatsu M (1963) Dosage effect of the spelta gene q of hexaploid wheat. Genetics 48:469–482.

Nelson J C, Autrique J E, Fuentes-D'vila, Sorrells M E (1998) Chromosomal location of genes for resistance to karnal bunt in wheat. Crop Sci 38:231-236.

Nelson J C, Singh R P, Autrique J E, Sorrells M E (1997) Mapping genes conferring and suppressing leaf rust resistance in wheat. Crop Sci 37:1928-1935.

Nelson J C, Sorrells M E, Van Deynze A E, Lu YH, Atkinson M, Bernard M, Leroy P, Faris J D, and Anderson J A (1995a) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141:721-731.

Nelson J C, Van Deynze A E, Autrique E, Sorrells M E, Lu Y H, Merlino M, Atkinson M, Leroy P (1995b) Molecular mapping of wheat homoeologous group 2. Genome 38:516-524.

Nelson J C, Van Deynze A E, Autrique E, Sorrells M E, Lu Y H, Negre S, Bernard M, Leroy P (1995c) Molecular mapping of wheat homoeologous group 3. Genome 38:525-533.

Nishikawa K, Furuta Y, Yamada T, Kudo S (1992) Genetic studies of a-amylase isozymes in wheat. VII. Variation in diploid ancestral species and phylogeny of tetraploid wheat. Jpn J Genet 67:1–15.

Ogihara Y, Hasegawa K, Tsujimoto H (1994) High-resolution cytological mapping of the long arm of chromosome 5A in common wheat using a series of deletion

lines induced by gametocidal (Gc) genes of *Aegilops speltoides*. Mol Gen Genet 244:253-259.

Otto C D, Kianian S F, Elias E M, Stack R W and Joppa L R (2002) Genetic dissection of a major Fusarium head blight QTL in tetraploid wheat. Plant Mol. Biol. 48:625-632.

Paterson A H, Damon S, Hewitt J D, Zamir D, Rabinowitch H D, Lincoln S E, Lander E S, Tanksley S D (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics 127:181-197.

Peng J, Ronin Y, Fhima T, Röder M S, Li Y, Nevo E and Korol A (2003) Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. Proc Natl Acad Sci 100: 2489-2494.

Pestsova E, Ganal M W, Röder M S (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689-697.

Phillips R L and Vasil I K (1994) Introduction: molecular marker maps of major crop species. In: Phillips R L and Vasil I K (Eds) DNA-based markers in plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp.421-442.

Qi X, Stam P, Lindhout P (1996) Comparison and integration of four barley genetic maps. Genome 39: 379-394.

Rafter A J, Abell M L, Braselton P J (2002) Multiple comparison methods for means. SIAM Rev. 40(2):259-278.

Rao M V P (1977) Mapping of *sphaerococcum* gene 'S' on chromosome 3D of wheat. Cer Res Commun, 5:15-17.

Rao M V P (1972) Mapping of *compactum* gene 'C' on chromosome 2D of wheat. Wheat Inf Serv, 35:9.

Riera-Lizarazu O, Vales M I, Ananiev E V, Rines H W and Phillips R L (2000) Production and characterization of maize chromosome 9 radiation hybrids derived from an oat-maize addition line. Genetics 156:327-339.

Riley R G, Kumber G and Law C N (1966) Correspondence between wheat and alien chromosomes. Ann Rep Plant Breed Inst 1964-65:108-109.

Riley R., Unrau J. and Chapman V (1958) Evidence on the origin of the B genome of wheat. J Hered 49: 91-98.

Röder M S, Korzun V, Wendehake K, Plaschke J, Tixier M H, Leroy P, Ganal M W (1998) A microsatellite map of wheat. Genetics 149:2007-2023.

Salamini F, Özkan H, Brandolini A, Schäfer-Pregl R, and Martin W (2002) Genetics and geography of wild cereal domestication in the near east. Nature Rev Genet 3:429-441.

SAS Institute, Inc. (1989) SAS/STAT Users Guide: version 6, volume 2, 4th ed. SAS Institute, Cary, North Carolina.

Sax K (1923) The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552.

Sears E R (1966) Nullisomic-tetrasomic combinations in hexaploid wheat. In: Lewis D R (Ed) Chromosome manipulation and plant genetics pp 29-47. Oliver and Boyd, London.

Sears E R (1954) The aneuploids of common wheat. Columbia, Mo., University of Missouri: 3-58.

Sears E R (1946) The sphaerococcum gene in wheat. Rec Genet Soc Amer 15:65-66.

Shao Q, Li C and Basang C (1983) Semi-wild wheat from Xizang (Tibet). Proc 6th Intern Wheat Genet Symp Kyoto, Japan 111-114.

Sharma, H C and Waines, J G (1980) Inheritance of tough rachis in crosses of *Triticum monococcum* and *T. boeticum*. J. Hered. 71: 214 - 216.

Simonetti M C, Bellomo M P, Laghetti G, Perrino P, Simeone R, Blanco A (1999) Quantitative trait loci influencing free-threshing habit in tetraploid wheats. Genet Res Crop Evol 46:267-271.

Smilde W D, Haluśkova J, Sasaki T and Graner (2001) New evidence for the synteny of rice chromosome 1 and barley chromosome 3H from rice expressed sequence tags. Genome 44:361-467.

Somers D J, Issac P and Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109: 1105-1114.

Sorrells M E, La Rota M, Bermudez-Kandianis C E, Greene R A, Kantety R, Munkvold J D, Miftahudin, Mahmoud A, Ma X, Gustafson P J, Qi L L, Echalier B, Gill BS, Matthews DE, Lazo GR, Chao S, Anderson OD, Edwards H, Linkiewicz A M, Dubcovsky J, Akhunov E D, Dvorak J, Zhang D, Nguyen HT, Peng J, Lapitan N L, Gonzalez-Hernandez J L, Anderson J A, Hossain K, Kalavacharla V, Kianian S F, Choi D W, Close T J, Dilbirligi M, Gill KS,

Steber C, Walker-Simmons M K, McGuire P E, Qualset CO (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818-1827.

Sourdille P, Perretant M R, Charmet G, Leroy P, Gautier M F, Joudrier P, Nelson J C, Sorrells M E, Bernard M (1996) Linkage between RFLP markers and genes affecting kernel hardness in wheat. Theor Appl Genet 93(4): 580-586.

Staub J E, Serquen F C, Gupta M (1996) Genetic markers, map construction, and their application in plant breeding. HortScience 31: 729-741.

Szabó A T and Hammer K (1995) In: Padulosi S, Hammer K and Heller J (Eds) Hulled Wheats. Promoting the Conservation and Use of Underutilized and Neglected Crops. 4. Proc. First Int. Workshop on Hulled Wheats. Castelvecchio Pascoli, Tuscany, Italy. 2–30.

Taenzler B, Esposti R F, Vaccino P, Brandolini A, Effgen S, Heun M, Schafer-Pregl R, Borghi B, Salamini F (2002) Molecular linkage map of Einkorn wheat: mapping of storage-protein and soft-glum genes and bread-making quality QTLs. Genet Res Camb 80: 131-143.

Takahashi R and Hayashi J (1964) Linkage study of two complementary genes for brittle rachis in barley. Ber Ohara Inst Landw Biol, Okayama Univ. 12:99-105.

Ternowskaya T K and Zhirov E G (1993) Bread wheat genome D. Genetic control of tender glume and depression at its base. Tsitologiya I Genetica 27:78–83.

Tsunewaki K. and Ogihara Y (1983) The molecular basis of cytoplasmic diversity among cytoplasms of *Triticum* and *Aegilops* species. II. On the origin of polyploid wheat cytoplasms as suggested by chloroplast DNA patterns. Genetics 104: 155-171.

Tsunewaki K (1966) Comparative gene analysis of common wheat and its ancestral species. II. Waxiness, growth habit and awnedness. Japan J Bot 19:175-229.

Unrau J (1950) The use of monosomic and nullisomics in cytogenetic studies of common wheat. Sci Agr 30:66-89.

Urbano M, Rest P, Benedettelli S and Blanco A (1988) A Dasypyrum cillosum (L.) Candargy chromosome related to homoeologous group 3 of wheat. In. T E Miller and R M D Koebner (Eds), Proc 7th Internatl Wheat Genet Symp:169-173. IPSR Cambridge Lab, Cambridge, UK.

Van Deynze A E, Dubcovsky J, Gill K S, Nelson J C, Sorrels M E, Dvořák J, Gill B S, Lagudah E S, McCouch S R, Appels R (1995) Molecular-genetic maps for

group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. Genome 38:45-59.

Villareal R L, Mujeeb-Kazi, Rajaram S (1996) Inheritance of threshability in synthetic hexaploid (*Triticum turgidum* x *T. tauschii*) by *T. aestivum* crosses. Plant Breeding 115:4407-4414.

Watanabe N, Sugiyama K, Yamagashi Y and Sakata Y (2002) Comparative telosomic mapping of homoeologous genes for brittle rachis in tetraploid and hexaploid wheats. Hereditas 137:180-185.

Watanabe N and Ikebata, N (2000) The effects of homoeologous group 3 chromosomes on grain color dependent seed dormancy and brittle rachis in tetraploid wheat. Euphytica 115: 215-220.

Whittaker J, Thompson R and Visscher P M (1996) On the mapping of QTL by regression of phenotype on marker-type. Heredity **77**:23-32.

Witcombe J R (1983) A guide to the species of Aegilops. L. I. B. P. G. R. Secretariat, Rome.

Xiong L Z, Liu K D, Dai X K, Xu C G and Zhang Q (1999) Identification of genetic factors controlling domestication-related traits of rice using a F_2 population of a cross between *Oryza sativa* and *O. rufipogon*. Theor Appl Genet 98:243-251.

Zeng Z (1994) Precision mapping of quantitative trait loci. Genetics 136:1457-1468.

Zeng Z (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc Natl Acad Sci USA. 90:10972-10976.

Zohary D and Hopf M (1993) Domestication of plants in the old world – The origin and spread of plants in West Asia, Europe and the Nile Valley. Claredon Press, Oxford.

Appendices

Appendix 1 Microsatellite segregation data for the RICL-3A mapping population. A score of 0 represents allele from LDN, 1 from LDN (Dic-3A) and '-' represents missing data. RL indicates Recombinant Inbred Chromosome Line

Lines	*Xbarc294*	*Xbarc310*	*Xbarc12*	*Xgwm369*	*Xcdo395*	*Xcdo1435*	*Xbcd1532*	*Xbarc45*	*Xgwm2*	*Br-A2*	*Xgwm666.1*	*Xbarc19*	*Xcfa2164b*	*Xbarc356*
RL01	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL02	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL03	1	1	1	1	1	1	1	1	1	1	0	0	0	0
RL04	1	1	1	0	0	0	0	0	0	-	0	0	0	0
RL05	1	0	0	0	0	0	0	0	0	0	0	-	0	0
RL06	-	0	0	0	0	0	0	0	0	-	0	0	0	0
RL07	1	1	1	1	1	1	1	1	0	0	0	0	0	0
RL08	0	0	0	-	1	1	1	1	1	0	1	1	1	1
RL09	0	0	0	-	0	0	0	0	0	0	0	0	0	0
RL10	0	0	0	0	1	1	1	1	1	1	1	1	1	1
RL11	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL12	0	0	-	0	0	0	0	0	0	0	0	0	0	0
RL13	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL14	0	0	0	1	1	1	1	1	1	1	1	1	1	1
RL15	1	1	1	1	1	1	1	1	0	0	0	0	0	0
RL16	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL17	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL18	1	1	1	0	0	0	0	0	0	0	0	0	0	0
RL19	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL20	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL21	0	0	0	0	0	-	0	0	0	0	0	0	0	0
RL22	0	0	0	1	1	1	1	1	1	1	1	1	1	1
RL23	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL24	1	1	1	1	1	1	1	1	1	0	0	0	0	0
RL25	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Appendix 1 (Continued)

Lines	*Xbarc294*	*Xbarc310*	*Xbarc12*	*Xgwm369*	*Xcdo395*	*Xcdo1435*	*Xbcd1532*	*Xbarc45*	*Xgwm2*	*Br-A2*	*Xgwm666.1*	*Xbarc19*	*Xcfa2164b*	*Xbarc356*
RL25	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL26	1	1	1	-	0	0	0	0	0	0	0	0	0	0
RL27	-	1	1	0	1	0	0	0	-	0	0	0	0	0
RL28	0	0	0	0	0	0	0	0	0	0	-	1	1	1
RL29	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL30	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL31	0	0	0	0	0	0	0	0	1	0	0	0	0	0
RL32	0	0	0	1	1	1	1	1	1	1	1	1	1	1
RL33	1	1	1	1	1	1	1	1	0	0	1	1	1	1
RL34	1	1	1	0	0	0	0	0	1	0	0	0	0	0
RL35	1	1	1	0	0	0	0	0	1	0	1	1	1	1
RL36	0	0	0	0	0	0	0	0	0	0	1	1	1	1
RL37	0	0	0	0	0	0	0	0	0	0	0	0	-	0
RL38	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL39	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL40	0	0	0	1	1	1	1	1	-	1	1	1	1	1
RL41	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL42	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL43	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL44	1	1	-	1	0	0	0	0	0	0	0	0	0	0
RL45	1	1	1	1	0	0	0	0	0	0	0	0	0	0
RL46	1	1	1	0	1	1	1	1	1	0	0	0	0	0
RL47	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL48	-	0	-	0	0	0	0	-	-	0	-	-	-	0
RL49	1	1	0	1	1	1	1	1	0	0	1	1	1	0
RL50	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 1 (Continued)

Lines	*Xbarc294*	*Xbarc310*	*Xbarc12*	*Xgwm369*	*Xcdo395*	*Xcdo1435*	*Xbcd1532*	*Xbarc45*	*Xgwm2*	*Br-A2*	*Xgwm666.1*	*Xbarc19*	*Xcfa2164b*	*Xbarc356*
RL51	0	0	0	-	0	0	0	0	0	0	0	0	0	0
RL52	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL53	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL54	0	0	1	0	0	0	0	0	1	0	0	0	0	1
RL55	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL56	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL57	0	0	1	0	0	1	1	0	1	1	0	1	1	1
RL58	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL59	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL60	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL61	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL62	0	0	1	1	1	1	1	-	1	1	0	1	1	1
RL63	0	0	1	1	1	1	1	0	1	1	0	1	1	1
RL64	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL65	1	1	0	0	0	0	0	1	0	0	1	0	-	0
RL66	1	0	0	0	0	0	0	0	-	0	1	0	1	0
RL67	0	0	1	0	0	1	1	0	1	1	0	1	1	1
RL68	1	0	1	0	1	1	1	0	1	1	1	1	1	1
RL69	0	0	0	0	0	0	0	0	0	0	0	-	0	0
RL70	0	1	0	1	0	0	0	1	0	0	0	0	0	0
RL71	-	0	1	1	1	1	1	0	1	1	-	1	1	1
RL72	1	0	1	0	0	1	1	0	-	1	1	1	1	1
RL73	0	1	0	1	1	1	1	1	0	1	0	1	1	0
RL74	0	1	0	0	1	1	1	1	0	0	0	0	0	0
RL75	1	0	0	0	0	0	0	0	0	0	1	0	0	0
RL76	0	1	0	1	0	0	0	1	0	0	0	0	0	0

Appendix 1 (Continued)

Lines	*Xbarc294*	*Xbarc310*	*Xbarc12*	*Xgwm369*	*Xcdo395*	*Xcdo1435*	*Xbcd1532*	*Xbarc45*	*Xgwm2*	*Br-A2*	*Xgwm666.1*	*Xbarc19*	*Xcfa2164b*	*Xbarc356*
RL77	1	0	0	0	0	0	0	0	0	0	1	0	0	0
RL78	1	0	0	0	0	0	0	0	0	0	1	0	0	0
RL79	1	0	0	0	0	0	0	0	0	0	1	0	0	0
RL80	0	1	0	0	1	0	0	1	0	0	0	0	-	0
RL81	1	0	1	0	0	0	0	0	1	1	1	0	0	1
RL82	1	0	0	0	0	0	0	-	0	0	1	0	-	0
RL83	0	1	1	1	1	1	1	1	1	1	0	1	1	1

Appendix 1 (Continued)

Lines	*Xbarc67*	*Xbcd828*	*Xcfa2134*	*Xcfa2037*	*Xbcd115*	*Xbcd2044*	*Xwmc264*	*Xcfa2193*	*Xwmc153*	*Xbarc51*	*Xcfa2076*	*Xwmc169*	*Xgwm666.2*	*Xgwm391*
RL01	0	0	0	0	0	0	0	1	1	1	1	1	1	1
RL02	0	0	0	0	0	0	1	1	1	1	1	1	1	1
RL03	0	0	0	0	0	0	0	0	0	0	0	0	0	1
RL04	0	0	0	0	1	1	-	1	1	1	1	1	1	0
RL05	0	0	0	0	1	1	1	1	1	1	1	1	1	1
RL06	0	0	0	0	1	1	0	0	0	0	0	0	0	0
RL07	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL08	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL09	0	0	0	0	1	1	1	0	1	1	1	1	1	1
RL10	1	1	1	1	0	0	0	0	1	0	0	0	0	0
RL11	1	1	1	1	1	1	-	1	0	0	0	0	0	-
RL12	0	0	0	0	0	0	1	1	1	1	1	1	1	1
RL13	1	1	1	1	1	1	1	1	0	0	0	0	0	0
RL14	1	1	1	1	1	1	1	1	1	1	1	1	1	0
RL15	-	0	0	0	1	1	1	1	1	1	1	1	1	1
RL16	1	1	1	1	1	1	1	1	1	0	0	0	0	0
RL17	0	0	1	1	1	1	1	0	1	1	1	1	1	1
RL18	0	0	0	0	0	0	0	1	1	1	1	1	1	1
RL19	0	0	0	0	0	0	0	1	1	1	1	1	1	1
RL20	1	1	1	1	1	1	1	0	0	0	0	0	0	1
RL21	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL22	1	1	1	1	1	1	0	0	0	0	0	0	0	0
RL23	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL24	0	0	0	0	0	0	1	1	1	1	1	1	1	1
RL25	1	1	1	1	0	0	1	1	1	1	0	0	0	0
RL26	0	0	0	0	0	0	0	1	1	1	1	1	0	0

Appendix 1 (Continued)

Lines	*Xbarc67*	*Xbcd828*	*Xcfa2134*	*Xcfa2037*	*Xbcd115*	*Xbcd2044*	*Xwmc264*	*Xcfa2193*	*Xwmc153*	*Xbarc51*	*Xcfa2076*	*Xwmc169*	*Xgwm666.2*	*Xgwm391*
RL27	0	0	0	0	0	0	0	1	1	1	1	1	1	1
RL28	1	1	1	1	1	1	1	0	0	0	0	0	-	0
RL29	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL30	1	1	1	1	1	1	1	0	0	0	0	0	0	1
RL31	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL32	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL33	1	1	1	1	0	0	-	0	0	0	0	0	0	0
RL34	0	0	0	0	0	0	0	-	0	0	0	0	0	0
RL35	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL36	1	1	1	1	1	1	1	1	0	0	0	0	0	0
RL37	0	1	1	1	1	1	1	-	1	1	1	1	1	1
RL38	0	0	0	0	0	0	1	1	-	1	1	1	1	1
RL39	0	0	0	0	1	1	1	1	-	1	1	1	1	0
RL40	1	1	1	1	1	1	1	1	1	0	0	0	0	0
RL41	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL42	1	1	0	0	0	0	0	0	0	0	0	1	0	-
RL43	1	1	1	1	1	1	1	1	1	1	1	1	1	0
RL44	0	0	0	0	0	0	0	0	0	1	1	1	1	1
RL45	0	0	0	0	0	0	0	1	1	1	1	1	1	0
RL46	0	0	0	0	0	0	0	0	0	?	0	0	0	0
RL47	0	0	0	0	0	0	0	0	0	0	1	1	1	1
RL48	-	0	-	-	0	0	1	-	-	-	-	1	-	1
RL49	0	0	0	0	0	0	0	1	1	1	1	1	1	0
RL50	0	1	1	1	1	1	1	1	1	?	1	1	1	-
RL51	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL52	1	1	1	1	1	1	0	0	0	0	0	0	0	1

Appendix 1 (Continued)

Lines	*Xbarc67*	*Xbcd828*	*Xcfa2134*	*Xcfa2037*	*Xbcd115*	*Xbcd2044*	*Xwmc264*	*Xcfa2193*	*Xwmc153*	*Xbarc51*	*Xcfa2076*	*Xwmc169*	*Xgwm666.2*	*Xgwm391*
RL53	1	1	1	1	1	1	1	1	1	1	1	1	1	0
RL54	1	1	1	1	1	1	1	1	1	1	0	0	0	0
RL55	0	0	0	0	1	1	1	1	1	1	1	1	1	1
RL56	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL57	1	1	1	1	1	1	1	1	1	1	0	0	0	0
RL58	1	1	1	1	1	1	1	1	1	1	1	1	1	0
RL59	0	0	0	0	0	0	0	1	1	1	1	1	1	0
RL60	0	0	1	0	1	1	1	1	1	1	1	1	1	0
RL61	0	0	1	0	0	0	0	1	1	1	0	0	0	0
RL62	1	1	1	1	0	0	0	0	0	0	0	0	0	1
RL63	1	1	1	1	1	1	1	1	1	1	1	1	1	0
RL64	1	1	1	1	1	1	1	-	1	0	0	0	0	0
RL65	0	0	0	0	0	0	0	0	0	0	0	0	0	-
RL66	0	0	0	0	0	0	0	1	1	1	1	1	-	0
RL67	1	1	1	1	1	1	1	1	1	1	1	1	1	1
RL68	1	1	1	1	0	0	1	1	1	1	1	1	1	0
RL69	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL70	0	1	1	1	1	1	-	1	-	1	1	1	0	1
RL71	1	1	1	1	0	0	1	1	1	1	1	-	1	1
RL72	1	1	1	1	0	0	0	0	0	0	0	0	-	0
RL73	0	0	0	0	0	0	1	1	1	1	1	1	1	1
RL74	0	0	0	0	0	0	1	1	1	1	1	1	1	1
RL75	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL76	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RL77	0	1	1	1	1	1	1	1	1	1	1	1	1	1
RL78	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 1 (Continued)

Lines	*Xbarc67*	*Xbcd828*	*Xcfa2134*	*Xcfa2037*	*Xbcd115*	*Xbcd2044*	*Xwmc264*	*Xcfa2193*	*Xwmc153*	*Xbarc51*	*Xcfa2076*	*Xwmc169*	*Xgwm666.2*	*Xgwm391*
RL79	0	0	0	0	1	1	1	1	1	1	1	1	1	1
RL80	0	0	0	0	0	0	-	1	1	1	1	1	1	1
RL81	1	1	1	1	0	0	0	0	0	0	0	0	0	0
RL82	0	0	0	0	0	0	0	0	0	0	0	0	0	-
RL83	1	-	1	1	0	0	0	0	0	0	0	0	1	0

Appendix 2 Microsatellite segregation data for the RICL-3B mapping population. A score of 0 represents allele from LDN, 1 from LDN(Dic-3B) and '-' represents missing data. RL indicates Recombinant Inbred Chromosome Line.

Lines	**Xgwm389*	**Xbarc133*	**Xbarc147*	**Xgwm493*	**Xbarc102*	**Xwmc78*	**Xksum45*	**Xwmc43*	**Xbarc218*	**Br-A3*	**Xwmc540*	**Xwmc777*	**Xgwm264*
RL01	0	0	0	0	0	0	0	0	0	0	0	0	0
RL02	1	1	1	1	1	1	1	1	1	1	1	1	1
RL03	0	0	0	0	1	1	1	1	1	1	1	1	1
RL04	0	0	0	0	0	0	0	0	0	-	0	0	0
RL05	0	0	0	0	0	1	1	1	1	1	1	1	-
RL06	1	1	1	1	1	0	0	0	0	0	0	0	0
RL07	1	1	1	0	0	0	0	0	0	-	0	0	0
RL08	0	0	0	0	0	0	0	0	0	0	0	0	0
RL09	0	0	0	0	0	0	0	0	0	0	0	0	0
RL10	0	0	0	1	1	1	1	1	1	1	1	1	1
RL11	0	0	0	1	1	0	-	0	0	0	0	0	0
RL12	1	1	1	1	1	0	0	0	0	1	0	0	0
RL13	0	0	0	0	0	0	0	0	0	0	1	0	0
RL14	1	1	1	1	1	1	1	1	1	0	1	1	1
RL15	0	1	1	1	1	1	1	1	1	1	1	1	1
RL16	0	0	0	0	0	0	0	0	0	0	0	0	0
RL17	1	1	1	1	1	0	0	0	0	0	0	0	0
RL18	1	1	1	1	1	1	1	1	1	1	1	1	1
RL19	1	1	1	1	1	0	0	0	0	0	0	0	0
RL20	1	-	-	-	-	1	-	-	-	0	0	0	0
RL21	1	1	1	1	1	1	1	1	1	1	0	0	0
RL22	1	1	1	1	0	0	0	0	0	0	0	0	0
RL23	1	1	1	0	0	0	0	0	0	0	0	0	0
RL24	0	0	0	0	0	1	1	1	1	1	1	1	1

Appendix 2 (Continued)

Lines	**Xgwm389*	**Xbarc133*	**Xbarc147*	**Xgwm493*	**Xbarc102*	**Xwmc78*	**Xksum45*	**Xwmc43*	**Xbarc218*	**Br-A3*	**Xwmc540*	**Xwmc777*	**Xgwm264*
RL25	0	0	0	0	0	0	0	0	0	1	1	1	1
RL26	1	1	1	1	1	1	-	1	1	0	0	0	0
RL27	0	0	0	0	0	1	1	1	1	1	1	1	1
RL28	1	1	1	1	1	0	0	0	0	0	0	0	0
RL29	1	1	1	1	1	1	1	1	1	1	1	1	0
RL30	0	1	1	1	1	1	1	1	1	0	0	0	0
RL31	0	1	1	1	1	1	1	1	1	1	1	1	1
RL32	0	1	1	1	1	1	1	1	1	0	1	1	1
RL33	1	1	1	1	1	1	1	1	1	1	1	1	1
RL34	1	1	1	1	1	1	1	1	1	1	1	1	1
RL35	0	0	0	0	0	1	1	1	1	0	0	0	1
RL36	0	0	0	0	0	1	1	1	1	-	1	1	1
RL37	0	0	0	0	0	0	0	0	0	0	0	0	0
RL38	0	1	1	1	1	1	1	1	1	0	0	0	0
RL39	0	1	1	1	1	1	1	1	1	1	1	1	1
RL40	0	0	0	0	0	0	0	0	0	0	0	0	0
RL41	1	1	1	1	1	0	0	0	0	0	0	0	0
RL42	1	1	1	1	1	0	0	0	0	-	0	0	0
RL43	1	1	1	1	1	1	1	1	1	-	1	1	1
RL44	0	0	0	0	0	0	0	0	0	0	0	0	0
RL45	-	0	0	0	0	0	0	0	0	0	0	0	0
RL46	-	1	1	1	1	0	0	-	0	0	0	0	0
RL47	0	0	0	0	1	1	1	1	1	0	0	0	0
RL48	0	0	0	0	0	0	0	0	0	-	1	1	1
RL49	1	1	1	1	1	1	1	1	1	1	1	1	1

Appendix 2 (Continued)

Lines	**Xgwm389*	**Xbarc133*	**Xbarc147*	**Xgwm493*	**Xbarc102*	**Xwmc78*	**Xksum45*	**Xwmc43*	**Xbarc218*	**Br-A3*	**Xwmc540*	**Xwmc777*	**Xgwm264*
RL50	0	0	0	0	0	0	0	0	0	0	0	0	0
RL51	0	0	0	0	0	0	0	0	0	0	0	1	0
RL52	1	-	1	1	1	1	1	1	1	1	0	1	0
RL53	0	0	0	0	0	0	0	1	1	1	1	1	1
RL54	1	1	1	1	1	1	1	1	1	0	0	0	0
RL55	0	0	0	0	0	0	-	0	0	0	0	0	0
RL56	1	1	1	1	1	1	1	1	1	1	1	1	1
RL57	1	1	1	1	1	1	1	-	1	1	1	1	1
RL58	0	0	0	-	0	0	0	0	0	0	0	0	1
RL59	1	-	1	1	1	1	1	1	1	0	0	0	0
RL60	1	1	1	1	1	0	0	0	0	0	0	0	0
RL61	0	1	1	-	1	1	1	1	1	0	0	0	0
RL62	0	1	1	1	1	-	1	1	1	0	0	0	0
RL63	0	-	0	0	0	0	-	0	0	0	1	1	1
RL64	1	1	1	1	1	1	1	1	1	1	1	0	0
RL65	1	1	1	1	1	1	1	1	1	-	0	0	0
RL66	0	1	1	0	0	0	0	0	0	0	0	0	0
RL67	0	-	-	0	-	0	-	0	0	1	1	1	1
RL68	1	1	1	1	1	1	1	1	1	1	1	1	1
RL69	0	1	1	1	1	1	1	1	1	0	1	1	1
RL70	1	1	1	1	1	1	1	1	1	1	1	1	1
RL71	0	0	0	0	0	0	0	0	0	0	0	0	0
RL72	1	-	0	0	0	0	0	0	0	0	0	0	0
RL73	1	1	1	0	0	0	0	0	0	0	0	0	0
RL74	1	1	1	-	1	1	-	-	1	0	0	0	0

Appendix 2 (Continued)

Lines	**Xgwm389*	**Xbarc133*	**Xbarc147*	**Xgwm493*	**Xbarc102*	**Xwmc78*	**Xksum45*	**Xwmc43*	**Xbarc218*	**Br-A3*	**Xwmc540*	**Xwmc777*	**Xgwm264*
RL75	0	0	0	0	0	0	0	0	0	0	0	0	0
RL76	1	1	1	1	1	1	1	1	1	0	0	0	0
RL77	0	0	0	0	0	0	0	0	0	0	0	0	0
RL78	0	0	0	0	0	0	0	0	0	0	0	0	0
RL79	0	0	0	1	1	1	1	1	1	1	1	1	1
RL80	0	0	0	0	0	0	0	0	0	0	0	0	0
RL81	-	0	0	0	0	0	0	0	0	-	1	1	1
RL82	1	1	1	1	1	0	0	0	0	0	0	0	0
RL83	0	0	-	0	0	0	0	0	0	0	0	0	0
RL84	1	1	1	1	1	1	1	1	1	0	1	1	1
RL85	0	1	1	1	1	0	1	1	0	0	0	0	0
RL86	1	1	1	0	0	0	0	0	0	0	0	0	0
RL87	1	1	1	1	1	1	1	1	1	-	0	0	0
RL88	0	1	1	1	1	1	1	1	-	1	1	1	1
RL89	1	1	1	1	1	1	1	1	1	0	0	0	0
RL90	1	1	1	1	1	1	1	1	1	0	1	1	0
RL91	1	-	1	1	-	1	1	-	-	0	1	1	1

Appendix 2 (Continued)

Lines	**Xwmc612*	**Xwmc366*	**Xgwm376*	**Xgwm77*	**Xbarc73*	**Xwmc1*	**Xwmc527*	**Xwmc471*	**Xbarc344*	**Xbarc229*	**Xwmc291*	**Xbarc84*	**Xwmc326*
RL01	0	0	0	0	0	0	0	0	0	0	0	0	0
RL02	1	1	1	1	1	1	1	1	1	1	1	1	1
RL03	1	1	1	1	1	1	1	1	1	1	1	1	1
RL04	0	0	0	0	0	0	0	0	0	0	0	0	0
RL05	1	1	1	1	-	0	0	0	0	0	0	0	0
RL06	0	0	0	0	0	0	0	0	0	0	0	0	0
RL07	0	0	0	0	0	0	0	1	1	1	-	1	1
RL08	0	0	0	0	0	0	0	0	0	0	0	1	1
RL09	0	0	0	0	0	0	0	0	0	0	0	1	1
RL10	1	1	1	1	1	1	1	1	1	1	1	-	1
RL11	0	0	0	0	0	0	0	0	1	1	1	1	1
RL12	0	0	0	0	0	0	0	0	0	0	0	0	0
RL13	0	1	1	1	1	1	1	0	0	0	0	0	1
RL14	1	1	1	1	1	1	1	0	0	0	0	0	0
RL15	1	1	1	1	1	0	0	0	0	1	1	0	0
RL16	0	0	0	0	0	0	0	0	0	0	0	0	0
RL17	0	0	0	0	0	0	0	1	1	1	1	1	1
RL18	1	1	1	1	1	1	1	1	1	1	1	1	1
RL19	0	0	0	0	0	0	0	0	1	1	1	1	1
RL20	0	0	0	0	0	0	0	1	1	0	0	-	-
RL21	0	0	0	0	0	0	0	0	0	1	1	1	1
RL22	-	0	0	0	0	0	0	0	0	0	0	0	0
RL23	0	0	0	0	0	0	0	0	0	0	0	0	0
RL24	1	1	1	1	1	0	0	1	1	1	1	1	1
RL25	1	1	-	1	1	1	1	1	0	0	0	-	0
RL26	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 2 (Continued)

Lines	**Xwmc612*	**Xwmc366*	**Xgwm376*	**Xgwm77*	**Xbarc73*	**Xwmc1*	**Xwmc527*	**Xwmc471*	**Xbarc344*	**Xbarc229*	**Xwmc291*	**Xbarc84*	**Xwmc326*
RL27	1	1	1	1	1	1	1	0	0	0	0	1	1
RL28	0	0	0	0	0	1	1	1	1	1	1	1	1
RL29	0	0	0	0	0	-	0	1	1	1	1	1	1
RL30	0	0	0	0	0	0	0	0	0	0	0	0	0
RL31	1	1	1	1	1	1	1	1	1	1	1	1	1
RL32	1	0	0	0	0	-	0	1	1	0	0	0	0
RL33	1	1	1	1	1	1	1	1	1	-	1	1	1
RL34	1	1	1	1	1	1	-	1	1	-	1	1	1
RL35	0	0	0	-	0	0	0	0	0	0	0	0	0
RL36	1	0	0	0	0	0	0	0	0	0	0	0	0
RL37	0	0	0	0	0	0	0	0	0	0	0	0	0
RL38	-	0	0	0	0	0	0	0	0	0	0	0	0
RL39	1	1	1	1	1	0	0	0	0	-	0	0	0
RL40	0	0	0	0	0	0	0	0	0	0	0	0	0
RL41	0	0	0	0	0	0	0	1	0	1	0	0	0
RL42	0	0	0	0	0	0	0	0	0	0	0	0	0
RL43	1	1	-	0	0	1	1	1	1	1	-	1	1
RL44	0	0	0	-	0	0	0	0	0	0	0	0	0
RL45	0	0	0	0	0	0	0	0	0	0	0	1	1
RL46	0	0	0	0	0	0	0	0	0	0	0	0	0
RL47	0	0	0	0	0	0	0	0	0	0	0	0	0
RL48	1	1	1	1	1	1	1	1	1	0	0	0	0
RL49	1	1	1	1	1	1	1	0	0	1	1	1	1
RL50	0	0	0	0	0	0	0	0	0	-	1	1	1
RL51	0	0	0	0	0	0	0	0	0	0	0	0	0
RL52	0	0	0	0	0	0	0	0	0	1	1	1	1
RL53	1	-	-	1	1	1	1	1	1	0	0	0	0

Appendix 2 (Continued)

Lines	**Xwmc612*	**Xwmc366*	**Xgwm376*	**Xgwm77*	**Xbarc73*	**Xwmc1*	**Xwmc527*	**Xwmc471*	**Xbarc344*	**Xbarc229*	**Xwmc291*	**Xbarc84*	**Xwmc326*
RL54	0	0	0	0	0	0	0	0	0	0	0	0	-
RL55	0	0	0	0	0	0	0	0	0	0	0	0	0
RL56	1	1	1	1	1	1	1	0	0	0	0	0	0
RL57	1	1	1	-	1	1	1	0	0	1	1	1	1
RL58	1	1	1	1	1	1	1	1	1	1	1	1	1
RL59	0	0	0	0	0	0	0	0	0	0	-	0	0
RL60	0	0	0	0	0	0	0	0	0	0	0	0	0
RL61	0	0	0	0	0	0	0	1	1	1	1	1	1
RL62	0	0	0	0	0	0	0	0	0	0	0	0	0
RL63	1	1	1	1	1	1	1	1	1	1	1	1	0
RL64	0	0	0	0	1	0	0	1	1	1	1	1	1
RL65	0	0	-	0	0	0	0	1	1	1	1	0	0
RL66	0	0	0	0	0	0	0	0	0	0	0	1	1
RL67	1	1	1	1	1	1	1	1	1	1	1	-	1
RL68	1	1	1	1	1	1	1	1	1	0	0	0	0
RL69	1	0	0	-	0	0	0	0	0	0	0	0	0
RL70	1	0	0	0	0	0	0	-	0	0	0	0	0
RL71	0	0	0	0	0	-	0	0	0	-	0	1	1
RL72	0	0	0	0	0	0	0	0	0	0	0	0	0
RL73	0	1	1	1	1	1	1	1	1	1	1	1	1
RL74	0	0	0	0	0	0	-	0	0	0	0	0	0
RL75	0	0	0	0	0	0	0	1	1	1	1	1	1
RL76	0	0	0	0	0	0	0	0	0	0	0	0	0
RL77	0	0	0	0	0	0	0	0	0	0	0	0	0
RL78	0	0	0	0	0	0	0	0	0	0	0	0	0
RL79	1	1	1	1	1	1	1	0	0	0	0	0	0
RL80	0	0	0	0	0	0	0	0	1	1	1	1	1

Appendix 2 (Continued)

Lines	**Xwmc612*	**Xwmc366*	**Xgwm376*	**Xgwm77*	**Xbarc73*	**Xwmc1*	**Xwmc527*	**Xwmc471*	**Xbarc344*	**Xbarc229*	**Xwmc291*	**Xbarc84*	**Xwmc326*
RL81	1	1	1	1	1	1	1	0	0	0	0	0	0
RL82	0	0	0	0	0	0	0	0	1	1	1	1	1
RL83	0	0	0	0	0	0	0	1	1	1	1	1	1
RL84	1	1	1	1	1	1	1	1	1	1	1	1	1
RL85	0	0	0	0	0	0	0	0	0	0	0	0	-
RL86	0	0	0	0	0	0	0	0	0	0	0	0	0
RL87	0	0	0	0	0	0	0	1	1	1	1	1	1
RL88	1	1	1	1	1	1	1	1	1	1	1	0	0
RL89	0	0	0	0	0	0	0	0	0	0	0	0	0
RL90	0	0	0	0	0	0	0	0	0	0	0	0	0
RL91	1	1	-	1	1	1	1	1	1	1	1	1	1

Appendix 2 (Continued)

Lines	**Xbarc77*	**Xbarc1124*	**Xwmc632*	**Xgwm247*	**Xgwm181*	**Xgwm340*	**Xgwm547*
RL01	0	0	0	0	0	0	0
RL02	1	1	1	1	1	1	1
RL03	1	1	1	1	1	1	1
RL04	1	1	1	1	1	1	1
RL05	0	0	0	0	0	0	0
RL06	0	1	1	1	1	1	1
RL07	1	1	1	1	1	1	1
RL08	0	0	0	-	0	0	0
RL09	1	1	1	1	1	1	1
RL10	1	1	1	1	1	1	1
RL11	1	1	1	1	1	1	1
RL12	0	0	0	0	0	0	0
RL13	1	1	1	1	1	1	1
RL14	0	0	0	0	0	0	0
RL15	0	0	0	0	0	0	0
RL16	0	0	0	0	0	0	0
RL17	1	1	0	0	0	0	0
RL18	1	1	1	1	1	1	1
RL19	1	1	1	1	1	1	1
RL20	-	-	0	0	0	0	0
RL21	1	1	1	1	1	1	1
RL22	0	0	0	0	0	0	0
RL23	0	0	0	0	-	0	0
RL24	1	1	1	1	1	1	1
RL25	0	1	1	1	1	1	1
RL26	0	0	0	0	0	0	0

Appendix 2 (Continued)

Lines	**Xbarc77*	**Xbarc1124*	**Xwmc632*	**Xgwm247*	**Xgwm181*	**Xgwm340*	**Xgwm547*
RL27	1	1	1	1	1	1	1
RL28	0	0	1	1	1	1	1
RL29	1	1	1	1	1	1	1
RL30	0	0	0	0	0	0	0
RL31	1	1	0	0	0	0	0
RL32	0	0	1	1	1	1	1
RL33	1	1	1	-	1	1	1
RL34	1	1	1	1	1	1	1
RL35	0	0	0	0	0	0	0
RL36	0	0	0	0	0	0	0
RL37	0	0	0	0	0	0	0
RL38	0	0	0	0	0	0	0
RL39	0	0	0	0	0	0	0
RL40	0	0	0	0	0	0	0
RL41	0	0	0	-	0	0	0
RL42	0	1	1	1	1	1	1
RL43	-	1	1	1	1	1	0
RL44	0	0	0	0	0	0	0
RL45	1	-	1	1	1	-	0
RL46	0	0	0	0	0	0	0
RL47	0	0	0	0	0	0	0
RL48	0	0	0	0	0	0	0
RL49	1	1	1	1	1	1	1
RL50	1	1	0	0	0	0	0
RL51	0	0	0	0	0	0	-
RL52	1	1	1	1	1	1	1
RL53	0	0	0	0	0	0	0

Appendix 2 (Continued)

Lines	**Xbarc77*	**Xbarc1124*	**Xwmc632*	**Xgwm247*	**Xgwm181*	**Xgwm340*	**Xgwm547*
RL54	0	0	1	1	1	1	1
RL55	0	0	0	0	0	-	0
RL56	0	0	0	0	0	0	-
RL57	1	1	-	1	1	1	1
RL58	1	1	1	-	1	1	-
RL59	0	0	0	0	0	0	0
RL60	0	0	0	0	0	0	0
RL61	1	1	1	-	1	1	-
RL62	0	0	0	0	0	0	0
RL63	0	0	0	0	-	0	0
RL64	1	1	1	1	1	1	1
RL65	0	0	1	1	1	1	1
RL66	1	1	0	0	0	0	-
RL67	-	-	1	-	1	1	1
RL68	0	0	0	0	0	0	0
RL69	0	1	1	1	1	1	1
RL70	0	0	0	0	0	0	0
RL71	1	1	1	1	1	1	1
RL72	0	0	0	0	0	0	0
RL73	1	1	1	1	1	1	-
RL74	1	1	1	1	1	1	1
RL75	1	1	1	1	1	1	1
RL76	0	0	1	1	1	1	1
RL77	0	0	0	0	0	-	0
RL78	0	0	0	0	0	0	0
RL79	0	0	0	0	0	0	0
RL80	1	1	1	1	1	1	1

Appendix 2 (Continued)

Lines	**Xbarc77*	**Xbarc1124*	**Xwmc632*	**Xgwm247*	**Xgwm181*	**Xgwm340*	**Xgwm547*
RL81	0	0	0	0	0	0	-
RL82	1	1	1	1	1	1	1
RL83	1	1	1	1	1	1	1
RL84	1	1	0	0	0	0	0
RL85	0	0	-	0	0	0	0
RL86	1	1	1	1	1	1	1
RL87	1	1	1	1	1	1	1
RL88	0	0	0	0	0	0	0
RL89	0	0	0	0	0	0	0
RL90	0	0	0	0	0	0	0
RL91	1	1	1	1	-	1	1

Appendix 3 Microsatellite segregation data for the ITMI RIL population. A score of 0 represents allele from Opata-85, 1 from W-7984 and '-' represents missing data. RIL #061 was not used in the analysis.

Lines	*Xgwm296*	*Xgwm261*	*Xgwm484*	*Xgwm455*	*Xgdm5*	*Xgdm107*	*Xgwm210*	*Xgwm102*	*Xgwm515*	*Xwmc25*	*Tg1*	*Tg3*	*Iw2*
Opata	0	0	0	0	0	0	0	0	0	0	0	0	0
W-7984	1	1	1	1	1	1	1	1	1	1	1	1	1
RIL #001	1	1	1	1	1	1	1	1	0	1	1	1	1
RIL #002	0	0	1	0	0	0	0	0	0	0	0	0	0
RIL #003	0	0	0	0	0	0	0	0	0	0	-	0	0
RIL #004	-	1	0	1	1	0	1	1	1	1	1	1	1
RIL #005	0	0	0	1	0	0	0	0	0	0	0	-	0
RIL #006	1	1	0	1	1	1	1	-	0	1	1	1	1
RIL #007	0	1	1	0	0	1	0	1	1	1	1	0	0
RIL #008	0	0	0	0	0	0	0	0	0	0	1	0	0
RIL #009	0	0	0	0	0	0	0	0	-	0	-	0	0
RIL #010	0	0	1	0	0	0	0	1	1	0	0	0	0
RIL #011	-	1	1	1	1	1	1	1	1	1	1	1	1
RIL #012	0	0	0	0	0	0	0	0	0	0	-	0	0
RIL #013	0	0	1	0	0	1	0	0	0	0	0	0	0
RIL #014	0	0	0	0	0	0	0	0	1	0	0	0	0
RIL #015	1	1	1	1	0	1	1	1	1	1	1	-	1
RIL #016	-	1	0	1	1	1	1	0	0	1	1	1	1
RIL #017	-	1	0	1	1	1	1	0	-	1	1	1	1
RIL #018	0	0	0	0	0	0	0	0	0	0	0	0	0
RIL #019	1	1	1	1	1	1	1	0	0	1	1	1	1
RIL #020	1	1	0	1	1	0	0	1	1	0	1	1	1
RIL #021	0	0	1	0	0	0	0	0	0	0	1	0	0

Appendix 3 (Continued)

Lines	*Xgwm296*	*Xgwm261*	*Xgwm484*	*Xgwm455*	*Xgdm5*	*Xgdm107*	*Xgwm210*	*Xgwm102*	*Xgwm515*	*Xwmc25*	*Tg1*	*Tg3*	*Iw2*
RIL #022	-	0	0	0	0	0	1	0	0	1	0	0	0
RIL #023	1	1	0	1	1	1	1	0	0	1	1	1	1
RIL #024	-	1	0	1	1	1	1	0	0	1	1	1	1
RIL #025	0	0	0	0	0	0	0	0	-	0	0	0	0
RIL #026	0	0	0	0	0	0	0	1	0	0	0	0	0
RIL #027	0	0	0	0	0	0	0	0	0	0	0	0	0
RIL #028	0	0	0	0	0	0	0	0	0	0	0	0	0
RIL #029	0	0	0	0	0	0	0	0	0	0	0	0	0
RIL #030	0	0	0	0	0	0	1	0	0	0	0	0	-
RIL #031	0	0	1	0	0	-	0	1	1	1	1	0	-
RIL #032	0	0	1	0	0	1	0	1	1	0	0	0	0
RIL #033	0	0	1	0	0	1	0	1	-	1	0	0	0
RIL #034	0	0	1	0	0	1	0	1	1	0	0	0	0
RIL #035	1	1	0	1	1	0	1	0	0	1	0	0	1
RIL #036	0	0	0	1	0	0	1	0	1	0	0	-	1
RIL #037	1	1	1	1	-	1	1	1	1	1	1	-	1
RIL #038	1	1	0	1	1	0	0	1	0	1	-	1	0
RIL #039	-	1	1	1	1	1	1	1	1	1	1	1	1
RIL #040	0	0	1	0	0	1	0	1	1	0	0	0	0
RIL #041	0	0	0	0	0	0	1	0	0	0	0	0	1
RIL #042	-	-	0	0	1	-	-	-	1	0	1	-	0
RIL #043	0	0	1	0	0	1	0	1	1	0	1	0	0
RIL #044	1	1	1	1	-	1	0	1	1	1	1	-	0
RIL #045	1	1	1	1	1	1	0	1	1	1	1	1	0

Appendix 3 (Continued)

Lines	*Xgwm296*	*Xgwm261*	*Xgwm484*	*Xgwm455*	*Xgdm5*	*Xgdm107*	*Xgwm210*	*Xgwm102*	*Xgwm515*	*Xwmc25*	*Tg1*	*Tg3*	*Iw2*
RIL #046	1	1	1	1	1	1	0	1	1	1	0	0	0
RIL #047	0	0	1	0	0	1	0	0	0	0	0	0	0
RIL #048	0	0	0	0	0	0	0	0	0	0	0	0	0
RIL #049	0	0	0	0	0	0	0	1	1	0	0	0	0
RIL #051	-	0	1	0	0	0	0	0	0	1	0	1	0
RIL #052	0	0	0	0	0	0	1	-	-	0	0	0	1
RIL #053	1	1	1	1	1	1	1	0	0	0	-	0	0
RIL #054	1	1	1	1	-	1	1	1	0	1	1	1	1
RIL #055	1	1	1	1	1	1	1	1	0	1	1	-	1
RIL #056	0	0	-	0	0	0	0	1	1	1	1	1	1
RIL #057	0	0	0	0	0	0	1	1	1	0	0	0	0
RIL #058	0	0	0	0	0	0	0	1	-	0	0	0	1
RIL #059	0	0	0	0	0	0	0	0	0	0	0	0	0
RIL #060	0	0	0	0	0	0	0	0	1	0	0	0	0
RIL #062	0	0	0	0	0	0	1	0	0	0	-	0	0
RIL #063	-	-	0	1	-	1	1	1	1	0	0	0	1
RIL #064	0	0	0	0	0	0	0	-	-	1	1	-	1
RIL #065	0	0	0	0	0	0	0	1	0	0	0	0	0
RIL #066	0	0	0	0	0	0	0	0	0	0	0	0	0
RIL #067	1	1	0	0	0	0	0	0	0	0	0	0	0
RIL #068	1	1	0	1	1	0	1	0	0	1	1	0	0
RIL #069	0	0	1	1	0	1	0	0	0	1	0	1	1
RIL #070	0	0	1	0	0	1	0	1	0	1	0	-	0
RIL #071	0	0	0	0	0	0	0	1	1	0	0	0	0

Appendix 3 (Continued)

Lines	*Xgwm296*	*Xgwm261*	*Xgwm484*	*Xgwm455*	*Xgdm5*	*Xgdm107*	*Xgwm210*	*Xgwm102*	*Xgwm515*	*Xwmc25*	*Tg1*	*Tg3*	*Iw2*
RIL #072	1	1	1	1	1	1	-	0	0	0	1	0	0
RIL #073	0	0	0	0	0	0	0	1	-	1	1	1	1
RIL #074	0	0	0	0	0	0	0	0	0	0	0	0	0
RIL #075	1	1	1	1	1	1	1	0	0	0	1	0	0
RIL #076	-	1	1	1	1	1	1	0	-	1	0	1	1
RIL #077	0	0	1	0	0	1	0	1	-	1	0	0	1
RIL #078	1	1	1	1	-	1	1	1	1	0	1	-	0
RIL #079	0	0	0	0	0	0	1	1	1	1	0	0	1
RIL #080	1	1	1	1	1	1	1	0	0	0	1	1	1
RIL #081	1	1	1	1	1	1	1	1	1	1	1	1	1
RIL #082	0	0	0	0	0	0	0	1	0	1	0	0	1
RIL #083	1	1	1	1	1	1	0	0	0	0	1	0	0
RIL #084	-	-	-	-	-	1	0	1	1	1	1	-	0
RIL #085	1	1	1	1	1	1	0	1	0	1	1	1	0
RIL #086	1	1	1	1	1	1	1	0	0	1	1	1	0
RIL #087	0	0	0	0	0	0	1	1	1	1	0	0	1
RIL #088	-	1	1	1	1	1	1	1	1	0	1	1	1
RIL #089	0	0	0	0	0	0	0	1	1	1	0	0	1
RIL #090	0	0	1	0	1	1	0	0	1	0	0	-	0
RIL #091	0	0	0	1	1	0	1	1	1	0	0	0	0
RIL #092	0	0	0	1	1	0	1	0	1	0	0	1	1
RIL #093	0	0	0	0	0	0	0	0	0	0	0	0	1
RIL #094	1	1	0	1	1	0	0	0	1	0	0	1	0
RIL #095	1	1	1	1	1	1	1	1	1	1	-	0	0

Appendix 3 (Continued)

Lines	*Xgwm296*	*Xgwm261*	*Xgwm484*	*Xgwm455*	*Xgdm5*	*Xgdm107*	*Xgwm210*	*Xgwm102*	*Xgwm515*	*Xwmc25*	*Tg1*	*Tg3*	*lw2*
RIL #096	0	0	1	0	0	1	0	1	1	1	0	0	1
RIL #097	0	0	0	0	0	0	0	1	1	0	1	0	0
RIL #098	1	1	0	1	1	0	1	0	0	0	1	1	0
RIL #099	0	0	0	0	0	0	0	1	-	1	0	1	1
RIL #100	1	1	1	1	1	1	1	0	0	0	1	1	0
RIL #101	0	0	0	0	0	0	0	1	1	1	0	0	1
RIL #102	0	0	0	0	0	0	0	0	0	0	0	0	0
RIL #103	1	1	1	1	1	1	1	0	0	0	1	1	0
RIL #104	1	1	0	1	1	0	1	1	1	1	1	1	1
RIL #105	-	1	1	1	1	1	1	0	0	1	1	1	1
RIL #106	0	0	0	0	0	0	0	1	0	1	0	0	1
RIL #107	0	1	1	1	0	1	1	0	0	0	1	-	0
RIL #108	-	-	-	-	-	-	-	-	-	-	-	-	-
RIL #109	-	-	-	-	-	-	-	-	-	-	0	0	-
RIL #110	1	1	0	1	1	0	1	-	-	-	1	1	-
RIL #111	1	1	1	1	1	1	1	0	0	1	1	1	1
RIL #112	1	1	1	1	1	1	1	1	1	1	1	1	1
RIL #113	1	1	1	1	1	1	0	1	1	1	1	1	1
RIL #114	-	1	-	1	1	-	-	0	1	1	1	1	0
RIL #115	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 4 Microsatellite segregation data for CS/CS2D F_2 mapping population. A score of 0 represents allele from Chinese Spring, 1 represents allele from 2D2, 2 represents a heterozygote, '-' represents missing value, 4 indicates that the individual is not a homozygote for allele from Chinese Spring (*tauschii* 2D) and 5 indicates that the individual is not a homozygote for allele from Chinese Spring.

Lines	*Iw2*	*Xgwm296*	*Xgwm261*	*Xgwm484*	*Xgwm455*	*Xgdm5*	*Xgwm515*	*Xwmc25*	*Xbarc124*	*Xbarc168*	*Xbarc228*	*Xcfd43*	*Xwmc245*
CS	0	0	0	0	0	0	0	0	0	0	0	0	0
CS2D	1	1	1	1	1	1	1	1	1	1	1	1	1
F2 001	0	2	2	2	2	2	2	2	0	2	1	2	2
F2 002	4	2	2	1	2	2	1	2	2	1	1	1	1
F2 003	4	1	1	1	1	1	1	1	1	1	1	1	1
F2 004	4	0	0	0	0	0	0	0	2	0	0	0	0
F2 005	4	2	2	1	1	1	1	2	1	1	-	1	1
F2 006	0	0	0	0	0	0	2	0	0	0	2	2	2
F2 007	4	0	0	0	-	0	0	0	2	0	1	0	0
F2 008	4	1	1	1	1	1	2	-	2	1	1	1	2
F2 009	4	1	2	2	1	1	2	1	2	2	0	2	0
F2 010	4	2	2	2	2	2	2	2	1	2	2	2	2
F2 011	0	0	0	0	-	0	0	0	0	0	0	0	0
F2 012	4	2	2	0	-	2	2	2	2	0	0	0	2
F2 013	4	1	1	1	1	1	2	1	1	1	2	1	2
F2 014	0	2	2	2	2	2	2	2	0	2	2	2	2
F2 015	4	2	2	2	2	2	2	2	2	2	0	2	2
F2 016	0	0	0	0	0	0	2	0	0	0	2	0	2
F2 017	4	0	0	0	0	0	0	0	2	0	2	0	2
F2 018	0	0	0	2	0	0	2	0	0	0	2	2	2
F2 019	4	0	0	0	0	0	0	0	-	-	0	0	0
F2 020	4	2	2	2	2	2	2	2	2	2	0	2	2
F2 021	4	2	2	2	2	2	2	2	2	-	1	-	2
F2 022	4	2	2	2	2	2	2	2	2	1	2	2	2
F2 023	0	0	0	0	0	0	0	0	0	0	2	0	2

Appendix 4 (Continued)

Lines	*lw2*	*Xgwm296*	*Xgwm261*	*Xgwm484*	*Xgwm455*	*Xgdm5*	*Xgwm515*	*Xwmc25*	*Xbarc124*	*Xbarc168*	*Xbarc228*	*Xcfd43*	*Xwmc245*
F2 024	4	2	2	2	2	2	2	2	2	2	1	2	2
F2 025	4	2	2	2	2	2	2	2	2	2	0	2	2
F2 026	4	2	2	2	2	2	2	2	1	2	2	-	-
F2 027	4	1	1	1	1	1	1	1	2	1	1	1	1
F2 028	4	2	2	2	2	2	0	2	2	2	0	2	0
F2 029	4	2	2	2	2	2	2	2	2	2	2	2	2
F2 030	4	2	1	2	2	2	2	2	2	2	-	2	2
F2 031	4	1	1	2	1	1	0	1	1	2	0	2	0
F2 032	4	0	0	0	2	2	0	0	2	0	2	0	0
F2 033	4	2	2	0	2	2	0	2	2	0	0	0	0
F2 034	4	2	2	2	2	2	2	2	2	2	2	2	2
F2 035	0	2	2	2	2	2	2	2	2	2	1	2	2
F2 036	4	0	0	1	0	0	2	0	0	1	0	1	0
F2 037	0	2	2	2	2	2	2	2	2	2	2	2	2
F2 038	0	2	2	2	2	2	2	2	0	2	2	2	2
F2 040	4	2	2	0	2	2	2	2	2	0	1	0	2
F2 042	0	0	0	0	0	0	0	0	0	0	0	0	0
F2 043	4	2	2	2	2	2	2	2	2	2	2	2	2
F2 044	0	0	0	0	0	0	0	0	0	-	-	-	-
F2 045	0	2	2	2	2	2	0	2	0	2	0	2	0
F2 046	0	0	0	2	0	0	-	0	0	2	2	2	1
F2 047	4	2	2	2	2	2	2	2	2	2	2	2	2
F2 048	4	2	2	2	2	2	0	2	2	2	2	-	0
F2 049	4	1	1	1	1	1	1	1	1	1	1	1	1
F2 050	4	2	2	2	2	2	2	2	2	2	1	2	2
F2 051	4	1	1	2	1	1	2	1	1	2	0	2	0
F2 052	4	2	2	2	2	2	2	2	2	2	2	0	2
F2 054	4	0	0	0	0	0	2	0	1	0	2	0	2

Appendix 4 (Continued)

Lines	*lw2*	*Xgwm296*	*Xgwm261*	*Xgwm484*	*Xgwm455*	*Xgdm5*	*Xgwm515*	*Xwmc25*	*Xbarc124*	*Xbarc168*	*Xbarc228*	*Xcfd43*	*Xwmc245*
F2 055	4	2	2	0	2	2	0	2	2	0	-	0	0
F2 056	4	1	2	2	1	1	2	-	2	2	1	2	2
F2 057	4	1	1	1	1	1	2	1	1	1	1	1	2
F2 058	4	1	1	2	1	1	2	1	1	2	2	2	2
F2 059	4	2	2	1	2	2	2	2	2	1	2	1	2
F2 060	4	2	2	2	2	2	2	2	2	2	2	2	2
F2 061	0	2	2	2	2	2	2	2	0	2	2	2	2
F2 062	4	2	2	2	2	2	1	2	2	1	2	2	2
F2 063	4	2	2	2	2	2	2	2	1	2	2	2	2
F2 064	4	2	2	2	2	2	2	2	1	2	1	2	0
F2 065	4	2	2	2	2	2	1	2	2	2	2	2	1
F2 066	0	0	0	0	0	0	0	0	0	0	2	0	0
F2 067	0	0	0	0	0	0	0	0	0	0	2	0	0
F2 068	4	2	2	2	2	2	2	2	2	2	1	2	1
F2 069	0	0	0	0	0	0	0	0	0	0	0	0	0
F2 070	4	2	2	2	2	2	2	2	2	2	0	2	2
F2 071	4	1	1	1	1	1	1	1	2	1	1	1	1
F2 072	4	2	2	1	2	2	1	2	2	1	1	1	1
F2 073	4	0	0	0	0	0	0	0	2	0	0	0	0
F2 074	0	0	0	0	0	0	0	-	0	2	0	0	0
F2 075	4	2	2	0	2	2	0	2	2	0	0	0	0
F2 076	0	2	2	2	2	2	1	2	0	2	1	2	1
F2 077	0	0	0	2	0	0	2	0	0	2	2	2	2
F2 078	4	1	1	0	1	1	2	1	1	0	2	2	2
F2 079	4	2	2	2	2	2	0	2	-	2	0	2	0
F2 080	0	0	0	2	0	0	2	0	-	2	2	2	2
F2 081	0	2	0	0	2	2	0	2	2	0	0	0	0
F2 082	4	2	2	2	2	2	2	2	1	2	-	2	2

Appendix 4 (Continued)

Lines	*Iw2*	*Xgwm296*	*Xgwm261*	*Xgwm484*	*Xgwm455*	*Xgdm5*	*Xgwm515*	*Xwmc25*	*Xbarc124*	*Xbarc168*	*Xbarc228*	*Xcfd43*	*Xwmc245*
F2 083	0	0	0	0	0	0	2	0	0	0	1	0	1
F2 084	0	0	0	2	0	0	-	0	0	2	2	2	2
F2 085	4	0	2	2	0	0	0	0	-	2	2	2	0
F2 086	4	2	2	2	2	2	2	2	2	2	2	2	2
F2 087	4	2	2	2	2	2	2	2	2	2	2	2	2
F2 088	4	1	1	1	1	1	1	1	2	1	1	1	1
F2 090	4	2	2	2	1	1	2	2	1	2	0	0	2
F2 091	0	0	0	2	0	0	2	0	0	2	1	2	2
F2 092	4	1	1	2	1	1	2	1	1	2	2	2	2
F2 093	0	0	0	0	0	0	2	0	0	0	1	2	2
F2 094	4	2	2	2	2	2	2	2	2	2	1	2	2
F2 095	4	2	2	0	2	2	0	2	2	0	0	0	0
F2 096	4	1	1	2	1	1	1	1	1	2	1	2	1
F2 097	0	0	0	0	0	0	0	0	0	-	0	0	2

Appendix 4 (Continued)

Lines	*Tg1*	*Xgwm311*	*Xpsr928*	*Xgwm301*	*Xgwm320*	*Xgwm349*	*Xbcd175.2*	*Xbcd1970.2*	*Xwmc503*	*Xgwm157*	*Xgwm539*	*Xcfd168*
CS	0	0	0	0	0	0	0	0	0	0	0	0
CS2D	1	1	1	1	1	1	1	1	1	1	1	1
F2 001	5	2	2	2	2	5	-	5	2	2	5	1
F2 002	-	1	2	1	1	1	-	5	2	1	1	1
F2 003	1	1	1	1	1	1	-	1	1	1	5	1
F2 004	0	0	0	0	0	5	-	5	0	0	5	0
F2 005	-	1	1	2	2	1	-	1	2	2	5	2
F2 006	5	2	0	2	2	5	0	5	0	2	5	2
F2 007	-	1	0	2	1	1	0	5	0	2	5	1
F2 008	4	2	1	2	2	5	1	1	1	2	5	1
F2 009	-	1	1	1	1	1	1	1	2	0	5	0
F2 010	4	2	2	2	2	5	-	1	2	2	5	2
F2 011	0	2	0	2	2	5	0	5	0	0	5	0
F2 012	5	2	2	2	2	5	-	5	2	2	5	0
F2 013	4	1	1	1	1	1	-	1	1	2	5	2
F2 014	4	0	0	0	0	5	2	5	2	2	5	2
F2 015	5	2	2	1	2	5	2	5	2	2	5	0
F2 016	5	0	0	0	0	5	0	5	0	2	5	2
F2 017	5	1	0	1	1	1	0	5	0	2	5	2
F2 018	5	1	0	1	1	1	0	5	0	2	5	2
F2 019	-	2	2	2	2	5	0	5	0	1	5	0
F2 020	-	0	2	2	2	5	-	5	2	1	5	0
F2 021	4	-	-	-	2	1	-	-	2	2	5	1
F2 022	4	0	2	0	0	5	2	1	1	2	5	2
F2 023	5	1	0	1	1	1	0	5	0	2	5	2
F2 024	4	0	2	0	0	5	2	5	2	2	1	1
F2 025	5	0	2	0	0	5	-	5	2	2	5	0
F2 026	-	2	1	2	2	1	1	1	2	2	5	2

Appendix 4 (Continued)

Lines	*Tg1*	*Xgwm311*	*Xpsr928*	*Xgwm301*	*Xgwm320*	*Xgwm349*	*Xbcd175.2*	*Xbcd1970.2*	*Xwmc503*	*Xgwm157*	*Xgwm539*	*Xcfd168*
F2 027	1	2	1	2	2	5	1	1	1	1	1	1
F2 028	4	0	2	0	0	5	-	5	2	0	5	0
F2 029	4	0	-	2	2	5	-	5	2	2	5	2
F2 030	4	2	2	2	2	5	2	5	1	2	5	2
F2 031	4	2	1	2	2	1	1	1	1	0	5	2
F2 032	0	2	2	0	0	1	2	5	0	0	5	1
F2 033	0	2	2	2	2	1	-	5	2	0	5	0
F2 034	4	1	2	1	1	1	-	5	2	2	5	2
F2 035	-	2	2	2	2	5	2	5	2	1	1	1
F2 036	0	0	0	0	0	5	-	5	0	0	5	0
F2 037	4	2	1	0	2	5	-	5	2	2	5	2
F2 038	4	1	0	1	-	5	0	5	2	2	5	2
F2 040	5	2	2	2	2	5	2	5	2	2	1	1
F2 042	0	0	0	0	0	5	0	5	0	0	5	0
F2 043	4	2	2	2	2	5	2	1	2	2	5	2
F2 044	-	-	-	-	-	-	0	1	2	0	5	2
F2 045	4	2	0	0	0	1	0	5	2	0	5	0
F2 046	5	2	0	2	2	5	0	5	2	1	1	0
F2 047	5	1	2	1	1	5	-	5	2	2	5	0
F2 048	-	0	-	0	0	5	2	5	2	2	5	2
F2 049	1	1	1	1	1	1	1	1	1	1	1	1
F2 050	4	1	2	1	1	1	2	5	2	2	5	1
F2 051	4	0	1	0	0	5	1	1	1	0	5	0
F2 052	-	2	2	0	0	5	2	5	2	2	5	2
F2 054	-	1	1	1	1	1	-	1	0	2	5	2
F2 055	4	2	2	2	2	5	2	5	2	0	5	0
F2 056	4	2	1	2	2	5	-	1	2	2	5	1
F2 057	-	2	1	2	2	1	1	1	1	1	1	1

Appendix 4 (Continued)

Lines	*Tg1*	*Xgwm311*	*Xpsr928*	*Xgwm301*	*Xgwm320*	*Xgwm349*	*Xbcd175.2*	*Xbcd1970.2*	*Xwmc503*	*Xgwm157*	*Xgwm539*	*Xcfd168*
F2 058	4	0	1	0	0	5	1	1	1	2	5	2
F2 059	4	2	2	2	2	5	2	5	2	2	-	2
F2 060	4	2	2	2	-	5	2	5	2	2	5	2
F2 061	4	1	0	1	1	1	2	5	2	2	5	2
F2 062	-	2	2	2	2	5	2	5	2	2	5	2
F2 063	4	2	1	2	2	5	1	1	2	2	5	2
F2 064	4	1	2	1	1	1	-	5	2	0	5	1
F2 065	1	2	2	2	2	5	2	5	2	1	1	2
F2 066	0	1	0	1	1	1	0	5	0	0	5	2
F2 067	0	0	0	0	0	5	0	5	0	0	5	2
F2 068	1	2	2	2	2	1	2	5	2	1	1	1
F2 069	0	2	0	2	2	5	0	5	0	0	5	0
F2 070	4	0	2	0	0	5	2	5	2	2	5	0
F2 071	1	1	1	1	1	-	1	1	1	1	1	1
F2 072	1	1	2	1	1	1	-	5	2	1	1	1
F2 073	0	2	1	2	2	5	2	5	0	0	5	0
F2 074	0	0	0	0	0	5	0	5	0	0	5	0
F2 075	4	0	2	0	0	5	2	5	2	0	5	0
F2 076	1	0	2	2	2	5	2	5	2	1	1	1
F2 077	4	1	0	2	2	-	0	5	0	2	5	2
F2 078	4	2	1	2	2	5	1	1	1	2	5	2
F2 079	4	0	2	2	2	5	2	5	2	0	5	0
F2 080	5	2	0	1	2	5	-	5	0	2	5	2
F2 081	0	1	2	1	1	5	2	-	0	0	5	0
F2 082	4	0	2	0	0	5	2	5	2	2	5	0
F2 083	5	2	0	2	2	5	0	5	0	1	1	1
F2 084	4	2	0	1	0	5	0	5	0	2	5	2

Appendix 4 (Continued)

Lines	*Tg1*	*Xgwm311*	*Xpsr928*	*Xgwm301*	*Xgwm320*	*Xgwm349*	*Xbcd175.2*	*Xbcd1970.2*	*Xwmc503*	*Xgwm157*	*Xgwm539*	*Xcfd168*
F2 085	4	2	0	2	2	5	0	5	2	0	5	2
F2 086	4	2	2	2	2	1	2	5	2	2	5	2
F2 087	4	2	2	2	-	5	2	5	2	2	1	2
F2 088	1	2	2	2	2	5	2	5	1	1	1	1
F2 090	4	0	1	0	0	5	1	1	2	2	5	0
F2 091	5	1	0	2	1	1	0	5	0	2	5	1
F2 092	4	2	1	2	2	1	1	1	1	2	5	2
F2 093	-	1	0	1	0	1	0	5	0	1	1	1
F2 094	4	2	2	2	2	5	2	5	2	2	1	2
F2 095	4	0	2	0	0	5	2	5	2	0	5	0
F2 096	1	2	1	-	2	5	1	1	1	1	1	1
F2 097	5	1	0	1	1	5	0	5	0	2	5	0

Appendix 5 Percent threshability (%) data for the ITMI RIL population for three environments and the combined average. '-' represents a missing data point.

Line	Hyslop Farm 1999	East Farm 2000	Hyslop Farm 2001	Combined (Avg)
Opata	97.74	98.54	97.94	98.07
M6	26.62	32.73	33.59	30.98
Altar	85.16	87.79	93.63	88.86
RIL #001	77.78	80.51	30.45	62.92
RIL #002	87.14	81.12	92.42	86.89
RIL #003	50.56	87.12	52.41	63.36
RIL #004	54.30	59.51	47.01	53.60
RIL #005	77.93	87.67	82.49	82.70
RIL #006	36.24	58.44	51.03	48.57
RIL #007	39.10	61.55	59.89	53.51
RIL #008	46.89	-	34.34	40.62
RIL #009	56.54	85.32	46.96	62.94
RIL #010	75.15	88.49	84.46	82.70
RIL #011	60.82	57.46	61.18	59.82
RIL #012	68.75	80.94	51.95	67.21
RIL #013	95.05	93.96	92.52	93.85
RIL #014	82.04	86.97	67.34	78.78
RIL #015	52.26	66.29	43.30	53.95
RIL #016	56.15	79.64	61.16	65.65
RIL #017	69.32	73.72	59.52	67.52
RIL #018	84.24	90.62	54.81	76.56
RIL #019	26.03	68.71	45.60	46.78
RIL #020	71.81	70.33	75.07	72.41
RIL #021	60.21	80.24	70.73	70.39
RIL #022	81.58	-	70.73	76.16
RIL #023	53.25	55.90	39.09	49.41
RIL #024	51.79	51.88	49.79	51.15
RIL #025	83.12	93.50	64.56	80.39
RIL #026	71.57	74.40	54.11	66.69
RIL #027	68.92	76.68	58.49	68.03
RIL #028	86.25	87.76	84.76	86.26
RIL #029	86.72	77.89	67.27	77.29
RIL #030	84.62	-	45.45	65.03
RIL #031	64.14	92.87	11.76	56.26
RIL #032	93.78	88.19	60.93	80.97
RIL #033	91.27	93.40	80.00	88.22
RIL #034	93.57	83.12	64.10	80.26
RIL #035	84.73	82.74	94.03	87.17
RIL #036	94.06	83.61	55.04	77.57
RIL #037	34.57	-	43.37	38.97
RIL #038	52.15	34.50	38.10	41.58
RIL #039	22.47	29.65	16.75	22.96
RIL #040	92.74	93.25	96.37	94.12
RIL #041	89.10	94.27	60.73	81.36
RIL #042	79.66	69.94	68.47	72.69

Appendix 5 (Continued)

Lines	Hyslop Farm 1999	East Farm 2000	Hyslop Farm 2001	Combined (Avg)
RIL #043	51.63	42.47	60.52	51.54
RIL #044	57.22	49.62	59.09	55.31
RIL #045	50.87	38.68	28.45	39.33
RIL #046	77.29	77.41	95.02	83.24
RIL #047	81.54	85.02	67.68	78.08
RIL #048	91.86	-	54.25	73.05
RIL #049	100.00	97.35	94.26	97.20
RIL #050	81.47	92.50	83.76	85.91
RIL #051	89.77	93.13	92.56	91.82
RIL #052	53.37	78.59	72.14	68.03
RIL #053	63.96	46.51	54.68	55.05
RIL #054	78.21	67.02	76.33	73.85
RIL #055	50.43	48.83	43.63	47.63
RIL #056	90.75	92.01	81.45	88.07
RIL #057	78.49	70.84	85.10	78.14
RIL #058	80.00	77.77	85.11	80.96
RIL #059	85.96	85.13	87.57	86.22
RIL #060	67.05	66.81	74.44	69.43
RIL #062	95.74	97.63	94.56	95.98
RIL #063	27.90	34.43	42.69	35.01
RIL #064	92.46	83.22	78.47	84.72
RIL #065	98.04	-	77.00	87.52
RIL #066	78.33	80.68	67.05	75.35
RIL #067	62.50	35.66	47.46	48.54
RIL #068	52.94	56.16	72.92	60.67
RIL #069	80.86	76.07	67.68	74.87
RIL #070	84.47	86.47	72.20	81.05
RIL #071	83.74	72.10	56.58	70.81
RIL #072	69.18	54.87	51.55	58.53
RIL #067	62.50	35.66	47.46	48.54
RIL #068	52.94	56.16	72.92	60.67
RIL #069	80.86	76.07	67.68	74.87
RIL #070	84.47	86.47	72.20	81.05
RIL #071	83.74	72.10	56.58	70.81
RIL #072	69.18	54.87	51.55	58.53
RIL #073	97.46	90.22	84.96	90.88
RIL #074	82.42	96.82	81.66	86.97
RIL #075	41.78	36.28	41.85	39.97
RIL #076	94.57	67.54	85.66	82.59
RIL #077	89.74	96.80	96.09	94.21
RIL #078	33.33	49.44	20.72	34.50
RIL #079	84.12	91.20	69.23	81.51
RIL #080	-	41.92	68.86	55.39
RIL #081	44.27	46.86	71.10	54.08

Appendix 5 (Continued)

Lines	Hyslop Farm 1999	East Farm 2000	Hyslop Farm 2001	Combined (Avg)
RIL #082	90.36	91.36	82.86	88.19
RIL #083	64.93	57.15	46.08	56.05
RIL #084	60.68	54.94	48.60	54.74
RIL #085	56.41	41.27	38.89	45.52
RIL #086	45.97	46.56	50.00	47.51
RIL #087	87.35	80.03	78.38	81.92
RIL #088	69.48	67.28	73.28	70.01
RIL #089	88.57	95.44	98.76	94.26
RIL #090	82.81	86.57	94.72	88.03
RIL #091	96.32	90.43	81.25	89.33
RIL #092	92.57	96.46	84.21	91.08
RIL #093	82.21	85.65	78.91	82.25
RIL #094	93.05	88.65	68.46	83.38
RIL #095	51.59	-	77.30	64.45
RIL #096	81.05	88.69	73.17	80.97
RIL #097	80.81	72.83	72.22	75.29
RIL #098	53.79	40.02	47.91	47.24
RIL #099	67.48	88.45	74.55	76.83
RIL #100	68.27	64.04	76.44	69.59
RIL #101	83.02	83.85	64.75	77.21
RIL #102	79.69	84.19	60.27	74.72
RIL #103	68.69	71.28	71.13	70.37
RIL #104	77.87	65.80	77.78	73.82
RIL #105	33.18	38.98	41.70	37.96
RIL #106	86.13	95.90	94.53	92.18
RIL #107	58.46	.	77.13	67.80
RIL #108	-	-	-	-
RIL #109	-	-	-	-
RIL #110	71.33	70.86	72.22	71.47
RIL #111	42.39	63.13	59.71	55.08
RIL #112	76.38	66.83	68.98	70.73
RIL #113	25.27	.	73.71	49.49
RIL #114	61.16	67.65	66.84	65.22
RIL #115	-	-	-	-

Appendix 6 Glume tenacity (N) measurements in the ITMI RIL population. Glume tenacity measurements for three environments and the combined average are presented. '-' represents missing data points.

Lines	East Farm 2000	Greenhouse 2000	Hyslop Farm 2001	Combined (Avg)
Opata	0.35	0.26	1.02	0.55
M6	5.80	5.96	6.24	6.00
Altar	0.50	0.50	0.81	0.60
RIL #001	0.98	1.11	4.78	2.29
RIL #002	1.33	0.67	2.72	1.57
RIL #003	1.68	-	3.03	2.36
RIL #004	2.10	0.61	5.42	2.71
RIL #005	1.04	0.74	2.59	1.45
RIL #006	1.74	1.73	5.06	2.84
RIL #007	1.44	-	6.12	3.78
RIL #008	3.58	1.33	3.22	2.71
RIL #009	1.66	0.93	3.78	2.12
RIL #010	0.87	0.61	3.86	1.78
RIL #011	1.05	1.00	6.48	2.84
RIL #012	1.73	0.69	2.97	1.80
RIL #013	0.40	-	2.42	1.41
RIL #014	0.97	0.86	2.28	1.37
RIL #015	1.04	1.01	5.81	2.62
RIL #016	0.81	-	5.09	2.95
RIL #017	0.81	-	5.03	2.92
RIL #018	0.61	-	2.84	1.72
RIL #019	1.70	2.55	3.42	2.56
RIL #020	0.82	0.87	6.53	2.74
RIL #021	1.77	1.18	4.39	2.44
RIL #022	0.76	0.57	2.70	1.34
RIL #023	2.96	1.76	3.81	2.84
RIL #024	2.23	1.87	4.39	2.83
RIL #025	0.51	0.57	1.93	1.00
RIL #026	1.60	-	1.31	1.45
RIL #027	1.30	-	1.58	1.44
RIL #028	0.48	0.39	0.97	0.61
RIL #029	1.95	0.68	1.53	1.39
RIL #030	2.53	-	1.75	2.14
RIL #031	0.89	-	3.11	2.00
RIL #032	1.12	-	0.89	1.01
RIL #033	0.79	-	0.75	0.77
RIL #034	2.35	0.76	0.97	1.36
RIL #035	0.55	0.40	1.08	0.68
RIL #036	0.46	0.59	1.36	0.80
RIL #037	1.14	0.74	7.45	3.11
RIL #038	4.35	1.72	2.75	2.94
RIL #039	4.77	2.82	3.36	3.65
RIL #040	0.31	0.32	0.95	0.53

Appendix 6 (Continued)

Lines	East Farm 2000	Greenhouse 2000	Hyslop Farm 2001	Combined (Avg)
RIL #041	0.67	0.52	2.06	1.08
RIL #042	1.76	1.33	4.09	2.39
RIL #043	2.32	1.10	3.84	2.42
RIL #044	2.48	1.27	3.48	2.41
RIL #045	2.61	1.29	4.09	2.66
RIL #046	0.83	0.62	2.09	1.18
RIL #047	1.26	-	1.61	1.44
RIL #048	0.99	-	1.78	1.38
RIL #049	0.46	0.62	2.45	1.18
RIL #050	0.56	-	3.42	1.99
RIL #051	0.76	0.71	3.39	1.62
RIL #052	2.30	0.87	3.48	2.22
RIL #053	1.25	-	4.87	3.06
RIL #054	1.06	-	6.45	3.75
RIL #055	1.99	1.14	4.67	2.60
RIL #056	0.44	-	1.58	1.01
RIL #057	1.52	-	3.39	2.46
RIL #058	1.99	1.31	3.48	2.26
RIL #059	1.78	1.22	2.61	1.87
RIL #060	4.11	-	3.36	3.73
RIL #062	0.34	0.29	2.22	0.95
RIL #063	3.36	4.08	3.11	3.52
RIL #064	1.55	0.34	2.03	1.31
RIL #065	0.38	0.34	2.09	0.94
RIL #066	1.96	1.41	1.97	1.78
RIL #067	3.04	2.41	3.03	2.83
RIL #068	1.84	1.13	2.84	1.93
RIL #069	1.15	0.43	2.67	1.41
RIL #070	1.93	1.09	1.39	1.47
RIL #071	1.40	0.49	4.92	2.27
RIL #072	1.81	0.92	5.45	2.73
RIL #073	1.20	0.65	2.59	1.48
RIL #074	1.03	0.59	1.97	1.20
RIL #075	3.93	2.98	3.70	3.54
RIL #076	2.44	-	2.72	2.58
RIL #077	0.47	0.61	2.34	1.14
RIL #078	3.83	2.48	3.48	3.26
RIL #079	0.70	0.64	3.61	1.65
RIL #080	-	2.36	4.09	3.22
RIL #081	3.10	-	3.89	3.50
RIL #082	0.47	0.63	1.67	0.92
RIL #083	1.57	0.81	4.03	2.14
RIL #084	1.65	-	4.31	2.98
RIL #085	2.10	-	4.95	3.53
RIL #086	3.82	-	3.61	3.72
RIL #087	1.57	1.18	2.39	1.71

Appendix 6 (Continued)

Lines	East Farm 2000	Greenhouse 2000	Hyslop Farm 2001	Combined (Avg)
RIL #088	0.93	0.96	5.56	2.49
RIL #089	0.39	0.34	1.64	0.79
RIL #090	0.48	0.34	1.86	0.90
RIL #091	0.66	0.39	3.45	1.50
RIL #092	0.66	0.32	3.11	1.36
RIL #093	1.82	0.91	2.95	1.89
RIL #094	0.78	0.54	2.06	1.13
RIL #095	1.27	0.63	3.20	1.70
RIL #096	1.38	0.66	2.81	1.62
RIL #097	2.20	1.00	4.23	2.47
RIL #098	3.96	2.95	6.53	4.48
RIL #099	1.48	-	2.39	1.93
RIL #100	0.88	0.68	6.01	2.52
RIL #101	2.43	-	3.20	2.81
RIL #102	1.36	0.78	2.59	1.58
RIL #103	1.00	0.79	6.06	2.62
RIL #104	1.11	-	5.28	3.20
RIL #105	2.29	1.51	3.64	2.48
RIL #106	0.70	0.47	2.14	1.10
RIL #107	-	-	4.14	4.14
RIL #108	-	-	3.20	3.20
RIL #109	-	-	2.50	2.50
RIL #110	1.86	0.98	5.12	2.65
RIL #111	1.03	0.93	6.62	2.86
RIL #112	0.73	0.52	5.25	2.17
RIL #113	2.70	.	3.95	3.32
RIL #114	1.18	0.72	5.28	2.39
RIL #115	-	-	-	-

Appendix 7 Glume tenacity (N) for the CS/CS2D F_2 population for one environment (Greenhouse 2003). Data for individual spikes and total spike average are provided '-' represents missing data points.

Lines	Spike1	Spike2	Spike3	Spike4	Total Avg.
CS	0.75	0.86	1.06	0.88	0.89
CS	0.75	0.75	0.84	1.41	0.94
CS2D	1.85	2.71	2.86	3.78	2.80
CS2D	3.03	2.70	3.71	2.86	3.08
F2 001	0.49	0.71	1.10	2.05	1.09
F2 002	2.14	1.99	2.40	3.15	2.42
F2 003	3.18	2.60	2.78	3.19	2.93
F2 004	0.09	0.41	0.65	0.84	0.50
F2 005	1.44	1.18	1.10	1.26	1.24
F2 006	1.13	1.25	1.31	0.76	1.11
F2 007	1.08	0.76	0.75	0.79	0.84
F2 008	2.65	2.05	1.45	2.00	2.04
F2 009	1.15	1.19	1.26	2.25	1.46
F2 010	2.01	1.65	1.91	1.38	1.74
F2 011	0.90	0.91	0.93	0.95	0.92
F2 012	1.04	0.39	0.80	-	0.74
F2 013	1.68	2.30	2.05	2.61	2.16
F2 014	2.78	2.33	1.64	1.99	2.18
F2 015	1.41	2.15	1.75	-	1.77
F2 016	0.81	1.10	0.93	0.93	0.94
F2 017	0.43	0.50	1.35	0.73	0.75
F2 018	1.15	1.28	1.43	0.96	1.20
F2 019	0.94	0.85	0.48	0.46	0.68
F2 020	1.56	1.91	1.20	0.74	1.35
F2 021	2.26	2.10	1.95	1.74	2.01
F2 022	1.98	2.86	2.28	2.84	2.49
F2 023	0.85	1.06	0.68	1.00	0.90
F2 024	3.30	2.35	1.96	2.66	2.57
F2 025	1.54	1.85	1.14	2.11	1.66
F2 026	2.80	2.96	3.31	2.63	2.93
F2 027	3.53	4.64	2.96	5.19	4.08
F2 028	1.61	1.74	2.14	1.73	1.80
F2 029	2.39	1.65	2.44	1.83	2.08
F2 030	3.90	3.18	4.25	3.25	3.64
F2 031	1.86	2.48	2.55	2.16	2.26
F2 032	0.95	1.00	1.23	1.24	1.10
F2 033	0.91	1.19	0.94	0.95	1.00
F2 034	2.74	1.73	2.30	2.94	2.43
F2 035	1.91	2.06	2.05	3.09	2.28
F2 036	1.58	1.11	1.56	1.23	1.37
F2 037	2.60	2.89	3.16	3.44	3.02
F2 038	2.40	1.89	1.60	-	1.96

Appendix 7 (Continued)

Lines	Spike1	Spike2	Spike3	Spike4	Total Avg.
F2 040	0.63	0.76	0.95	1.04	0.84
F2 042	1.11	1.24	0.89	0.76	1.00
F2 043	2.28	1.71	1.11	1.89	1.75
F2 044	0.80	0.96	0.75	0.93	0.86
F2 045	1.79	2.81	2.36	3.16	2.53
F2 046	1.56	1.91	1.28	1.66	1.60
F2 047	1.60	0.98	1.50	1.29	1.34
F2 048	2.39	3.81	2.64	2.60	2.86
F2 049	3.55	2.80	-	-	3.18
F2 050	2.36	1.84	2.09	1.99	2.07
F2 051	1.80	1.08	2.21	1.88	1.74
F2 052	-	-	-	-	0.00
F2 054	-	-	-	-	0.00
F2 055	1.61	1.41	1.70	1.90	1.66
F2 056	1.43	2.16	1.41	3.45	2.11
F2 057	2.85	2.64	2.65	3.71	2.96
F2 058	2.53	2.45	3.35	.	2.78
F2 059	2.29	2.35	2.06	1.96	2.17
F2 060	2.61	2.35	2.09	2.14	2.30
F2 061	3.10	3.63	3.33	2.50	3.14
F2 062	-	-	-	-	0.00
F2 063	2.88	3.48	3.33	3.29	3.24
F2 064	1.94	2.01	2.31	3.36	2.41
F2 065	3.20	4.16	4.34	3.96	3.92
F2 066	1.05	1.01	0.93	0.79	0.94
F2 067	0.74	1.18	1.38	1.13	1.10
F2 068	2.56	3.25	2.41	-	2.74
F2 069	0.95	1.05	0.98	0.86	0.96
F2 070	2.33	2.69	3.08	2.38	2.62
F2 071	3.83	4.60	4.08	5.03	4.38
F2 072	2.49	2.81	2.79	3.16	2.81
F2 073	1.05	1.48	1.20	0.99	1.18
F2 074	1.06	1.26	1.01	1.09	1.11
F2 075	2.11	1.30	2.21	1.63	1.81
F2 076	3.73	3.41	4.43	4.36	3.98
F2 077	2.39	2.61	2.30	2.13	2.36
F2 078	3.93	3.39	4.01	3.89	3.80
F2 079	1.78	3.03	1.59	1.95	2.08
F2 080	0.81	1.18	1.33	1.15	1.12
F2 081	1.04	1.03	0.91	0.83	0.95
F2 082	2.03	1.44	1.84	2.29	1.90
F2 083	1.46	1.46	1.25	1.65	1.46
F2 084	2.44	2.78	2.79	2.26	2.57
F2 085	2.35	1.90	2.16	2.19	2.15
F2 086	2.18	2.84	2.56	2.15	2.43

Appendix 7 (Continued)

Lines	Spike1	Spike2	Spike3	Spike4	Total Avg.
F2 087	2.36	2.40	2.85	1.93	2.38
F2 088	5.39	3.63	3.94	-	4.32
F2 090	2.54	2.24	2.24	2.54	2.39
F2 091	1.28	1.16	1.29	1.03	1.19
F2 092	4.25	3.54	2.63	3.78	3.55
F2 093	1.31	1.89	1.65	1.56	1.60
F2 094	2.11	2.66	-	-	2.39
F2 095	1.95	3.13	2.00	2.06	2.28
F2 096	3.30	4.53	5.66	-	4.50
F2 097	1.18	1.03	0.99	0.78	0.99